J Clin Pathol: Mol Pathol 1995;48:M342–M346

Circulating IgG autoanti-IgE antibodies in atopic patients block the binding of IgE to its low affinity receptor (CD23)

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Abstract

***Aims*—To investigate the ability of circulating IgG autoanti-IgE antibodies from atopic rhinitis patients to block the binding of IgE to its low affinity receptor (FcεRII), also termed CD23.**

***Methods*—This involved the use of a well validated flow cytometric method to detect inhibition of FITC labelled IgE binding to human B cells expressing CD23 (RPMI 8866 cell line).**

***Results*—Taking inhibition values greater than 20% as being significant, 15 out of 20 IgG anti-IgE containing sera inhibited the binding of IgE-FITC to the RPMI 8866 cells. The inhibitory effect was recoverable in the IgG fraction of serum, but was not related to the titre of either IgG1 anti-IgE or IgG4 anti-IgE, thus suggesting that it might be related to epitope specificity. No such inhibition was demonstrable with rheumatoid sera containing autoanti-IgG (that is, rheumatoid factor), but lacking autoanti-IgE.**

***Conclusions*—The capacity of anti-IgE to block the binding of IgE to CD23 has important implications, particularly in terms of upregulation of IgE synthesis and the consequent perpetuation of the inflammatory response.**

(*J Clin Pathol: Mol Pathol* 1995:**48**:M342–M346)

Keywords: Atopy, autoanti-IgE, CD23, IgE.

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Accepted for publication
21 July 1995

The low affinity receptor for IgE (FcεRII), also known as CD23, is found on a variety of cells, including lymphocytes, eosinophils, neutrophils, platelets, and macrophages. The binding of IgE to these cells and the subsequent interaction of receptor bound IgE with allergen leads to cellular activation and release of mediators of chronic allergic inflammation. The expression of CD23 on B lymphocytes is of particular importance, since it is involved in the regulation of IgE synthesis.[1 2] When CD23 is not occupied by IgE it undergoes proteolytic cleavage, releasing soluble CD23 (sCD23) fragments, which are known to upregulate IgE synthesis in an autocrine fashion.[3] Thus the binding of IgE to CD23 not only protects the receptor against proteolytic cleavage, but also allows membrane CD23 to deliver a negative IgE regulatory signal to the B lymphocyte.[4] Therefore the interaction of naturally occurring IgG autoanti-IgE antibodies with IgE could potentially enhance IgE synthesis by disrupting this vital regulatory mechanism.

IgG autoanti-IgE is detectable in the serum of individuals showing enhanced IgE production, namely those with allergic asthma,[5] allergic rhinitis,[6] and parasitic infestations.[7] Investigations of the subclass profile of IgG autoanti-IgE have revealed that most of the activity is found in IgG1 and IgG4, with IgG4 showing a disproportionately high percentage of the activity.[8] The autoantibody response appears to be directed against epitopes located within either the Cε2–Cε3 interdomain region (aa301–339) or the Cε4 domain of IgE.[9]

We have previously shown that autoanti-IgE of the IgG1 subclass blocks the binding of IgE to the high affinity receptor (FcεRI), found on mast cells, basophils, and eosinophils.[10] Given that IgE binds to both types of receptor through sites within its Cε3 domain,[11] it would be interesting to examine the effect of autoanti-IgE on IgE binding to CD23. The availability of a human lymphoblastoid B cell line expressing CD23 (RPMI 8866 cells) provides a direct system for studying such molecular interactions by flow cytometry. In this study we demonstrate the ability of IgG anti-IgE containing sera, obtained from atopic rhinitis patients, to block the binding of IgE to CD23.

