Reprinted from THE LANCET, *August* 12, 1967, *pp.* 330-332

SPECIFIC INHIBITION OF THE PRAUSNITZ-KÜSTNER REACTION BY AN ATYPICAL HUMAN MYELOMA PROTEIN

D. R. STANWORTH
D.Sc. Birm.
OF THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY, UNIVERSITY OF BIRMINGHAM

J. H. HUMPHREY
M.D. Cantab., F.R.S.
OF THE NATIONAL INSTITUTE OF MEDICAL RESEARCH, MILL HILL, LONDON

H. BENNICH
M.B., B.Sc. Göteborg
OF THE INSTITUTE OF BIOCHEMISTRY, UNIVERSITY OF UPPSALA, SWEDEN

S. G. O. JOHANSSON
M.B. Uppsala
OF THE BLOOD CENTRE, UNIVERSITY HOSPITAL, UPPSALA, SWEDEN

Summary Results obtained from inhibition Prausnitz-Küstner (P.-K.) tests in a healthy person provide strong evidence of a similarity in biological activity between myeloma-IgND and human reagin (to horse-dandruff allergen). These findings are believed to mark an important turning-point in the elucidation of the mechanism of immediate hypersensitivity reactions.

Introduction

RECENT studies by Johansson and Bennich (1967a) of an atypical human myeloma globulin (myeloma-IgND), and by Johansson, Bennich, and Wide (1967,) of its counterpart in normal serum, have revealed the existence of a new class of immunoglobulin (tentatively designated IgND). Myeloma-IgND, which is characterised by an 8S sedimentation coefficient and fast electrophoretic mobility, appears to have no antigenic determinants in common with human $\alpha-$, $\delta-$, $\gamma-$ or $\mu-$ Ig polypeptide chains.

The resemblance of the physicochemical properties of

myeloma-IgND to those reported for skin-sensitising antibodies, its apparent inability to be transported through the placenta (Johansson and Bennich 1967b), and the detection by immunochemical means of a similar immunoglobulin at raised concentration (mean 1191 ng. per ml., range 133–5850 ng. per ml.) in sera of patients with asthma and hay-fever caused by proven allergy (Johansson and Bennich 1967b) as compared with the concentration found by Johnasson, Bennich, and Wide (1968) in normal human sera, suggested that it might represent a class of immunoglobulins associated with at least some kinds of skin-sensitising antibodies. This possibility seemed even likelier as a result of preliminary comparative immunodiffusion studies (Johansson, Bennich, and Ishizaka 1967) which indicated that γE-globulin (Ishizaka et al. 1966) might be antigenically related to myeloma-IgND.

Technical Details

The reagin-allergen system employed was the well-defined horse-dander system which has been used (Stanworth 1957, 1959, Stanworth and Kuhns 1965) in P.-K. testing for some years and which has proved safe in that it has never transferred hepatitis virus. The allergic serum (donated by their usual horse-dander-sensitive donor, " P.R.") was prepared by reconstituting material in sterile distilled water which had been stored for 19 days at 4°C in lyophilised form under nitrogen in a sealed ampoule. A horse-dandruff/protein mixture containing 10 mg. protein per ml. of saline solution, which had been stored frozen at −20°C, was used as challenging allergen. The control human IgG preparation, comprising only the 7S form as indicated by analytical ultracentrifugation, was obtained by gel-filtration (on ' Sephadex G200 ') of a Squibb " immune serum-globulin " preparation (kindly supplied by the American National Red Cross). All solutions were sterilised by filtration through cellulose-acetate membranes, in Swinny filters. The standard P.-K. test procedure, evolved as a result of previous extensive quantitative studies (Stanworth and Kuhns 1965), was employed throughout. In the first series of inhibition tests, duplicate sites on the back of a healthy control recipient were sensitised by intradermal 0·1 ml. injections of allergic serum —myeloma-IgND (or control) mixture. After an interval of 18 hours, these sites were challenged by pricking in the horse-dandruff/allergen solution with sterile sewing-needles. The outlines of the weals thus produced were traced on thin transparent sheeting, their areas being determined by the method of " counting squares ".

