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Experimental allergic encephalomyelitis (EAE) in mice selectively bred to produce high affinity (HA) or low affinity (LA) antibody responses

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SUMMARY

Induction of experimental allergic encephalomyelitis (EAE) in mice genetically selected to produce either high affinity (HA) or low affinity (LA) antibody responses has revealed significant differences in disease susceptibility between the two lines. HA mice were highly susceptible to EAE following subcutaneous sensitization to mouse central nervous system (CNS) tissue emulsified in Freund's complete adjuvant (FCA). Furthermore, of HA mice surviving acute EAE, up to 93% subsequently developed chronic relapsing disease (CREAE) characterized by variable demyelinating inflammatory changes within the spinal cord. In contrast, LA mice, despite having a major histocompatability complex (MHC) haplotype associated with susceptibility to EAE, were highly resistant to the disease and showed no signs of CREAE when observed for up to 100 days post-sensitization. Antibodies to myelin basic protein (MBP) were detected in both lines but rising titres of high functional affinity antibodies were only seen in HA mice. These HA and LA lines of mice provide a new approach to the study of EAE and, in particular, the role of antibody and antibody affinity in the chronic relapsing form of the disease.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a prototypic autoimmune disease induced in experimental animals by sensitization to central nervous system (CNS) myelin or myelin basic protein (MBP) in adjuvant (reviewed by Alvord, Kies & Suckling, 1984; Paterson & Swanborg, 1988). EAE may be limited to an acute, monophasic form of disease or follow a chronic, exacerbating or progressive course as in chronic relapsing EAE (CREAE), which is characterized by recurrent episodes of clinical signs in association with conspicuous focal areas of CNS demyelination (Raine *et al.*, 1980; Brown & McFarlin, 1981; Lassman, 1983; Alvord *et al.*, 1984). EAE in general, and CREAE in particular, are accepted as promising animal model systems for studying immune responses believed to be important in multiple sclerosis (MS), a major demyelinating inflammatory CNS disorder of humans (Peterson, 1979; Lassmann, 1983; Alvord *et al.*, 1984).

Whilst the precise pathogenesis of EAE remains to be defined, prevailing opinion holds that the disease is mediated by sensitized T cells (Paterson, 1983; Paterson, 1984; Alvord *et al.*, 1984) of the helper-inducer (CD4) lineage (Brostoff & Mason, 1984). Transfer of EAE with T lymphocytes (Paterson, 1960; Ben-Nun, Wekerle & Cohen, 1981) and the lack of successful passive transfer by immune serum (Chase, 1959) are the strongest points supporting this view. However, indirect evidence implicating a role for antibody in EAE has been reported (Gausas *et al.*, 1982; Willenborg & Prowse, 1983; Paterson & Halberg, 1989). One observation underscoring the potential importance of antibody is the transitory appearance of high-affinity anti-MBP antibodies in the sera of Lewis rats just prior to or in conjunction with the onset of clinical neurological signs of acute EAE and in a limited number of sera of MS patients experiencing early exacerbations of their disease (Paterson & Day, 1981).

Antibody affinity is an important qualitative parameter of the immune response under genetic controls similar to, but independent of, those governing antibody levels (Katz & Steward, 1975). Progressively higher affinity antibodies are generated with time during an immune response, by a combination of somatic mutation of germ line genes and antigen selection of high-affinity B cells. Affinity maturation of autoantibodies, reflecting a T-cell-driven response to an autoantigen, may be a key event in the development of many autoimmune diseases. The HA and LA mouse lines originated from an outbred (TO) population that had been selectively bred for either high affinity (HA) or low affinity (LA) antibody

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responses against protein antigens injected in saline (Katz & Steward, 1975). Significant differences in antibody affinity were demonstrated by generation 10, and H-2 typing showed both lines were similar to H-2^s (A.SW) mice (Steward, Reinhardt & Staines, 1979). The HA and LA mice provide a novel means for studying the role of antibodies in general, and high-affinity antibodies in particular, in the immunopathogenesis of murine EAE. To the best of our knowledge, this is the first study of EAE or any other autoimmune disease in HA and LA mice or in any other species with gene-specified distinctions concerning antibody-binding affinities.