Methods

ANTIBODY REAGENTS

Mouse monoclonal (mAb) anti-human IgE antibodies were obtained from Dr P E Brenchly, Department of Immunology, St Mary's Hospital, Manchester, UK (clones E1 and E3), Dr S Harada, Shionogi Institute for Medical Science, Osaka, Japan (clones HE-35A, HE-5B, HE-69B, HE-50H and HE-56A), and Prof B M Stadler, Institute of Clinical Immunology, Bern, Switzerland (clones BSW17 and Le27). The plasma of an IgE myeloma patient (WT) was kindly provided by Prof D R Stanworth, Peptide Therapeutics Ltd, Birmingham, UK. The mouse anti-human IgG mAb antibodies [anti-IgG1 (clone NL-16), anti-IgG2 (clone GOM1), anti-IgG3 (clone ZG4), and anti-IgG4 (clone GB7B)] were purchased from Unipath (Basingstoke, UK). The alkaline phosphatase conjugated goat anti-mouse IgG was purchased from Sigma (Poole, UK). The FITC labelled mAb anti-human CD23 (clone Bu38) was purchased from The Binding Site Ltd (Birmingham, UK). The recombinant human IgE-Fc domains were kindly provided by Dr B Sutton, Molecular Biology

and Biophysics Group, The Randall Institute, King's College, London, UK. The single IgE domains were expressed in *E coli* and refolded; the IgE-Fc fragment (Cε2–Cε4) was expressed in NSO cells and was glycated as described elsewhere.[12]

MAPPING THE EPITOPE LOCATION OF mAb ANTI-IgE

A range of chimaeric mouse-human IgE antibodies was used for mapping the epitope location of anti-IgE antibody. The chimaeric antibodies were constructed, as described elsewhere,[13] by interchanging constant region domains between human and mouse IgE anti-NIP molecules. This epitope mapping strategy is based on the fact that mAb anti-human IgE does not react with the equivalent domains of mouse IgE.

Wells of Nunc Maxisorp microtitre plates were coated with 100 µl of 10 mg/ml NIP-BSA (bovine serum albumin) in 0·05 M carbonate-bicarbonate buffer pH 9·6 and incubated overnight at 4°C. The plate was washed three times with phosphate buffered saline (PBS)/Tween20 pH 7·2 buffer and 100 µl aliquots of 1:10 dilution of the chimaeric mouse-human IgE anti-NIP antibodies were added to the appropriate wells and the plate incubated for one hour at 37°C. The plate was then washed and 100 µl of mAb anti-human IgE was added to each well and the plate incubated for one hour at 37°C. The plate was washed three times, 100 µl of alkaline phosphatase conjugated goat anti-mouse IgG (1/500) was added to each well, and the enzymatic colour reaction was developed as described elsewhere.[57]

PURIFICATION OF HUMAN IgE (WT)

IgE was isolated from plasma by euglobulin precipitation and affinity chromatography. The purity of the IgE preparation was assessed by immunoelectrophoresis, and its concentration determined by a Milenia IgE kinetic EIA (DPC Ltd, Gwynedd, UK).

FITC LABELLING OF IgE

One millilitre of the IgE protein (2·5 mg/ml) was incubated for three hours at 37°C with FITC isomer 1 on 10% Celite (Calbiochem, Nottingham, UK) in 0·15 M sodium chloride containing 0·1 M sodium hydrogen carbonate. The samples were passed through a Sephadex G-25 column (Pharmacia, Milton Keynes, UK) to remove unbound FITC. Fractions containing FITC labelled IgE were collected and pooled, and the FITC to protein ratio was determined by measuring optical density at 280 nm and 495 nm.

IgG SUBCLASS ANTI-IgE MEASUREMENT

IgG1 and IgG4 anti-IgE antibodies were measured in serum samples using a previously described enzyme linked immunoassay (ELISA).[57] Briefly, the procedure involved incubating a 1/5 dilution of serum on IgE or IgE-Fc coated microtitre plates and detecting bound IgG1 and IgG4 anti-IgE with mouse monoclonal anti-human IgG1 and IgG4, followed by alkaline phosphatase conjugated goat anti-mouse IgG. Results were expressed in OD405 units by taking the mean value of duplicate estimations, corrected for non-specific binding (that is, samples added to wells not coated with IgE).

TOTAL IgE ASSAY

Total IgE levels were measured in serum using a Milenia IgE kinetic EIA (DPC Ltd, Gwynedd, UK).

SERUM SAMPLES

Blood samples were obtained, as detailed elsewhere,[6] from atopic rhinitis patients attending the ENT outpatient clinic at University Hospital, Queen's Medical Centre, Nottingham. Samples which were found to be positive for IgG anti-IgE were used in the IgE blocking experiments (see below). Sera from rheumatoid arthritis patients containing autoantibodies to IgG (that is, rheumatoid factor) were used as controls. Such rheumatoid factor positive sera did not have detectable levels of IgG anti-IgE.

