

Characterization of IgE and IgG Antibody Responses to *Dermatophagoides farinae* in Rats

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Rats (Brown Norway/Wistar Fu) that were pretreated or not pretreated with cyclophosphamide were immunized with varying amounts of *Dermatophagoides farinae* allergen extract with alum as adjuvant. Sera were analysed by RAST and by crossed radioimmuno-electrophoresis (CRIE). The highest IgE antibody responses were recorded in animals pretreated with cyclophosphamide that had received low immunizing doses of antigen. The IgE antibody pattern in CRIE was, however, not influenced by the dose of antigen or by cyclophosphamide pretreatment. The IgG pattern in CRIE closely resembled the IgE pattern, demonstrating a similar specificity of the antibody responses of the two isotypes. This indicates non-class specific control of the specificity of antibody responses.

Key words: CRIE; *Dermatophagoides farinae*; IgE; IgG; rat.

Accepted for publication 2 August 1984

Complex substances, e.g. pollens, animal danders and mould spores, containing a variety of different antigens are common causes of allergy. Some of the antigens may induce synthesis of IgE antibodies in humans and they have in a more restricted sense been defined as allergens (1). It has been suggested that allergens are characterized by special structural features (5, 29). The now predominant view is, however, that they are protein antigens, usually of a molecular weight ranging between 10000 and 65000 (1, 21), but otherwise without any special characteristics.

The genetic regulation of antibody responses may be non-class specific (26). The close correlation obtained between IgG and IgE antibody responses to purified allergens (7), as well as recent work in the human genetic field (22), lend support to this interpretation. This means that the IgG and IgE antibody responses in an individual would be expected directed against similar structures. We have studied this, comparing mite antigen specific IgE and IgG anti-

body responses in rats by crossed radioimmuno-electrophoresis (CRIE). The antibody responses in the animals were also compared with those found in allergic humans.

MATERIAL AND METHODS

Animals

F₁ hybrid rats of congenic rat strains (Brown Norway/Wistar Fu), known to be good IgE responders, were obtained from Professor O. Sjögren, Institute of Tumour Immunology, University of Lund, Sweden.

D. farinae extract

Isolated and crushed *D. farinae* mites (Allergon AB, Sweden) were extracted overnight at 6°C as a 2.5% (w/v) solution in a buffer of 0.125 mol/l ammonium bicarbonate with 2% v/v n-butanol (pH 8.0, 25°C). The suspension was centrifuged (27000 g for 1 h at 0°C) and fil-

tered (Acropore 0.8 μ). The clarified filtrate was stored freeze-dried in vials. The protein content was about 20% of the dry weight.

Immunization and bleedings of the rats

The rats were divided into two groups of eight animals each. Four rats in each group received cyclophosphamide 50 mg/kg body weight intraperitoneally (i.p.) one day before the primary immunization. This was given as a subcutaneous (s.c.) injection in the neck with the allergen extract dissolved in 0.25 ml 0.13% aluminium hydroxide. The given dosages of protein were 30 μ g to the rats in one group and 1 mg to those in the other. These injections were repeated 3 weeks later. After another 4 weeks 1 μ g protein in aluminium hydroxide was given as a second booster injection to all animals. Retro-orbital bleedings were performed under light ether anaesthesia 7 days after the last injection. The sera were frozen and analysed individually.

Anti-rat IgE and anti-rat IgG antibodies

Antibodies with rat Fc-IgE specificity, isolated from rabbit antiserum against rat IgE myeloma IR162 (4) were used. Sepharose coupled rat IgG, F(ab')₂ fragments from rat IgE myeloma IR2 and intact IgE myeloma IR2 were used for the isolation procedure (18). Anti-rat IgG specific antibodies were obtained by immunosorbent purification using IgG as the antigen. The isolated antibodies were radiolabelled using the chloramine-T procedure (16). These antisera were obtained from Dr. Torbjörn Karlsson, Biocell AB, Uppsala, Sweden.