MATERIALS AND METHODS

Mice

Male HA and LA mice of 9–12 weeks of age, at generation 29–31 of selective breeding, were bred and maintained at the London School of Hygiene and Tropical Medicine, London, U.K. Mice were selected for breeding at each generation on the basis of the affinity of antibodies to human serum albumen or human serum transferrin given at alternate generations. Typing for class I and II MHC antigens was performed on spleen cells by complement-dependent cytotoxicity assays using a panel of monoclonal antibodies (mAb) that collectively covered a range of possible haplotypes (ATCC, Rockville, MA) and, subsequently, peripheral blood lymphocytes were analysed using a FACS. Mice used in this study were derived from breeding pairs that were apparently homozygous for the 's' haplotype, since lack of reactivity with appropriate mAb ruled out H-$2^{k,d,b,q,p,r,f}$ and the presence of an I-E molecule, whilst positive reactivity was obtained with mAb recognizing I-A^s (HB4 and TIB92). SJL/J mice (Harlan-Olac, Bicester, Oxon, U.K.) of 6–8 weeks of age were included as controls in some experiments as they are H-2^s and known to be susceptible to EAE (Raine *et al.*, 1980; Brown & McFarlin, 1981).

Sensitization

EAE was induced by the method of Brown & McFarlin (1981). Lyophilized SJL/J spinal cord was homogenized in phosphate-buffered saline (PBS) and emulsified in incomplete Freund's adjuvant supplemented with a mixture (8:1) of *Mycobacterium tuberculosis* (H37 Rv) and *Mycobacterium butyricum* (a gift to P. Y. P. Paterson from Dr J. Freud) using an ultrasonic probe. Mice were sensitized by subcutaneous injection of 0·3 ml emulsion containing 1 mg spinal cord and 33 μg mycobacteria in three sites in the lower back on Days 0 and 7.

Clinical assessment of disease

To minimize any possibility of subjective bias, all mice were assigned a code that was not broken until the clinical and histopathological scorings were complete. Sensitized mice were observed daily and clinical signs of EAE were scored as follows: loss of tail tonus 1+, weakness of one or both hind legs 2+, complete paralysis of both hind legs 3+ and death preceded by any of these neurological signs 4+. The maximum clinical score of each mouse was used in calculating mean scores for each group of animals. In some experiments, surviving mice were observed for up to 98 days for relapsing EAE, which was defined as an increase in clinical score after a period of stabilization or improvement.

Histopathological assessment of disease

Mice were killed when considered moribund during the second or third week post-sensitization. Surviving mice were killed on Day 30 (experiment 1), Day 79 (experiment 3) or on Day 98 (experiments 2 and 4). Brains and entire spinal columns were fixed in 10% formalin, sections were stained with haematoxylin and eosin, examined for EAE lesions as previously described (Carbone, Ovadia & Peterson, 1983) and graded on a scale of 0 (no lesions), 1+ (< 10 total lesions), 2+ (10–20 total lesions) to 3+ (> 30 total lesions) in routinely sectioned forebrain, midbrain, cerebellum-pons and entire length of spinal cord. Spinal cords were also removed from the cervical end of the dissected spinal column by exerting pressure from a 10-ml syringe filled with distilled water using a 20 g needle clamped onto the caudal end of the column. After consecutive fixation in 20% formalin, 3% gluteraldehyde and osmic acid, spinal cords obtained in this way were studied as described previously (Dal Canto & Lipton, 1975). Briefly, tissue was further fixed in chilled 3% gluteraldehyde in PBS (pH 7·3); 1-mm thick cross-sections were post-fixed in 1% osmic acid for 1 hr, dehydrated in alcohol, cleared in propylene oxide and embedded in Epon. One-micrometre thick sections were stained with toluidine blue and studied using a Zeiss light microscope.