CELL CULTURES

RPMI 8866 (kindly provided by Prof J Gordon, University of Birmingham, UK) and Raji (European Collection of Animal Cell Cultures, Porton Down, Salisbury, UK) cells were cultured in RPMI 1640 containing 10% heat inactivated fetal calf serum (FCS) (Sigma) and 100 U/ml penicillin + 100 µg/ml streptomycin. Both cell lines were cultured and split 1/3 every 2 d. Binding studies were carried out in PBS containing 1% BSA, 0·1% azide (NaN_3), and 1 mM $CaCl_2$ at a cell concentration of 10^6/ml.

EXPRESSION OF CD23 ON THE RPMI 8866 CELLS

A 100 µl volume of 10µg/ml FITC labelled IgE was incubated for one hour at 37°C, with 100 µl of either RPMI 8866 (with or without treatment with 2 µg of anti-CD23 mAb) or Raji cells (10^6 cells/ml) and 100 µl of incubation buffer, and then washed with 2 ml of PBS containing 0·1% azide (to prevent capping). Unlabelled IgE was used to assess autofluorescence. The cells were resuspended and fixed in 0·3 ml Sysmex cellpack containing 0·5% methanal. Cells were collected on a FACScan (Becton Dickinson, Oxford, UK) with a linear fluorescence setting of 660 V. The fluorescence (FL1) profile versus forward scatter (FSC) was used to monitor the cells, the amplification scale was altered according to the level of fluorescence. For each sample 4000 events were collected and then analysed using the flowMATE program (Dako, High Wycombe, UK).

DEMONSTRATION OF THE IgE BINDING SPECIFICITY OF THE RPMI 8866 CELLS

A 50 µl volume of 20 µg/ml (that is, $5{\cdot}32 \times 10^{-8}$ M) FITC labelled IgE (WT) was mixed with 50 µl of $10{\cdot}64$–$1{\cdot}33 \times 10^{-8}$ M of either unlabelled human IgE or recombinant human epsilon constant region domains (Cε2–Cε4, Cε2, Cε3) before incubation for one hour at 37°C, with 100 µl of RPMI 8866 cells (10^6 cells/ml) and 100 µl of PBS containing 0·1% BSA. The cells were subsequently prepared for FACS analysis, as described above. Furthermore, to ascertain the IgE binding specificity of RPMI 8866 cells, blocking experiments were performed whereby 100 µl volume of 10 µg/ml FITC labelled IgE (WT) was incubated for one hour at 37°C, with 1·0 or 0·5 µg of a range of antihuman IgE mAb and four anti-human IgG subclass mAb (controls). Each mixture was then incubated for one hour at 37°C with 100 µl of RPMI 8866 cells (10^6 cells/ml). The cells were subsequently prepared for FACS analysis as described previously.

THE USE OF IgG ANTI-IgE CONTAINING SERA TO BLOCK IgE-FITC UPTAKE BY RPMI 8866 CELLS.

Serum (100 µl) was incubated for 1 h at 37°C with 100 µl of IgE-FITC (5 µg/ml) and the mixture was then incubated for one hour at 37°C with 100 µl of RPMI 8866 cells (10^6 cells/ml), after which the cells were prepared for FACS analysis as described earlier. In subsequent blocking assays IgE-FITC was used at a concentration of 0·5 µg/ml.

PURIFICATION OF AUTOANTI-IgE CONTAINING IgG FRACTIONS

Euglobulin fractions were prepared from sera of rhinitis patients with high titres of IgG anti-IgE by precipitation with 33% saturated solution of ammonium sulphate. The IgG fraction was isolated on protein G-Sepharose and the preparations were tested for anti-IgE activity by ELISA.

INVESTIGATION OF THE EFFECT OF ANTI-IgE mAb AND HUMAN IgG ANTI-IgE CONTAINING SERA ON RPMI 8866 BOUND IgE-FITC

A 100 µl volume of 5 µg/ml FITC labelled IgE was incubated for one hour at 37°C with 100 µl of RPMI 8866 cells (10^6 cells/ml); cells were washed and then treated for 30 minutes at 37°C with 1 µg of mAb anti-IgE or 100 µl of serum. The cells were then prepared for FACS analysis as described earlier.

