

Research articles

A REAGIN-LIKE ANTIBODY IN HORSE SERUM: 1. OCCURRENCE AND SOME BIOLOGICAL PROPERTIES

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ABSTRACT

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The demonstration of a reagin-like antibody against Culicoides pulicaris extract in the serum of horses and ponies affected with recurrent seasonal dermatitis (sweet itch) is reported. This antibody can confer Prauznitz-Küstner (P-K) sensitivity on homologous skin for up to 5 days and, like human IgE, is thermolabile and susceptible to 2-mercaptoethanol reduction. It is eluted on diethylaminoethyl dextran-52 anion exchange chromatography independently of IgG, IgG(T) and IgM, and its elution characteristics indicate similarity in net molecular charge to human IgE.

The P-K response observed in horse skin is biphasic, and is morphologically similar to the late cutaneous anaphylactic response in man. Both phases of the P-K response are dependent upon the reagin-like antibody, although other serum factors appear involved in the delayed phase of the response.

INTRODUCTION

Human reagin (immunoglobulin E) is a passively transferable gamma globulin capable of sensitising circulating basophils and tissue mast cells and of mediating the release of intracellular vasoactive compounds upon subsequent antigen or antiglobulin challenge (Bennich et al., 1976). An IgE-like immunoglobulin has been described in a number of mammalian species (Neilsen, 1977). These immunoglobulins share a number of properties with human IgE, and in most instances satisfy the three orders of criteria proposed by Vaerman (1970) for the identification of interspecies protein homology. These are: immunological cross reactivity and amino acid homology, the association of a protein with a particular function, and a range of biological and physiological homologies.

Passive transfer of systemic and cutaneous anaphylactic hypersensitivity has been described in the horse (Ritzenthaler, 1924; Eyre, 1972; Schatzman et al., 1973) and, in the United Kingdom, recurrent seasonal dermatitis of horses (RSD) is believed to be a cutaneous hypersensitivity to the biting midge Culicoides pulicaris (McCaig and Mellor, 1974).

This paper reports on investigations into some biological and physicochemical properties of a reagin-like antibody found in the serum of RSD-affected horses and ponies. Evidence of antigenic cross reactivity between human IgE and an equine reagin-like antibody is the subject of a second paper.

MATERIALS AND METHODS

Serum

Serum (active stage serum) was obtained from 12 horses and ponies showing typical signs of RSD and from 7 horses and ponies unaffected with the disease but with a history of the disease during previous summers (inactive stage serum). Serum was stored at -20°C until used.

Antigen

20 mg of whole C. pulicaris was macerated in 5 ml sterile 0.01M phosphate buffered saline (pH 7.4) (PBS). After centrifugation, the supernatant was diluted 1:5 in sterile PBS and passed through a 0.22 μm membrane filter, giving a final protein concentration of 200 $\mu\text{g}/\text{ml}$. Aliquots of antigen were stored at -20°C until used.

Prauznitz-Küstner (P-K) test

0.1 ml of test serum was injected intradermally (i/d) over the clipped lateral cervical region of recipient horses having no known history of RSD. Sensitised sites were challenged 24 hours later by i/d injection of 0.1 ml of antigen, placed so that the centre of the resulting dermal bleb lay as close as possible to an ink spot marking the centre of the sensitisation bleb. Sites which were injected subcutaneously were excluded from further consideration. The horizontal diameters (mm) of the resulting reactions were measured at 5, 30, 60, 120, 360 minutes and 24 hours.

Foal serum and physiological saline were included as negative sensitisation controls. Antigen and PBS were included as negative challenge controls. To avoid error arising from variation in recipient each P-K procedure was carried out on a single animal.

Persistence of the reagin-like antibodies in homologous skin

Antigen challenge of sites that had been sensitised with pooled P-K positive serum was carried out 2, 4, 24, 48 hours and 5 and 9 days after sensitisation.

Heat and thiol sensitivity of the reagin-like antibodies

Each of two 0.5 ml aliquots of pooled P-K positive serum was either heated to 56°C for 2 hours or dialysed against 250 ml of 0.1M 2-mercaptoethanol (2-ME) for 3 hours at room temperature. The latter was subsequently dialysed against 500 ml 0.02M iodoacetamide for 4 hours at room temperature and then against PBS for 24 hours at 4°C. Control serum was dialysed against iodoacetamide and PBS only.