Radioallergosorbent test (RAST)

This test was performed as previously described with sera diluted 1/10 (24) and commercially available Phadebas RAST® (Pharmacia Diagnostics AB, Sweden) discs. IgE antibody levels were arbitrarily expressed as the ratio between the radioactive uptake on discs incubated with test serum and that on discs incubated with a pooled control serum from non-immunized animals.

Rabbit antiserum to D. farinae

Rabbit antiserum to *D. farinae* was prepared basically as described by Harboe & Ingild (14) using the crude *D. farinae* extract at a concentration of about 50 g/l dry weight for immunization of three rabbits. The antiserum used in this investigation showed close similarity to that described by Dale & Landmark (11).

Crossed immunoelectrophoresis (CIE)

CIE was performed essentially as described by Weeke (33), using 1% agarose gel for immunoelectrophoresis, 170–3003, (BIO-RAD Laboratories, California, USA) containing a buffer of 0.08 M Tris, 0.024 M Tricine, 0.3 M calcium lactate and 0.02% sodium azide (pH 8.6, 22°C). The first and second dimension electrophoreses were performed at 12°C at 10 V $\times$ cm⁻¹ and at 2 V $\times$ cm⁻¹, respectively. CIE was performed on two types of glass plates, 5 $\times$ 7 cm and 3.5 $\times$ 5 cm, using the following gel:

Glass plate 5 $\times$ 7 cm: (5 $\times$ 3.5 $\times$ 0.11), (5 $\times$ 1 $\times$ 0.17), (5 $\times$ 1 $\times$ 0.19) and (5 $\times$ 1.5 $\times$ 0.13) cm, respectively, denoted from the anode. 500 μ l and 250 μ l of anti-*D. farinae* were added to the gel sections 1 and 4 from the anode. 135 μ g crude *D. farinae* in 15 μ l was applied for the first dimension electrophoresis. The first and second dimension electrophoreses were run for 33 min and 19 h respectively.

Glass plate 3.5 $\times$ 5 cm: (3.5 $\times$ 2.5 $\times$ 0.11), (3.5 $\times$ 0.7 $\times$ 0.17), (3.5 $\times$ 0.8 $\times$ 0.18) and (3.5 $\times$ 1.0 $\times$ 0.13) cm, respectively, denoted from the anode. 250 μ l anti-*D. farinae* was added to the gel section 1 and 4 from the anode. 72 μ g crude *D. farinae* in 8 μ l was applied for the first dimension electrophoresis. The first and second dimension electrophoreses were run for 24 min and for 19 h, respectively, giving immunoprecipitation patterns corresponding to those obtained on the 5 $\times$ 7 cm plate in miniature.

The slides were pressed, washed and dried without heating. They were then stored dry at 5°C until used and stained with Coomassie Bril-

liant Blue® after completion of the autoradiography.

Crossed radioimmuno-electrophoresis (CRIE)

Allergen-specific IgE detection. The CRIE was performed essentially as previously described (24), but with a higher serum concentration. Briefly, the 3.5×5 cm CIE plates were incubated in small plastic boxes over night in 2 ml serum from a single rat diluted in 2 ml of a phosphate-dextran-Tween (PDT) buffer solution containing 0.05 mol/l phosphate buffer, pH 7.4, with 0.3% Tween and 0.3% dextran 10. After incubation, the plates were washed three times for 10 min using 10% PDT buffer solution in 0.9% sodium chloride. The plates were then incubated in ^{125}I -anti-rat IgE $\sim 72,000$ cpm/ml in buffer over night and the washing procedure was repeated. After drying the plates, autoradiography was performed with the rapid procedure (32) using Du Pont Cronex-4 X-ray film and Du Pont lightning plus X-ray intensifying screen in Kodak X-Omatic cassette at low temperature (-70°C), and developed. Serial exposures were performed and the intensity of the radiostaining was compared with that of reference discs in a reference system.

Reference system for IgE-CRIE. The reference system was based on similar principles as previously described by Aukrust & Aas (3). Briefly, a system analogous to the Phadebas PRIST® in humans was used with rat-F(ab')₂-specific rabbit antibodies bound to paper discs as reference discs. As reference serum, a pool from five non-immunized rats diluted 1/5 was used. Radiostaining of the precipitates was compared with that of the reference discs and semiquantified in CRIE classes (A–G).