STATISTICAL ANALYSIS

The Spearman correlation was used to test for the existence of any correlation between IgG subclass anti-IgE titres, or IgE levels, and the ability to inhibit the binding of FITC labelled IgE to the RPMI 8866 cells.

Results

IgE BINDING SPECIFICITY OF RPMI 8866 CELLS

A dose-response curve was produced from a plot of the median channel number against the concentration of IgE-FITC per 10^5 RPMI 8866 cells. The observed increase in fluorescence was due to the binding of FITC-IgE to the cells, as no such increase in fluorescence was seen with unlabelled IgE. There was no significant binding of IgE-FITC to the Raji cells at antibody concentrations below 1 µg/ml per 10^5 cells. In addition, the binding of IgE-FITC to the RPMI 8866 cells was shown to be due to uptake by CD23, as this effect was completely blocked by previous treatment of the cells with anti-CD23 mAb (fig 1).

The specificity of the interaction of IgE with the RPMI 8866 cells was further ascertained in a competitive assay using purified human IgE and recombinant epsilon constant region domains.The binding of IgE-FITC to the RPMI 8866 cells was inhibited in a dose-dependent manner by unlabelled human IgE (WT) and by a recombinant fragment spanning

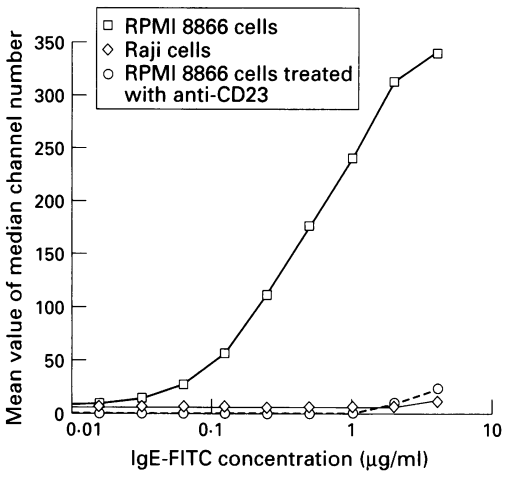

Figure 1 The IgE binding specificity of the RPMI 8866 B cell line. The dose-response curve shows the uptake of IgE-FITC by CD23 on B cells. No such binding was demonstrable by the Raji cells, or when the B cells were pretreated with anti-CD23 mAb (clone Bu38).

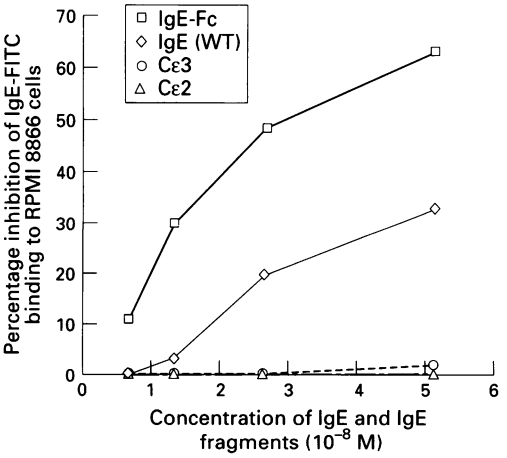

Figure 2 Dose-dependent inhibition of binding of IgE-FITC to the RPMI 8866 B cell line using unlabelled IgE (WT) and a recombinant IgE-Fc (Cε2–Cε4). No such inhibition was demonstrable with recombinant Cε2 or Cε3 fragments.

Table 1 The effects of anti-IgE mAb and anti-IgG1-4 mAb (i.e. tested at two concentrations) on IgE-FITC binding to the RPMI 8866 cells. The results show inhibition (−) and enhancement (+) of IgE-FITC binding

		Effect on IgE-FITC binding (%)	
Monoclonal antibody	*Domain specificity*	*1·0 μg*	*0·5 μg*
HE-69B	Cε2	+93	+212
HE-5B	Cε2	+140	+187
BSW17	Cε2	−93	−90
HE-35A	Cε3	−94	−91
E3	Cε3	−54	−10
Le27	Cε4	+164	+117
HE-50H	Cε4	+33	+73
HE-56A	Cε4	+74	+122
E1	Cε4	+74	+69
NL16	Cγ2–Cγ3	−10	−8
GOM1	Cγ2–Cγ3	−4	−4
ZG4	Cγ2–Cγ3	−8	−3
GB7B	Cγ2–Cγ3	−6	−3

the Fc portion of IgE (that is, Cε2–Cε4). However, the binding of IgE to the RPMI 8866 cells was not affected by adding the same molar concentrations of either recombinant Cε2 or Cε3 domains (fig 2).