Antibody assays

Antibodies to MBP were measured by ELISA using polystyrene microtitre plates coated with 0·5 μg/ml MBP in 0·1 M carbonate buffer, pH 9·6, by overnight incubation at 4° and stored dry until used. Purified MBP (Batch LP 13c) was prepared from guinea-pig spinal cords as described by Swanborg, Swierkosz & Saieg (1974). MBP-coated plates were incubated with serial dilutions of sera in PBS/2% BSA/0·5% Tween 20 for 1 hr at 37°, washed and incubated with a peroxidase-conjugated antiserum to mouse IgG (Jackson Immuno Research Laboratories) at 1/10,000. After washing and addition of substrate (o-phenylenediamine and H_2O_2), the reaction was stopped after 10 min with 2 M H_2SO_4 and optical density (OD) measured at 492 nm. Freezing and thawing of sera was avoided as aggregated IgG has been shown to bind avidly to solid-phase MBP via Fc interactions (Sindic *et al.*, 1980). A mouse monoclonal antibody to MBP (clone 2, provided by Dr Nigel Groome, Oxford Polytechnic, Oxford, U.K.) and pooled sera from normal HA and LA mice were included on each plate as positive and negative contols, respectively. Results were expressed as an end-point titre (the $\log_{10}$ dilution giving an OD of 0·2).

Affinity assays

Functional antibody affinity was measured by modified ELISA using the chaotropic agent diethylamine (DEA) to inhibit low-affinity interactions with the solid-phase antigen, as described previously (Devey *et al.*, 1988). Assays were standardized using high and low affinity monoclonal antibodies to MBP (rat clone 12 and mouse clone 2, provided by Dr Nigel Groome). Dose–response curves were plotted and the fall in titre due to DEA measured at half maximal absorbance. Results were expressed as a ratio of the titre of each paired serum sample in the presence and absence of DEA. Thus, a high value represented a high functional affinity.

Statistics

Student's *t*-test, regression analysis and the chi-squared test were used where indicated.

Table 1. Occurrence of clinical signs of EAE in HA and LA mice

Exp.	Mouse line	Incidence	Mean day of onset*	Mean score†	Acute EAE‡	CREAE§	Mean histopathology score ± SD¶
1	HA	10/10 (100%)	17·3	3·10 ± 0·88			2·00 ± 0·47
	LA	5/10 (50%)	18·4	0·60 ± 0·70			0·39 ± 0·78
2	HA	13/15 (87%)	17·8	2·77 ± 1·25	6/15 (40%)	5/9 (56%)	
3	HA	25/28 (89%)	18·2	2·88 ± 1·29	12/28 (43%)	11/16 (69%)	
	LA	9/27 (33%)	16·8	0·41 ± 0·64	0/27	0/27	
4	HA	42/43 (98%)	17·3	3·58 ± 0·76	28/43 (65%)	14/15 (93%)	

* Time in days post-sensitization when definite neurological signs observed.

† Mean maximum clinical score for each group of mice (±SD).

‡ Incidence of death from acute EAE prior to Day 30.

§ Incidence of 1 or more relapses of EAE in surviving mice Days 30–98.

¶ Histopathological score of mice killed during acute EAE graded on a scale of 0 to 3 (see the Materials and Methods).

Significance of differences in severity and incidence of clinical disease between HA and LA mice: Exp. 1 $\chi^2 = 17{\cdot}00$, $P < 0{\cdot}005$; Exp. 3 $\chi^2 = 38{\cdot}98$, $P < 0{\cdot}0005$. Significant difference between severity of acute histopathological score between HA and LA: $t = 5{\cdot}211$, $P < 0{\cdot}001$.

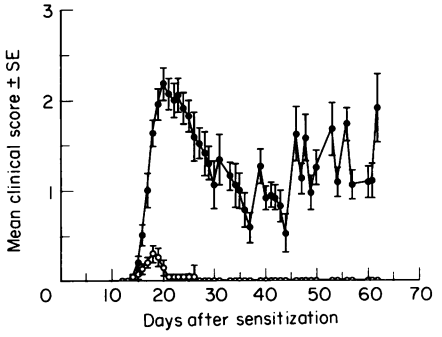

Figure 1. Clinical signs of EAE in HA(●) and LA (○) mice (cumulative data from several experiments: HA = 71 and LA = 43).