Allergen specific IgG detection. Rat IgG-CRIE was performed as previously described in detail for human sera (25) except that no reference system was used. Briefly, the 5×7 cm CIE plates were incubated in 7 ml test serum diluted 1/200. The second incubation step was performed with ^{125}I -labelled anti-rat IgG $\sim 72,000$ cpm/ml. As the negative control, serum from a pool of non-immunized rats was used. Washes

and autoradiography were performed as described for IgE-CRIE. Exposures were performed 4, 8 and 16 h and radiostaining of the precipitates was judged to be weak or strong.

RESULTS

The results of the rat CRIE and RAST assays after immunization with low doses of mite extract with and without pretreatment with cyclophosphamide are shown in Table 1. Pretreatment with cyclophosphamide gave the highest serum levels of mite-specific IgE antibodies. This pretreatment, however, did not influence the IgE responses qualitatively. Thus, all sera gave specific radiostaining of precipitates 2, 9, 19/20 and 30. Radiostaining of precipitates 10/11, 14 and 22 was also found but was not with certainty regarded to be specific. Since these precipitates were almost entirely enclosed in the precipitates of antigens 9 and 30, which both caused heavy radiostaining, there was a possibility of influence due to coprecipitation, i.e. incomplete separation. Such influence seemed likely in the case of antigen 22, since the faint degree of radiostaining of the small, unenclosed part of antigen 22 was weak and not clearly distinguishable from that of the background obtained with a negative serum.

The results obtained with sera from animals immunized with 1 mg of allergen are shown in Table 2. The levels of mite-specific IgE antibodies, as well as IgE antibodies against the individual components, were lower in these animals than in those immunized with low doses of antigen. Cyclophosphamide pretreatment did not influence the IgE responses as in the animals receiving low doses of antigen. Specific IgE antibody responses were found to precipitates 9, 19/20 and 30. For similar reasons as mentioned above, interpretation of the specificity of the radiostaining to precipitates 10/11, 14 and 22 was difficult, but that of antigen 22 was judged to be specific in one rat (rat No. 16). In this case the radiostaining of the enclosed antigen 22 was slightly heavier than that of antigen 30.