MODULATION OF IgE-FITC BINDING TO RPMI 8866 CELLS WITH ANTI-IgE mAb

The monoclonal anti-human IgE antibodies were tested for their reactivity with a range of chimaeric IgE anti-NIP, constructed by interchanging constant region domains between human and mouse IgE molecules. This has shown that the anti-IgE mAb recognise epitopes located within the Cε2 (HE-69B, HE-5B, and BSW17), Cε3 (HE-35A and E3), or Cε4 (E1, Le27, and HE-50H) domains respectively (table 1).

Anti-IgE mAb showed two opposing effects on IgE-FITC binding to the RPMI 8866 cells (table 1): three inhibited (HE-35A, E3, and BSW17) and five enhanced (E1, Le27, HE-50H, HE-69B, and HE-5B) the binding of IgE. These modulatory effects appeared to be related to the IgE domain specificity of the antibodies, with the inhibitory property being exhibited mainly by those recognising epitopes within Cε3. None of the four anti-human IgG subclass mAb affected the binding of IgE-FITC to the RPMI 8866 cells.

BLOCKING OF IgE-FITC BINDING TO RPMI 8866 CELLS WITH SERA CONTAINING IgG ANTI-IgE

IgE-FITC was routinely used at a concentration of 0·5 μg/ml per 10^5 cells. This concentration was chosen because it falls within the linear part of the binding curve (fig 1), thus ensuring optimal sensitivity of the assay.

Twenty rhinitis patients' sera (containing anti-IgE) and 15 rheumatoid sera (containing anti-IgG but lacking anti-IgE) were investigated for their effect on the binding of IgE-FITC to the RPMI 8866 cells. Up to 20% inhibition was obtained with the control (rheumatoid) sera (range 8–20%; mean 16%). Taking inhibition values greater than 20% as significant, 15 out of 20 IgG anti-IgE containing rhinitis sera inhibited the binding of IgE-FITC to the RPMI 8866 cells (range 3–48%; mean 31%). This inhibition was found to be independent of the levels of IgE, IgG1 anti-IgE, and IgG4 anti-IgE in the sera tested (table 2). Affinity purification of anti-IgE from sera of rhinitis patients, giving more than 40% inhibition, showed that the IgE blocking activity is fully recoverable in the IgG fraction of three (CD, DG, and LY) out of four serum samples tested.

EFFECT OF ANTI-IgE mAb AND HUMAN IgG ANTI-IgE CONTAINING SERA ON CELL BOUND IgE-FITC

Addition of mAb anti-IgE to RPMI 8866 cells which had already taken up IgE-FITC resulted in increased fluorescence of the cells. Such increase in detection of cell bound IgE-FITC was shown by all six (HE-56A, HE-5B, HE-50H, HE-69A, HE-35A, and E3) anti-IgE mAb that were tested (range 54–168%; mean 119%). However, the level of cell bound IgE-FITC was not affected by any of the eight IgG anti-IgE containing sera that were tested.

Discussion

The close proximity between the site on IgE to which CD23 binds (Cε3 domain)[14 15] and those recognised by circulating IgG anti-IgE

Table 2 Percentage inhibition of IgE-FITC binding to RPMI 8866 cells with IgG anti-IgE containing rhinitis sera