Results

The results obtained by determining the areas of weal

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

Test-solution no.	Composition one volume : one volume		Amount of added Ig injected (ng.)	Proportion of reagin-ND in total IgND injected* (%)	Results of duplicate P.-K. tests					
					10-min. weal areas (sq. mm.)			26-min. weal areas (sq. mm.)		
					1	2	mean	1	2	mean
1	Allergic serum (undiluted)	Saline solution	0	..	77	50	64	143	86	115
2	„	IgND (600 μg. ml.)	30,000	0·1	..	..	..	..	..	..
3	„	IgND (60 μg. ml.)	3000	1·0	..	..	..	..	..	..
4	„	IgND (6 μg. ml.)	300	9·0	10	13	12	28	44	36
5	„	IgG (600 μg. ml.)	30,000	0·1	43	44	44	119	106	113
6	„	IgG (60 μg. ml.)	3000	1·0	43	61	52	89	130	110

Allergic serum, " P.R."; Allergen, horse dander; normal recipient, " J.H."; test site, back; interval between transfer and challenge, 18 hours.
* Based on a mean (n = 6) level of 615 ng. ml. of IgND in the P.R. allergic serum, as estimated by the radioimmunosorbent assay.

tracings, 10 and 26 minutes after challenge, are shown in table I. Allergic serum containing myeloma-IgND (300 μg. or 30 μg. per ml.)—i.e., test solutions 2 and 3—failed to evoke any P.-K. response on subsequent challenge with horse-dander allergen. In contrast, pronounced weal reactions of similar size (after 26 minutes) were produced by samples of the allergic serum diluted to contain comparable amounts of normal human IgG (i.e., test solutions 5 and 6) and by the positive control (test solution 1) comprising allergic serum diluted with an equal volume of saline solution. On the other hand, the allergic serum containing the least amount of myeloma-IgND employed (3 μg. per ml.) only partly inhibited P.-K. activity. As can be seen from table I, such test solutions (4) evoked weals (after 26 minutes) which were about a third of the size of the weals evoked by the positive control. Hence, we deduced that the smallest amount of myeloma-IgND needed for complete inhibition of the P.-K. activity of the serum tested lay between 3 and 30 μg per ml. The mean level of IgND in the allergic serum was estimated to be 615 ng. per ml. (by means of a sensitive inhibition procedure, described in detail by Johansson, Bennich, and Wide [1968]) and based on the radioimmunosorbent technique of Wide and Porath (1966). From this it can be calculated that the proportion of reaginic IgND to total (reagin+myeloma) IgND at which P.-K. activity was completely inhibited lies between 1·0% and 9·0% (table I).

In a second series of duplicate tests on the forearms of the same control recipient, carried out at the same time as the first series of tests on the back (already described), passive transfer sites were first injected intradermally with 0·1 ml. of the various dilutions of myeloma-IgND, or IgG protein, or saline solution (table II). After 18 hours, each site (which had been carefully outlined with indelible ink) was injected intradermally with undiluted allergic serum (0·1 ml.). After a further 24 hours, each site was challenged by pricking in the horse-dandruff allergen solution (as already described). The weal areas, determined from 10-minute and 20-minute tracings (table II) demonstrated that the two most concentrated solutions (7 and 8) of myeloma-IgND completely inhibited subsequent sensitisation with the serum of the horse-dandruff-sensitive person, in contrast to the failure of transfer of IgG or saline solution to block subsequent P.-K. reactions. Moreover, the least concentrated myeloma-IgND solution

TABLE II—RESULTS OF P.-K. TESTING AT SITES INJECTED 18 HOURS PREVIOUSLY WITH Ig (OR SALINE SOLUTION)

Test solution no.	Composition of solution first injected (in duplicate as 0·1 ml. aliquots)	Ig-concentration (μg. protein/ml.)	Results of subsequent P.K. testing with undiluted allergic serum (24-hour 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
7	IgND	600	..	..	..	..	..	..
8	IgND	60	..	..	..	2	6	4
9	IgND	6	19	64	42	44	75	60
10	IgG	600	28	72	50	76	84	80
11	IgG	60	45	55	50	71	64	68
12	Saline	..	70	64	67	90	111	101

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

(9) (containing 6 μg. myeloma-IgND per ml.) was only partly successful in effecting inhibition.