The heat and 2-ME treated sera and controls were examined for P-K activity.

Elution of reagin-like activity from horse serum using ion-exchange chromatography

After dialysis against equilibrating buffer, 5 ml of pooled P-K positive serum was applied to a 1.5 cm x 25 cm diethylaminoethyl dextran-52 (DEAE-52) cellulose anion exchange column equilibrated with 0.04M Tris-HCl (pH 8.0). A Tris-HCl gradient of 0.04M (pH 8.0) to 0.5M (pH 8.0) was applied to the column over 16 hours giving a flow rate of 40 ml per hour. Individual protein peaks were collected, concentrated by evaporation to approximately 0.5 ml and dialysed against PBS at 4°C for 48 hours. After passage through a 0.22 µm membrane filter, the peaks were examined for P-K activity.

The distribution of IgG, IgM and IgG(T) in the column eluate was determined by slide double immunodiffusion employing 1% agar in 0.1M Tris-HCl (pH 8.0). IgG_{ab} and IgG_c were differentiated by their mobility after immunoelectrophoresis against anti-whole horse serum (Allen and Dalton, 1975). Immunoelectrophoresis was carried out as described by Hirschfeld (1960).

RESULTS

Praunizt-Küstner activity of sera from cases of equine recurrent seasonal dermatitis

Following antigen challenge, weal-like reactions were observed at 8 of the 12 sites sensitised with active stage sera. These reactions were apparent within 30-60 minutes and continued to enlarge for up to 6 hours after challenge. In all cases they had disappeared by 24 hours. Only transient dermal blebs were observed at the four remaining sites sensitised with active stage sera, the sites sensitised with inactive stage sera and the control sites. The mean and standard

deviation (SD) of the horizontal diameters of the dermal reactions observed at the 8 responding active stage sites (Group A), the 4 non-responding active stage sites (Group B) and the 7 inactive stage sites (Group C) are shown in Table 1 along with the horizontal diameters of the reactions at the control sites.

McPherson et al. (1979) deemed positive those responses following direct i/d challenge of horses where the diameter exceeded the mean plus 2 SD of the control diameters. In the present study, the reactions at sites sensitised with Group A sera (Table 1) were in excess of the mean plus 2 SD of the reactions at the sites sensitised with inactive stage sera (Group C). The former were deemed positive P-K responses, indicative of reagin-like antibodies in the sensitising sera. The responses at sites sensitised with Group B sera (Table 1) did not exceed the mean plus 2 SD of the Group C sera sensitised sites and were deemed negative responses. Reactions at Group B and Group C sera sensitised sites did not differ from those at the negative control sites.

Persistence of the reagin-like antibodies in homologous skin

Typical positive P-K responses were elicited up to 5 days after sensitisation. A negative response was elicited 9 days after sensitisation.

Heat and thiol sensitivity of the reagin-like antibodies

The effects of heat and 2-ME reduction-alkylation on the P-K activity of pooled P-K positive serum are shown in Table 2. Both treatments resulted in the elimination of the P-K response. Alkylation alone had no effect on the P-K activity.

Elution of reagin-like activity from horse serum using ion-exchange chromatography

The distribution of positive P-K and immunoglobulin activity in the DEAE fractions of horse serum is shown in Figure 1. P-K activity was eluted in fractions 2-6, over a molar range of 0.05M - 0.14M Tris-HCl (pH 8.0). This activity was independent of the major immunoglobulin classes. However, the P-K responses at sites sensitised with DEAE fractions 2-6 reached a maximum 30-60 minutes after antigen challenge and did not progress from that time. At sites sensitised by fractions 2, 3 and 5 the reactions had dispersed by 360 minutes.

DISCUSSION

The occurrence of positive P-K responses at skin sites sensitised with active stage RSD sera after challenge with midge (C. pulicaris) extract indicates the