Patient	*IgG1 anti-IgE (OD_{405})*	*Ig4 anti-IgE (OD_{405})*	*IgE (IU ml^{-1})*	*Inhibition of IgE-FITC binding (%)*
PG	0·690	0·000	84·1	29
PF	0·176	0·053	34·2	10
JK	0·472	0·359	103·1	20
SJ	0·080	0·272	20·1	35
SS	0·028	0·125	395·3	38
SJ	0·191	0·425	53·0	38
DM	0·120	0·130	27·7	41
CD	0·135	0·354	44·4	48
KM	0·038	0·152	56·5	38
JC	0·057	0·215	20·1	12
LY	0·459	0·046	216·1	48
MC	0·424	0·222	195·8	15
ZK	0·042	0·239	20·1	12
RZ	1·033	0·114	385·8	26
HG	0·158	0·218	97·1	46
RO	0·166	0·355	45·2	26
CC	0·108	0·051	125·5	44
DG	0·229	0·239	401·0	45
AS	0·225	0·270	41·1	42
JR	0·286	0·015	56·6	3

antibodies (Cε2–Cε3 interface and Cε4 domain)[9] has prompted us to examine the effect of this autoantibody on the binding of IgE to CD23. These interactions were explored by flow cytometry using a human B cell line (RPMI 8866) expressing CD23.

We have initially demonstrated the IgE binding specificity of the B cell line. In addition, we have shown that the binding of IgE to CD23 occurs through the Fc portion of IgE, as evidenced by the competitive inhibition experiment using recombinant human IgE-Fc. As expected, such inhibition was not demonstrable with recombinant Cε2 or Cε3 domains because, although the CD23 binding site is located within the Cε3 domain,[14 15] the structural integrity of the entire Fc region is apparently required for receptor engagement.[11] The IgE binding specificity of the B cells was further ascertained by showing that a range of mouse monoclonal anti-human IgE, but not anti-human IgG, was able to inhibit this interaction.

Monoclonal anti-human IgE showed two different effects on IgE binding to the B cells: three inhibited and five enhanced the binding of IgE. These modulatory effects appeared to be related to the domain specificity of the anti-IgE, the inhibitory property being shown mainly by antibodies recognising epitopes within Cε3, which contains the CD23 binding site.[14 15] Presumably the inhibitory effect was a result of direct or steric blocking of the CD23 binding site on IgE, whereas the enhancing effect was caused by the formation of IgE anti-IgE complexes favouring binding of IgE to CD23. The latter effect may also have been responsible for the enhancement seen when the anti-IgE mAb were added to B cells that had already taken up IgE-FITC.

Having established the validity of our experimental system, we tested 20 serum samples, taken from rhinitis patients with positive titres of IgG1 or IgG4 anti-IgE, or both, for their ability to block the binding of IgE-FITC to the B cells. This revealed significant inhibition (greater than 20%) in 15 out of 20 sera tested. The inhibitory effect was recoverable in the IgG fraction of three out of four serum samples tested. However, the inhibitory effect was not related to the titres of IgG1 anti-IgE, IgG4 anti-IgE, or IgE, thus suggesting that it might be related to epitope specificity of the anti-IgE.[9] None of the 15 rheumatoid sera tested, containing anti-IgG but lacking anti-IgE, was able to cause more than 20% inhibition of IgE binding to the B cells.

Although the IgG anti-IgE containing patients' sera caused modest inhibition (up to 48%) of IgE binding to the B cells, we believe that the experimental conditions used in this study underestimate the IgE blocking effect of anti-IgE in vivo. For instance, the inhibtion values presented in this paper were obtained using relatively high concentrations of IgE, thereby making the ratio of IgE to anti-IgE much greater than that in the circulation.[16]

The demonstration that human autoanti-IgE blocks the binding of IgE to CD23 in vitro is of pathological significance, since this action will render CD23 available for enzymatic cleavage. This will lead to upregulation of IgE synthesis through both sCD23 release[3] and disruption of the negative IgE regulatory signal delivered by membrane CD23 to the B lymphocyte.[4] The net effect of such excessive IgE production is the perpetuation of an ongoing allergic inflammation. A positive correlation has been reported between circulating levels of IgE and anti-IgE,[5 7] providing in vivo evidence in support of a positive IgE regulatory role for autoanti-IgE.

We have recently shown that IgG autoanti-IgE blocks the binding of IgE to its high affinity receptor (FcεRI), found on mast cells, basophils, and eosinophils.[10] In this context, IgG anti-IgE appears to have an anti-allergic effect, which is in marked contrast to the pro-allergic effect reported here. However, we have previously argued that the existence of epitope specific subpopulations of autoanti-IgE is clearly suggestive of a multiple role in immunological events involving IgE.[8]

This work is supported by a grant from the Wellcome Trust (grant number 036661/Z/92/Z).

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