RESULTS

Occurrence of EAE

The results of four experiments are shown in Table 1. As expected, 20/20 SJL/J mice, included in two experiments as positive controls, developed severe clinical signs of acute EAE (data not shown). A high proportion of HA mice developed EAE of comparable clinical severity to SJL/J mice, with a mortality rate of about 50% during the acute phase of disease. Of surviving HA mice, up to 93% subsequently exhibited 1 and usually more recurrent episodes of disease (CREAE) up to Day 70–98 post-sensitization. Characteristic patterns of remission and relapse are shown in Fig. 4 and consisted of variable periods of recovery from either total or partial paralysis. A small proportion of mice (< 10%) showed a chronic progressive disease course of partial or complete paralysis without remission. In contrast, LA mice showed a low incidence of EAE and, when present, disease was significantly less severe compared to HA mice (Table 1). LA mice observed over the same time period showed no evidence of CREAE. Cumulative data from several experiments are shown in Fig. 1

Acute EAE histopathological changes

Microscopic examination of CNS tissue during acute EAE (Day 15–30) revealed very severe inflammatory cell infiltration in both the brain and spinal cord of HA but not LA mice (Table 1). In the brains of HA mice, perivascular inflammation presented as random discreet foci in both grey and white matter. In the spinal cord, the lesions predominantly involved the white matter columns, sometimes along their entire length, almost without interruption. Cellular infiltration of pia-arachnoid membranes, especially in spinal cord sections, was regularly observed. Infiltrates consisted predominantly of mononuclear cells but segmented neutrophils were also present, a feature consistent with murine acute phase EAE.

CREAE histopathological changes

Microscopic examination of 1-μm thick Epon-embedded sections from brains and spinal cords of sensitized LA mice failed to disclose either inflammatory infiltration or demyelination (Fig. 2a). In contrast, HA mice showed mild inflammatory changes in the brain and moderate to severe white matter lesions in spinal cords. These were characterized by the presence of both inflammatory changes and demyelination in chronic active lesions (Fig. 2b).

Antibody levels

Low levels of antibody to MBP were detected in HA and LA mice on Day 12, before the onset of clinical signs of EAE, and in HA mice there was a significantly inverse correlation betweeen titre and the subsequent severity of acute EAE (Fig. 3a). LA mice consistently had significantly higher titres of antibody to

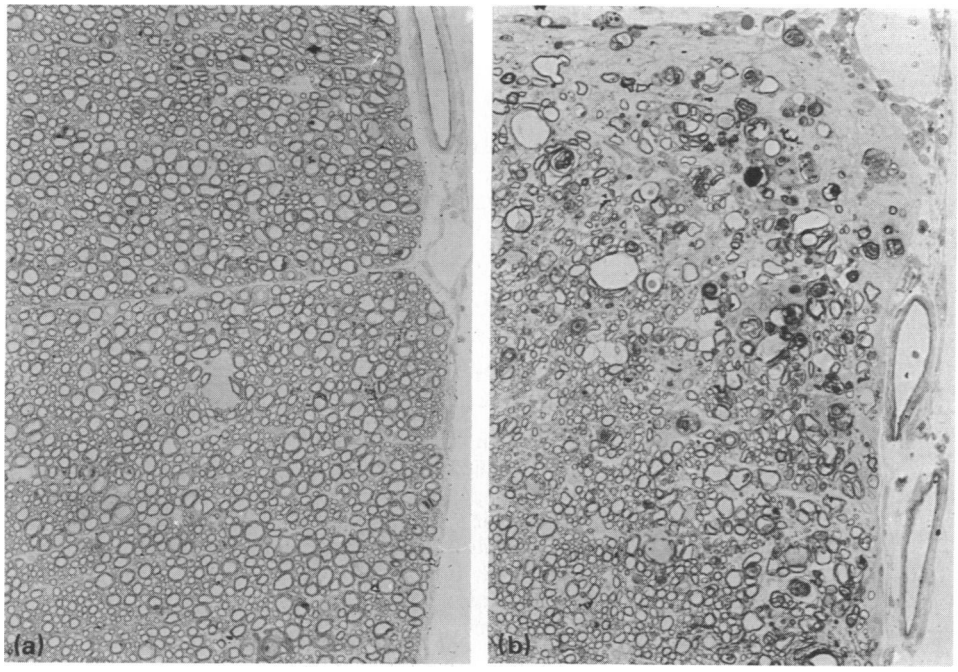

Figure 2. (a) Spinal cord section from LA mouse (Day 79 after sensitization) unaffected by either inflammation or demyelination. (b) Spinal cord from HA mouse with CREAE (Day 79) showing meningeal mononuclear inflammatory cell infiltration with extensive subpial demyelination. Sections are 1-μm Epon-embedded and stained with toluidine blue, $\times$ 160.

