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Detection of IgE-binding *Onchocerca volvulus* antigens after electrophoretic transfer and immuno-enzyme reaction[1]

Short communication

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IgE antibodies to filarial antigens can be quantified in sera of infected individuals using solid phase radioimmunoassays (Hamilton et al., 1981; Weiss et al., 1981). By cross-absorption experiments we explored the relative species specificities of the IgE and IgG antibody response to filarial infections in man (Weiss et al., 1982). From this study we concluded that IgE antibodies are more species-specific than IgG antibodies. Therefore, we became interested to analyze the IgE response not only quantitatively using a crude antigen preparation but also qualitatively by studying the IgE reactivity of selected sera against fractionated antigens. For this purpose *Onchocerca volvulus* antigens (a high and a low molecular weight fraction) were separated on SDS-gels, blotted electrophoretically onto nitrocellulose sheets and finally detected by an immuno-enzyme technique using a β-galactosidase labelled anti-human IgE antiserum.

Methodology

The *O. volvulus* antigens were prepared from adult worms after collagenase digestion of frozen nodules (Weiss et al., 1982). Crude extracts were dialyzed against 7 mM phosphate buffer pH 7.2 containing 18 mM NaCl, centrifuged (100,000 *g*, 2 h, 4° C), and the supernatant concentrated on an ultrafiltration membrane (PM 10, Amicon Corp.) to approx. 4 mg/ml protein (Biorad assay). An aliquot of this antigen was separated by ultrafiltration (XM 100A membrane, Amicon) into a low and a high molecular weight fraction (cut off approx. 100,000 daltons). Before electrophoresis, antigens were mixed with sample buffer (60 mM Tris-HCl pH 6.8, containing 79 mM SDS and 5% β-mer-

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captoethanol) and boiled for 5 min. Samples were run on SDS-polyacrylamide slab gels (separating gel: T = 12.5%, C = 0.83%; stacking gel: T = 4.0%, C = 3.33%; gel thickness 1.5 mm) at a protein concentration of 20 μg per mm of sample well width. For further details see Takacs (1979). The electrophoretic blotting procedure was as described by Towbin et al. (1979). Antigens were transferred to nitrocellulose membranes (HAWP 304FO, Millipore Corp.) using a Trans-Blot cell (BIO-RAD lab.) at 3.75 V/cm for 3 h followed by 7.5 V/cm (overnight). One part of the blot including molecular weight markers (LMW kit, Pharmacia AB) was stained with amidoblack. The blot was soaked in a 1% solution of polyvinylpyrrolidon in RAST-buffer (PBS pH 7.5, containing 0.4% Tween 20, 0.2% bovine serum albumin, 0.05% sodium azide) to saturate additional binding sites. Between each step of the following enzyme-immunoassay the nitrocellulose strips (0.6 $\times$ 10.5 cm) were carefully washed with RAST-buffer (3 $\times$ 10 min). Strips were incubated for 4 h (at room temperature) with approx. 200 μl of various human sera (diluted 1 in 2 with RAST-buffer), followed by a second incubation with a β-galactosidase labelled anti-human IgE antiserum (Phadezym RAST, Pharmacia Diagnostics) overnight (approx. 16 h) at room temperature. The strips were then covered with 1% agarose (in PBS pH 7.4 supplemented with 1 mM $MgCl_2$) containing 0.5 mg/ml 5-Bromo-4-chloro-indolyl-β-D-galactopyranoside (Sigma) as a substrate and incubated for 4 to 6 h at 37° C to reveal IgE-binding antigens (method by H. Schröder, Pharmacia Diagnostics). The enzyme reaction was stopped with 0.1 M sodium-glycine pH 10.4.

Alternatively, IgE binding antigens have also been detected autoradiographically using the same but 125-I labelled antihuman IgE antiserum. However, we preferred the enzyme-immuno method which turned out to be more sensitive, more reliable and less time-consuming than autoradiography.

Results

Patterns of the IgE-reactivity for some selected sera against the blotted high and low molecular weight fractions of *O. volvulus* antigens are given in Figs. 1 and 2. To allow comparison of serum reactivities, strips have been cut from the same transfer-membrane. Selected onchocerciasis sera with high RAST reactivities revealed complex patterns of up to 24 resp. 20 bands with the high and low molecular weight fractions (Figs. 1 and 2, b, c, e, f). Maximum reactivity has been found for a pool of selected sera from Tanzania (Figs. 1 and 2, b). Sera from noninfected donors with normal IgE levels showed faint background staining when incubation time for the enzyme reaction exceeded 6 h (Figs. 1 and 2, i). For better photographic documentation the enzyme reaction shown in Figs. 1 and 2 has been stopped after 18 h. To identify the specificity of fractionated antigens, selected filariasis sera from onchocerciasis-free areas, but highly reactive in RAST using a crude *O. volvulus* antigen, were tested (Figs. 1