In Table 3 the rat IgE responses are summarized and related to those detected in

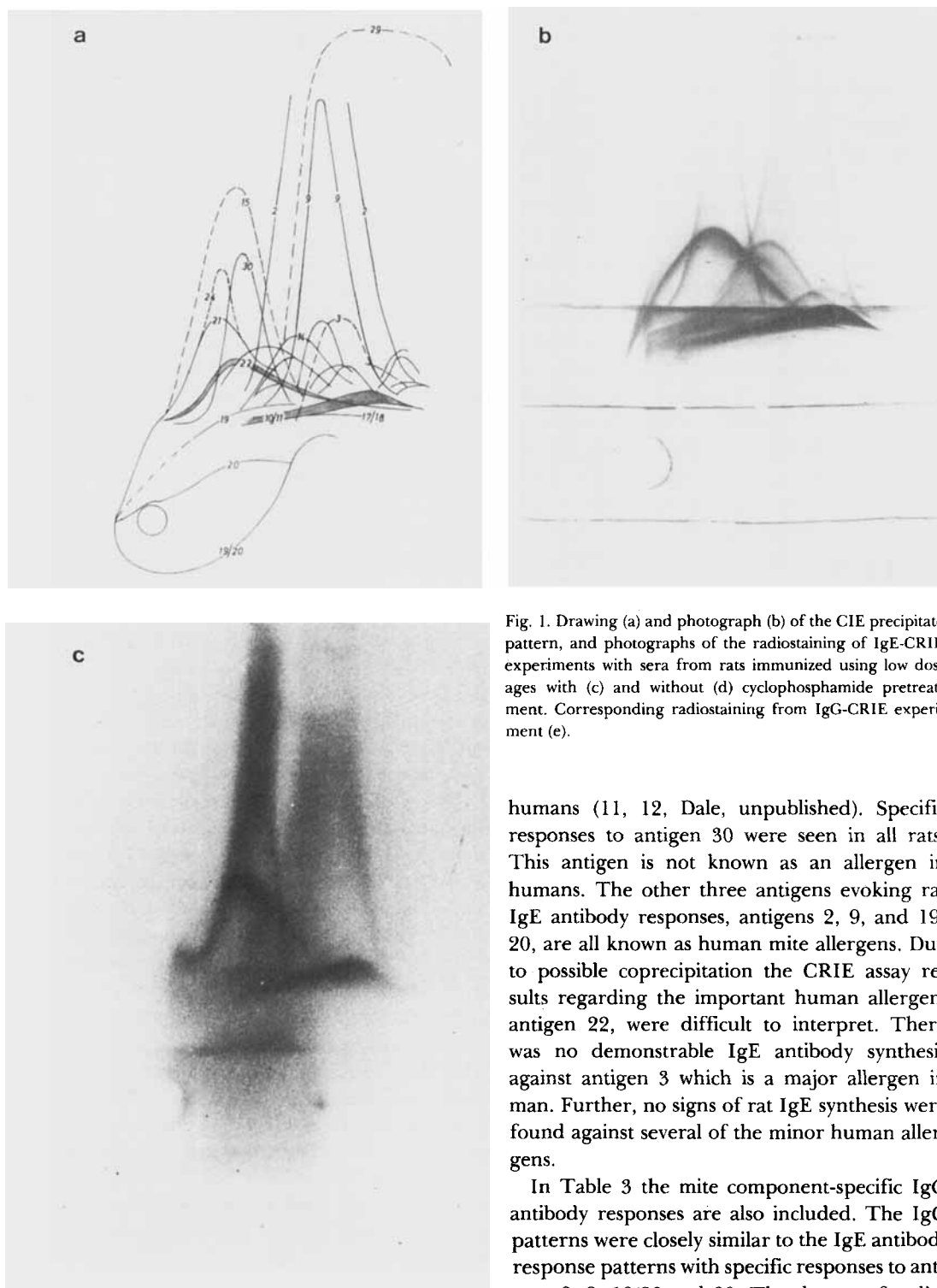
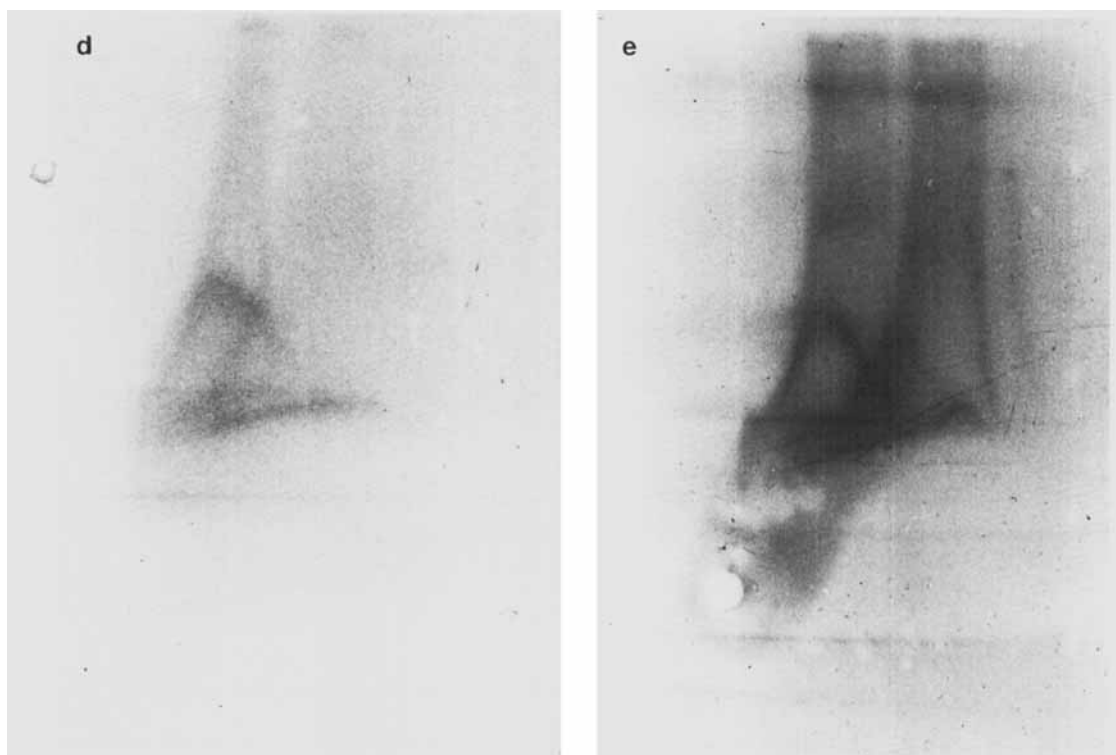