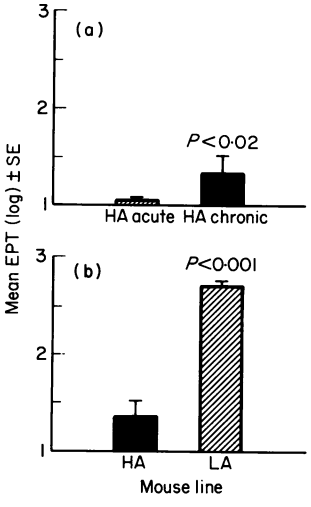

Figure 3. (a) Mean titre of antibody to MBP on Day 12 post-sensitization in HA mice (Exp. 4) subsequently dying from acute EAE (acute, $n=28$) and HA mice subsequently developing relapsing EAE (chronic, $n=14$). (b) Mean titre of antibody to MBP in HA and LA mice on Day 30 post-sensitization (Exp. 1). EPT, end-point titre

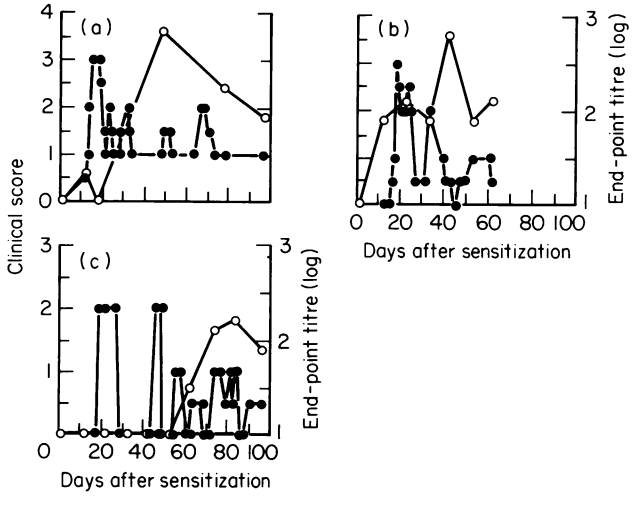

Figure 4. Anti-MBP titre (○) and clinical score (●) in individual HA mice with CREAE (a, b and c).

MBP compared to HA mice up to Day 30 post-sensitization (Fig. 3b). Thus, in the majority of HA mice, the early appearance of antibody was associated with survival from acute EAE and the later development of CREAE (Figs 3a, 4a, b), although in a smaller proportion of HA mice detectable antibody was not observed until after Day 40 (Fig. 4c). During CREAE in HA mice, serum levels of anti-MBP showed a positive correlation ($P<0{\cdot}005$) with the clinical score, although there was considerable scatter, particularly in mice during remission at the time of bleed (Fig. 5). Antibody was not detected in LA mice at this time (data not shown).

Antibody affinity

HA mice showed significant functional affinity maturation of antibodies to MBP between Days 30 and 98 post-sensitization (Table 2). Affinity maturation was not seen in LA mice and there was no correlation between titre and functional affinity in either line.

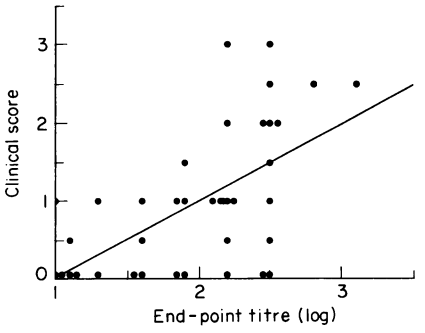

Figure 5. Positive correlation ($P < 0.005$) between anti-MBP titre and clinical score at time of bleed in HA mice with CREAE Days 70–90 after sensitization.

Table 2. Functional affinity of antibodies to MBP in HA and LA mice

		Day			
Line	*n*	30	48	78	98
LA	13	0·40±0·25*	0·23±0·13	ND	ND
HA	14	0·52±0·14	0·62±0·11	0·70±0·26	0·75±0·07

* Results expressed as the ratio of titre with and without DEA at 50% maximum OD±SD.