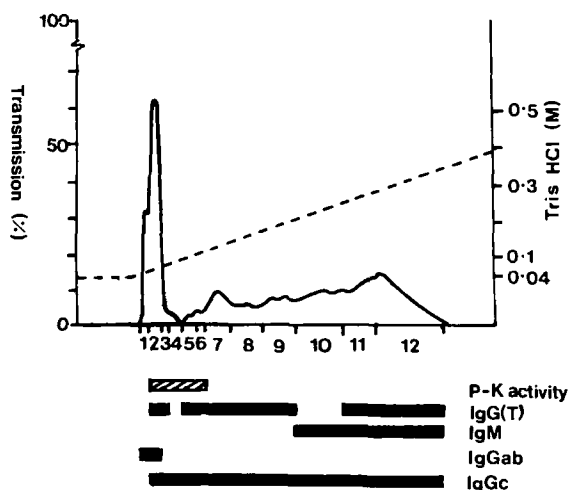


Fig. 1. Elution of Prauznitz-Kustner (P-K) activity and immunoglobulin classes Gab, Gc, M and G(T) from pooled P-K positive horse serum on DEAE-52 chromatography using a linear Tris-HCl (pH 8.0) gradient.

presence of reagin-like antibodies to the midge antigen in the sera of these affected animals. This is in accordance with the earlier observations of Baker (1978) on a single case of RSD and of Riek (1954) on a number of horses naturally hypersensitised to the bite of the midge C. robertsi.

The magnitude of the positive P-K responses are similar to those resulting from both direct i/d challenge of sensitised horses with fungal antigens (Halliwell et al., 1979; McPherson et al., 1979) and from P-K testing of sensitised horse serum and Parascaris equorum antigen (Mansman and Mansman, 1975). The persistence of swellings at negative test or negative control sites for up to 6 hours has been reported during both P-K and direct i/d testing of horses (Schatzman et al., 1973; McPherson et al., 1979) and probably reflects the extreme sensitivity of horse skin to relatively innocuous challenge noted by De Weck (1972).

The reaginic antibody in horse serum shares a number of physiological and biological properties with human IgE in accordance with Vaerman's third criterion of interspecies protein homology. The horse reagin remained detectable in

TABLE I

Mean and standard deviation of the horizontal diameters (mm) of dermal responses at Praunnitz-Küstner test sites and the horizontal diameters (mm) at control sites

Time (min)	Test Sites			Control Sites			
	Active stage serum Responding sites (Group A: n = 8)	Active stage serum Non-responding sites (Group B: n = 4)	Inactive stage serum (Group C: n = 7)	Sensitisation		Challenge	
				Saline	Foal serum	Antigen	PBS
5	11.6 ± 1.07	12.0 ± 1.63	11.9 ± 1.35	11	11	10	11
30	16.7 ± 1.58	12.0 ± 2.16	12.9 ± 1.07	12	12	9	10
60	16.5 ± 1.31	12.0 ± 1.41	11.7 ± 0.76	13	13	9	9
120	21.1 ± 2.47	13.0 ± 1.15	13.3 ± 0.95	D	14	D	D
360	22.9 ± 1.96	D	D	D	D	D	D
24 hr	D	D	D	D	D	D	D

D: reactions dispersing PBS: phosphate buffered saline

TABLE II

Heat and 2-ME reduction-alkylation of pooled P-K positive serum. Horizontal diameters (mm) of dermal responses following antigen challenge of sites sensitised with treated and untreated serum; reactions at control sites are also shown

Time (min)	Test Sites				Control Sites			
	Untreated P-K positive serum	56°C for 2 hours	2-ME/iodoacetamide	Iodoacetamide alone	Sensitisation		Challenge	
					Saline	Foal serum	Antigen	PBS
5	10	11	12	11	10	10	9	10
30	16	11	14	15	12	11	9	10
60	16	12	14	18	D	10	10	11
120	20	14	15	22	D	12	D	D
360	21	14	D	22	D	12	D	D
24 hr	D	D	D	D	D	D	D	D

D: reactions dispersing PBS: phosphate buffered saline

homologous skin for between 5 and 9 days. Although this is less than the latent period reported for the reaginic antibody in other species (Stanworth, 1973), the exponential decay of both reaginic antibody activity and IgE in homologous skin (Augustin, 1967; Ishizaka and Ishizaka, 1971) indicates that the duration of detectable levels will be dependent upon the initial antibody titre. Thus interspecies variation in latency is to be expected.

Like human IgE (Dorrington and Bennich, 1973), horse reagin is thermolabile at 56°C and is sensitive to thiol reduction. In contrast, IgG antibodies are typically thermostable at 56°C (Bryant et al., 1975) and retain their biological activity when dialysed against thiol reducing agents at neutral pH (Stanworth and Turner, 1973).