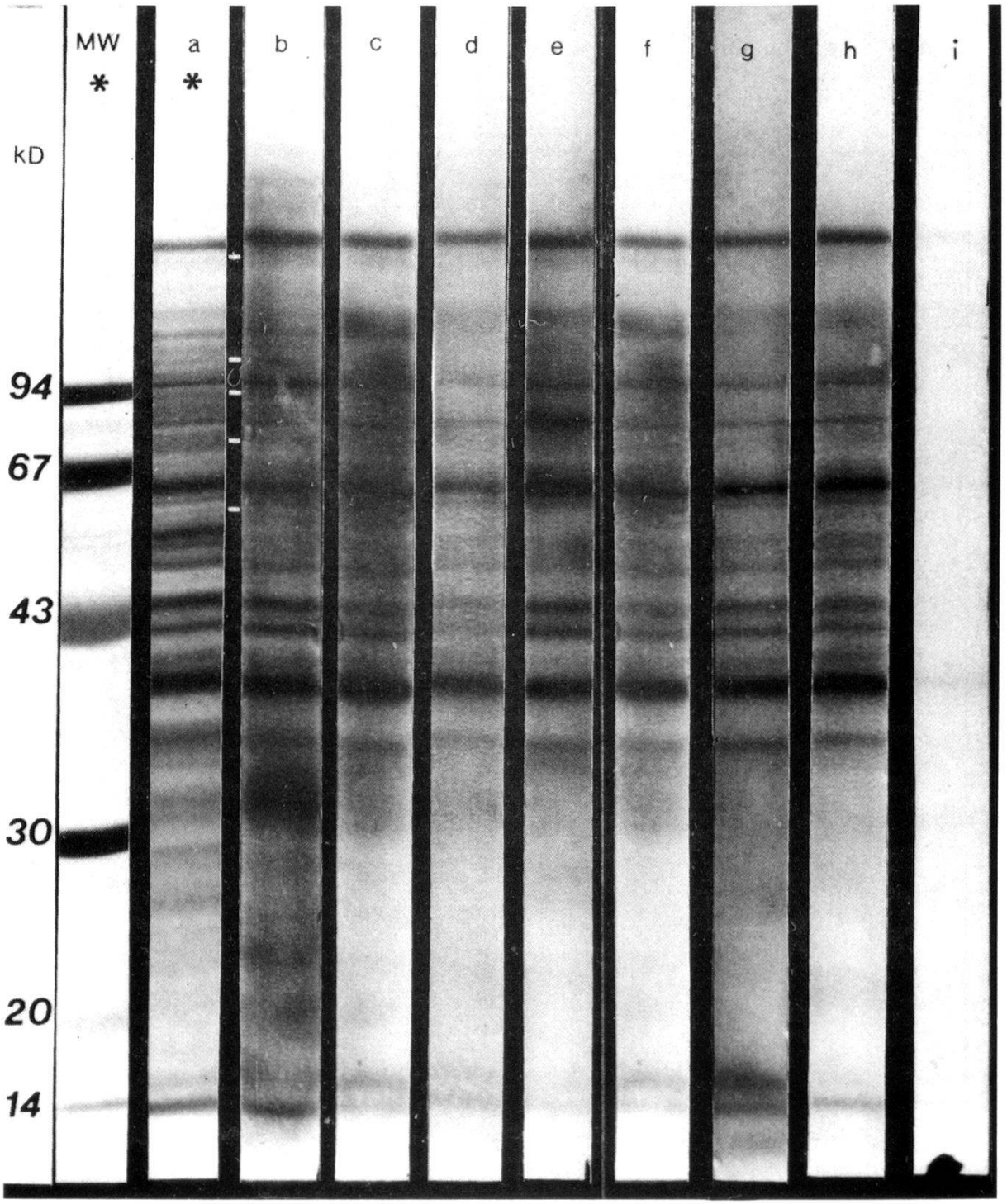

Fig. 1. Binding of IgE-antibodies to *O. volvulus* antigen (high molecular weight fraction). Amido-black staining (*) for molecular weight markers (MW) and antigen (a). Enzyme-reaction for serum pools (b-d) and individual sera (e-i).

b Onchocerciasis (4 sera, incl. serum e), from Tanzania; 19,000 I.U. IgE/ml
c Onchocerciasis (7 sera), from Cameroon: 16,000 I.U. IgE/ml
d Lymphatic filariasis (7 sera, incl. serum h), from India; 3,700 I.U. IgE/ml
e Onchocerciasis (individual serum of pool b), from Tanzania; 40,000 I.U. IgE/ml
f Onchocerciasis, Swiss returning from Cameroon; 2,200 I.U. IgE/ml
g Tropical pulmonary eosinophilia, from Bangla Desh, >20,000 I.U. IgE/ml
h Hydrocoele (individual serum of pool d), from India, 20,000 I.U. IgE/ml
i negative control, Swiss resident, 120 I.U. IgE/ml.