Fig. 1. Drawing (a) and photograph (b) of the CIE precipitate pattern, and photographs of the radiostaining of IgE-CRIE experiments with sera from rats immunized using low dosages with (c) and without (d) cyclophosphamide pretreatment. Corresponding radiostaining from IgG-CRIE experiment (e).

humans (11, 12, Dale, unpublished). Specific responses to antigen 30 were seen in all rats. This antigen is not known as an allergen in humans. The other three antigens evoking rat IgE antibody responses, antigens 2, 9, and 19/20, are all known as human mite allergens. Due to possible coprecipitation the CRIE assay results regarding the important human allergen, antigen 22, were difficult to interpret. There was no demonstrable IgE antibody synthesis against antigen 3 which is a major allergen in man. Further, no signs of rat IgE synthesis were found against several of the minor human allergens.

In Table 3 the mite component-specific IgG antibody responses are also included. The IgG patterns were closely similar to the IgE antibody response patterns with specific responses to antigens 2, 9, 19/20 and 30. The degree of radiostaining and the antibody specificities were not



influenced by dose of antigen or by pretreatment with cyclophosphamide. Similar to the evaluation of the IgE responses, the specificity of the radiostaining of precipitates 14 and 22 was difficult to evaluate, but probably at least some of the radiostaining of antigen 22 was specific.

Fig. 1 shows a reference drawing (1a) and a photograph (1b) of the antigens of the CIE plates and photographs of radiostaining obtained in IgE-CRIE experiments using sera from rats immunized with the low dose scheme with (1c) and without (1d) cyclophosphamide pretreatment. Fig. 1e shows the corresponding radiostaining from an IgG-CRIE experiment.

DISCUSSION

Low doses of the antigen and pretreatment with cyclophosphamide favoured IgE antibody syn-

thesis in the rats. This is in agreement with data obtained previously by other workers in the field (9, 17, 19). The immune responses in the rodents were only partly similar to those in atopic humans. Antigen 22 in this CIE system of *Dermatophagoides farinae* most probably represents the major allergen, shown to be partially identical with allergen P1, the most important *Dermatophagoides pteronyssinus* allergen (8, 15, 20). The rat response to this antigen and to antigen 3, which is also a major human allergen, was slight or absent. Thus, even though there was some overlapping in the rodent IgE pattern as compared with that in humans, distinct differences were registered. This contrasts with our previous findings in rodents using timothy as antigen (24). The strong rat IgE response to *Dermatophagoides farinae* antigen 9 is of particular interest since this antigen is known to crossreact with a scabies antigen and

Table 1

Mite-specific IgE antibody responses in rats immunized 1 mg + 1 mg + 1 µg *Dermatophagoides farinae* extract in alum. A–G denote CRIE classes, see text

Rat No.	Cy-pre-treatment	RAST value ^a	Mite antigen number, CRIE class						
			2	9	10/11	14	19/20	22	30
1	–	2.2	G	F	(E)	(G)	F	(E)	E
2	–	2.3	E	D	(D)	(G)	F	(E)	D
3	–	1.9	H	F	(E)	(G)	F	(E)	E
4	–	2.2	H	F	(E)	(G)	F	(F)	F
5	+	6.8	F	C	(C)	(D)	E	(C)	D
6	+	7.9	F	D	(D)	(E)	D	(D)	C
7	+	3.1	F	D	(D)	(H)	D	(E)	C
8	+	2.4	F	E	(E)	(G)	F	(E)	E
Control serum			–	H	H	H	H	G	H

^a Ratio radioactive uptake on discs incubated with test serum/control serum.