Discussion

The reagin-binding tissue sites in the skin of the normal recipient appeared to be blocked by myeloma-IgND in both test systems. In the first case, this was presumably by the successful competition of myeloma-IgND for such sites with the reagin transferred simultaneously in the allergic-serum/myeloma-IgND mixtures. In the second experiment, there was additional evidence that myeloma-IgND had remained at the injection site for at least 24 hours in a concentration sufficient to prevent subsequent attachment of the reaginic antibody in the undiluted test-serum. The ability of myeloma-IgND to block the interaction of tissue-bound reagin with allergen was not tested during the experiments. However, further P.-K. testing in the same healthy control, " J.H." (in which sites passively sensitised with the serum of the horse-dandruff-sensitive subject were challenged with dander-allergen/myeloma-IgND mixtures or with allergen alone), gave no evidence of blocking at this stage of the P.-K. reaction. Hence, the myeloma-IgND shows similar biological activity to reagin, in that it has a strong affinity for the same sites in isologous tissue. We have no evidence whether or not it has specific antigen-binding activity as well. In this connection, it seems pertinent that the donor of the myeloma protein (patient N.D.) shows none of the symptoms of immediate hypersensitivity. With such a massive level of the tissue-binding myeloma protein (IgND) in his circulation, however, any reaginic antibodies he produced would be expected to be blocked similarly to passive skin-sensitisation (as already described).

The inhibition of P.-K. activity may have been caused by a trace contaminant in the myeloma-IgND preparation, although a comparison of the estimated contents of reaginic IgND and myeloma-IgND of the test mixtures (table I) suggests that this is unlikely. The smallest quantity of myeloma-IgND needed for complete inhibition of P.-K. activity—namely, between tenfold and hundred-fold the estimated level of 615 ng. per ml. of reaginic-IgND in the allergic serum used—appears to be consistent with inhibition being effected by the myeloma-IgND itself rather than by a minor constituent (undetected by sensitive immunodiffusion techniques). Obviously,

these observations should be confirmed by different test systems (incorporating, for instance, grass and ragweed pollen allergens) and by different assay procedures, such as passive in-vitro tissue and leucocyte sensitisation.

The implications of the findings are important; as further study of the readily available myeloma-IgND should undoubtedly throw considerable light on the structural peculiarities of reagins which, in turn, should greatly increase our understanding of the mode of production of these antibodies and the nature of their striking affinity for many tissues.

We are grateful to the donors of the allergic serum and myeloma-IgND, and to Mr. R. Keogh for help in the independent assessment of the weal reactions.

Requests for reprints should be addressed to D. R. S., University Department of Experimental Pathology, Medical School, Birmingham 15, England.

REFERENCES

Ishizaka, K., Ishizaka, T., Hornbrook, M. M. (1966) *J. Immun.* **97**, 840.
Johansson, S. G. O., Bennich, H. (1967a) *Immunology*, **13**, 381.
— — (1967b) *in* Nobel Symposium III (edited by J. Killander). Uppsala.
— — Ishizaka, T. (1967) Unpublished.
— — Wide, L. (1968) *Immunology* (in the press).
Stanworth, D. R. (1957) *Biochem J.* **65**, 582.
— (1959) *Immunology*. **2**, 384.
— Kuhns, W. J. (1965) *ibid.* **8**, 323.
Wide, L., Porath, J. (1966) *Biochem. biophys. Acta*, **130**, 257.

The Lancet Office,
7, Adam Street, Adelphi, London, W.C.2