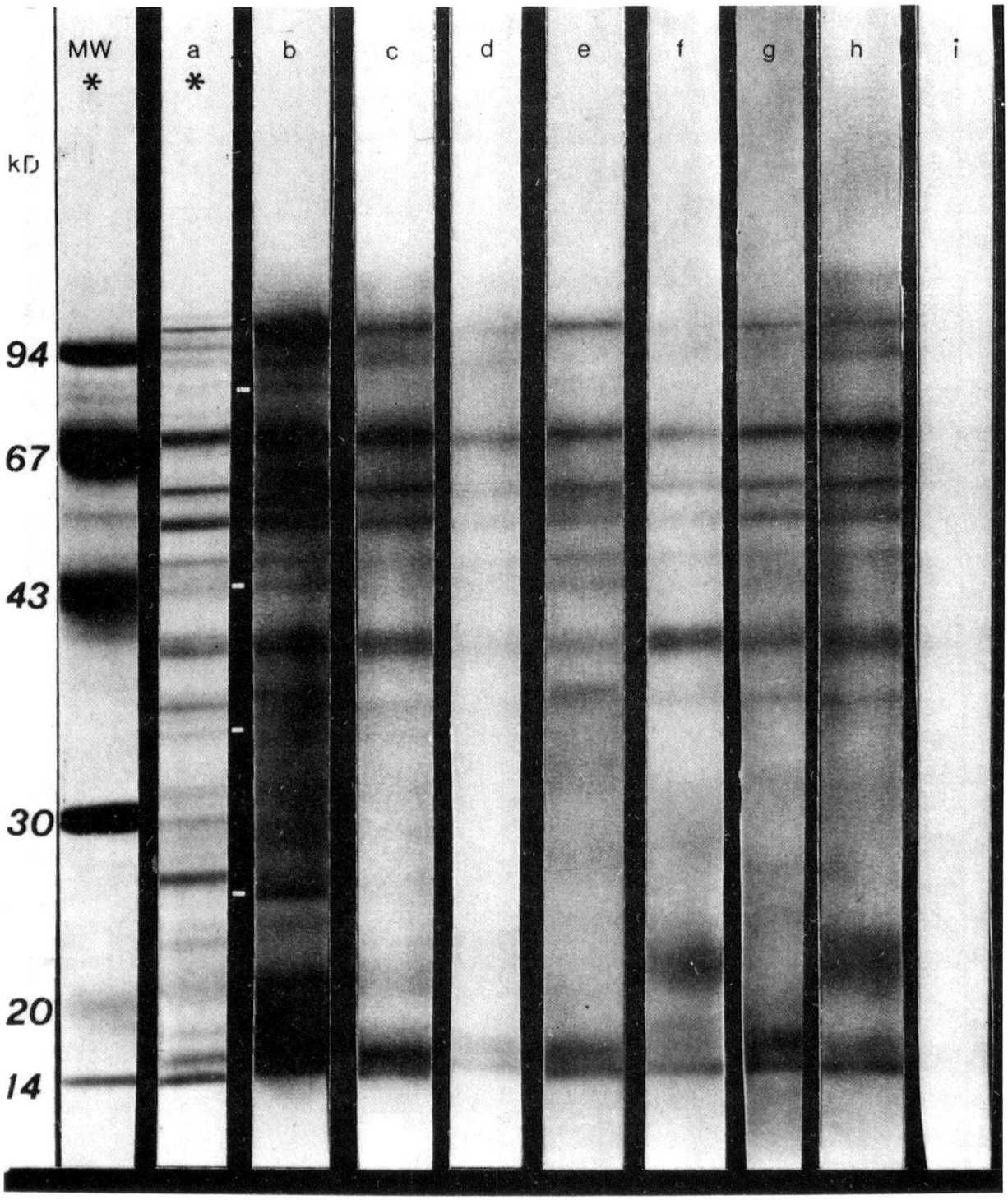

Fig. 2. Binding of IgE-antibodies to *O. volvulus* antigen (low molecular weight fraction). For details see Fig. 1.

and 2, d, g, h). IgE-antibodies of these sera reacted with the majority of the IgE-binding antigens revealed by onchocerciasis sera. Only few of these antigens (4 in the low, 5 in the high molecular weight fraction) gave no reaction with one of the few lymphatic filariasis sera tested so far (indicated by white marks between strips a and b on Figs. 1 and 2). In addition, some of these "specific"

antigen bands were only minor serological antigens detectable in serum pools but not in individual onchocerciasis sera (e.g. Fig. 2, f).

These preliminary results indicate for the first time that the allergen composition of adult *O. volvulus* worm extracts is very complex. Further studies, using different approaches for antigen fractionation, are needed to demonstrate if *Onchocerca*-specific allergens can be isolated. The described method does not only allow to monitor antigen fractions for immunodiagnostic relevant allergens but also to analyse comparatively individual IgE responses during helminth infections and therapy as well as to investigate the degree of cross-reactivity between allergens of taxonomically related helminth species.

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