Table 2

Mite-specific IgE antibody responses in rats immunized with 30 + 30 + 1 µg *Dermatophagoides farinae* extract in alum. A–G denote CRIE classes, see text

Rat No.	Cy-pre-treatment	RAST value ^a	Mite antigen number, CRIE class						
			2	9	10/11	14	19/20	22	30
9	–	2.4	–	G	(G)	–	H		G
10	–	2.1	–	G	(G)	–	G		H
11	–	3.5	–	F	(E)	(E)	F	(F)	H
12	–	1.9	–	G	(G)	–	G		H
13	+	1.9	–	E	(E)	–	H		E
14	+	2.2	–	F	(E)	–	G	(G)	F
15	+	2.2	–	E	(E)	–	E	(H)	G
16	+	1.1	–	E	(F)	–	–	F	H
Control serum				H	H	H	H	G	H

^a Ratio radioactive uptake on discs incubated with test serum/control.

Table 3

Rat IgE and IgG antibody responses in CRIE to mite antigens compared with IgE antibody responses described in humans

			1	2	3	4	5	6	7	8	9	10/11	12	13	14	15	16	17	18	19/20	21	22	23	24	25	26	27	28	29	30
Human			Mi	I	Ma	I	Mi				Mi				Mi	Mi	I			Ma	Ma	Mi								Mi
Rat	IgE		W								S	?			?					W	W?									S
	IgG		W								S	?			?					W	W?									S

Mi = minor; MA = major; I = intermediate; S = strong; W = weak.

scabies infestations may induce IgE antibody responses (12).

There was a close similarity between the rat IgG and IgE specificities to mite allergens in CRIE. In previous studies quantitative correlations between IgG and IgE antibody responses to particular antigens have been reported in humans (7, 22). Genetic analyses of immune responses in atopic patients (22) have shown, that the linkage between HLA DW 2 and immune response to the ragweed antigen Ra5 was similar for the IgG and the IgE isotypes. Our data in rats support these findings in humans and indicate a non-class-specific genetic control of the humoral immune response. This uniformity may not be restricted to the humoral immune response but include cellular responses as well (23). Thus, IgE antibody responses can be seen as only one part of the immune responses to potent protein antigens.

IgE antibody responses may appear early during immunization processes as a normal part of the immune response to protein antigens (2). In atopic patients IgE antibody responses are strong and long-lasting, which may be a consequence of defective suppression of the IgE synthesis (27). This situation is mimicked by pretreatment of animals with cyto-statics or by whole body irradiation, which are both thought to induce a defective suppressor T-cell activity (10, 30, 31). Although the magnitude of the IgE response was affected by pretreatment with cyclophosphamide it was not possible to manipulate the specificity of the response.

In atopic humans, non-genetic factors are known to influence the phenotypic expression of the individual IgE response and clinical allergy. Examples of such are month of birth (28), virus infections (13), degree of exposition (1, 21), exposure to cigarette smoke and to other air pollutants (34). The different expressions of atopic sensitization of the different members of atopic families may partly be explained by such influences which stand out most clearly in studies of monozygous twins (6).

In summary, our findings demonstrate a close similarity in the IgG and IgE antibody responses to a parenterally administered com-

plex mixture of antigens. This indicates that allergens may be strong antigens capable of inducing antibody responses in all immunoglobulin classes.

ACKNOWLEDGEMENT

This work was supported by a grant to L.N. from Meda/Dome, Gothenburg, Sweden.

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