P-K activity in the DEAE-cellulose eluate was detected overlapping in part the major IgG peak and extending into trailing IgG fraction, but not overlapping the IgM fraction. After DEAE-Sephadex chromatography of whole reaginic human serum or isolated immunoglobulin fractions using Tris-HCl (pH 8.0) buffer, the P-K activity is similarly distributed about the IgG and IgM peaks and is eluted in the molar range 0.1M - 0.3M (Ishizaka and Ishizaka, 1967; Johansson et al., 1968; Perelmutter and Liakopoulou, 1971). Although the Sephadex chromatography medium used by these authors differs from the cellulose medium used in the present study, the DEAE anion exchange group is the same and the similarity in elution characteristics of the homocytotropic antibody in horse and human serum, particularly in relation to the major immunoglobulin classes, indicates some similarity in net molecular charge.

The distribution of detectable P-K activity after DEAE chromatography of horse serum does not parallel that of the immunoglobulin classes Gab, Gc, G(T) and M, suggesting that this activity resides outside these classes. However, the possibility remains that the equine homocytotropic activity may be associated with the remaining immunoglobulin classes, namely IgA, AI (aggregating immunoglobulin) and 10S_{Y1} immunoglobulin. The respective biological roles of AI and the 10S_{Y1} immunoglobulin have not been elucidated. However, the latter immunoglobulin, occurring in anti-β lactoside sera, is sensitive to 2-mercaptoethanol reduction (Raynaud and Iskaki, 1970) and probably merits further investigation of its relationship with the reagin-like antibody.

Unlike the classical weal and flare response in human skin, the P-K response in horse skin elicited using whole serum appears biphasic. The initial weal-like response observed within 30 - 60 minutes is followed by a period during which the dermal response increases in size for up to 6 hours. Similar reactions have been observed in horse skin following both i/d challenge of sensitised individuals with parasitic antigens and i/d challenge with artificial mast cell degranulating compounds (Mansman and Mansman, 1975). Whether the prolonged phase of the P-K responses observed in the present study is the result of an Arthus-type response

mediated by non-homocytotropic antibodies is not known. However, the elimination of both phases of the P-K response following heating and thiol reduction of the sensitising serum suggests the entire response is dependent upon reaginic-type antibodies. Furthermore, the persistence of a biphasic response up to 5 days after sensitisation suggests that non-cytotropic IgG antibodies play no role in the genesis of the response, the estimated half-life of these antibodies in skin being 8-12 hours (Kuhns, 1961). The occurrence of isolated 30-60 minute P-K responses at sites sensitised by DEAE-52 peaks 2-6 indicates, however, that one or more additional serum factors may be involved in the genesis of the 2-6 hour phase of the reagin dependent response. Alternatively, should the delayed phase of the P-K response be dependent on high titres of sensitising antibody, then dilution and loss of the antibody during DEAE fractionation may have resulted in insufficient amounts of the antibody in the final fractions to initiate the delayed response.

The biphasic P-K response in horse skin is morphologically similar to the IgE dependent late cutaneous anaphylactic response (LCAR) in man, typically elicited by direct i/d challenge of allergic individuals with high doses of allergen (Umemoto et al., 1976). Less intense LCARs have been observed following P-K testing of human serum in homologous skin (Dolovich et al., 1973).

The absence of positive P-K responses at 4 sites sensitised with active stage RSD sera may have arisen due to the absence of detectable levels of reagin-like antibodies in the sensitising serum, or from the presence of antigen specific blocking antibodies arising from challenge during the previous summer. Antigen specific IgG antibodies have been induced in man following prolonged antigen exposure (Devey et al., 1976), although their biological role in the inhibition of reagin dependent hypersensitivity responses is not fully understood (Ishizaka and Ishizaka, 1973). Fisher and Connel (1962) have shown that antigen specific blocking antibodies in human sera can confer anaphylactic sensitivity on heterologous skin. In the horse, Cordal and Margni (1974) have demonstrated the occurrence of IgG antibodies in hyperimmune serum which are capable of conferring short-term anaphylactic sensitivity on heterologous skin, suggesting that horses may produce an antibody functionally similar to a human IgG blocking antibody.

The results of this study have shown the occurrence of a reagin-like antibody in horse serum functionally similar to human IgE, and sharing a number of biological properties in common with human IgE. In these respects this antibody fulfils Vaerman's second and third criteria of interspecies protein homology, suggesting the antibody may correspond to human IgE.

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