ND, not done.

Significant differences between HA and LA on Day 48 ($P < 0.001$) and for HA mice between Days 30 and 98 ($P < 0.01$).

DISCUSSION

These studies have yielded three major findings. First, HA mice were significantly more susceptible to EAE than LA mice and equalled the susceptibility of SJL/J mice to acute EAE after subcutaneous sensitization with murine CNS tissue in FCA. In addition, up to 93% of HA mice surviving acute EAE developed CREAE with demyelinating inflammatory changes within the spinal cord. Second, in all mice there was a significantly inverse correlation between the amount of antibody to MBP produced early in disease and the severity of acute EAE. Third, anti-MBP antibodies were produced by HA mice during CREAE and showed a positive correlation with clinical score. Although the question of proliferative responses have not been addressed in this paper, preliminary data (not shown) indicate that both HA and LA mice are able to respond *in vitro* to MBP, as shown by significant proliferation of lymph node cells taken 12 days after priming.

The cellular basis controlling the production of high and low affinity antibody responses in HA and LA mice remains to be fully established, although differences in T-cell and macrophage function have been demonstrated (Steward, Stanley & Furlong, 1986; Phillips, 1989) and differences between the lines are observed for T-dependent but not T-independent antigens (Steward *et al.*, 1986). In addition, these mice demonstrate marked differences in susceptibility to experimental malaria infection and to the adjuvant effect of interferon-gamma (IFN-γ) (Heath *et al.*, 1989). Thus, it is likely that antibody affinity, the basis for the selective breeding of the HA and LA mouse lines, may be a marker trait co-segregating with genes controlling other important aspects of immune responsiveness, e.g. antigen processing/presentation, CD4 T-cell activity, lymphokine production or, conceivably, factors independent of immune function important in the pathogenesis of EAE. One possibility is that the selective breeding for high affinity responses has resulted in the predominance in HA mice of TH1 cells, evidence for which has been suggested by Mossmann & Coffman (1987). Thus TH1, producing IFN-γ and delayed-type hypersensitivity (DTH), may be conducive to both high affinity antibody responses and the induction of EAE.

The major objective of the work reported here was to compare susceptibility to EAE between two lines of mice bred for differences in antibody affinity and not to establish a role, protective or otherwise, for antibody to MBP. However, the correlations observed between antibody and disease were of interest and will form the basis for further studies. The apparent protective effect of antibodies to MBP produced early in disease is reminiscent of a similar finding from a study in dogs (reviewed by Paterson & Halberg, 1989). The fact that LA mice produced the greatest amount of anti-MBP antibodies during the early phase of the disease and had evidence of the lowest susceptibility to acute EAE, once again raises the question as to whether certain types of anti-CNS antibodies, perhaps low affinity antibodies in particular, exert a protective role in neuroimmunological disease (Paterson, 1966). The mechanism by which this may be achieved is not entirely clear but passive blocking of the target autoantigen, active feed-back inhibition or idiotypic interactions are all possibilities (Cohen & Cooke, 1986). Alternatively, differences between anti-MBP titres may simply reflect activation of different T-cell subsets or differences in lymphokine production between the mouse lines.

Whilst the role of T cells is well documented in acute EAE, less is known about the immunopathological mechanisms operating during the chronic disease. The greater susceptibility of HA mice to CREAE, the demonstration of functional affinity maturation of antibodies to MBP and the correlation between titre and CREAE clinical score in HA mice provides additional and more direct evidence in support of earlier work (Paterson & Day 1981; Gausas *et al.*, 1982; Willenbourg & Prowse, 1983; Paterson, 1983, 1984) implicating a role for antibodies, and high affinity binding antibodies where this was investigated, in demyelinating processes in EAE as well as in MS. Recently other neural antigens, proteolipid protein (Trotter *et al.*, 1987) and myelin oligodendrocyte glycoprotein (Linington & Lassmann, 1987) have been implicated in EAE and antibodies have been shown to be capable of inducing demyelination by a complement-dependent mechanism (Linington & Lassmann, 1987). Thus, the role of antibodies in EAE appears to be complex and further studies are necessary to elucidate the target antigen(s) and whether antibodies to MBP, or some other neural antigen, play a direct role in demyelination in this new murine model of EAE.

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