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Interspecies Exchange of β_2-Microglobulin and Associated MHC and Differentiation Antigens

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Abstract. Radiolabeled human β_2-microglobulin (β_2m) can bind to mouse histocompatibility (H-2) antigens on the cell surface or to partially purified H-2 antigens in solution. The complexes containing human β_2m and H-2 antigens from C3H (*H-2*^{k}) mice could be immunoprecipitated specifically with alloantisera, rabbit anti-H-2 xenoantisera, and with monoclonal H-2-specific antibodies. Specific association with H-2 antigens was also observed with other haplotypes. The only exception was B10.D2 (*H-2*^{d}) from which complexes containing human β_2m could only be precipitated with anti-H-2 xenosera. Thus radiolabeled human β_2m can be used as a specific label for mouse H-2 antigens in precipitation and radioimmunoassays. The application of this finding extends to major histocompatibility complex antigens of other species, and to differentiation antigens with primary association with β_2m.

Introduction

The MHC gene products of all mammalian species studied so far have 45K heavy chain glycoprotein molecules encoded by homologues of the mouse *H-2K* and *H-2D* genes. These molecules are referred to as K/D/L or class I molecules (Klein 1975, Snell et al. 1976). Their chemistry has recently been elaborated by amino-acid sequencing and the primary structure of several of these molecules has now been established (Orr et al. 1979, Uehara et al. 1980). They are highly polymeric and are associated with a 12K β_2-microglobulin (β_2m) molecule which is usually non-polymorphic in each species (Peterson et al. 1974, Tanigaki et al. 1973, Robinson et

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Abbreviations used in this paper: MHC = major histocompatibility complex; β_2m = β_2-microglobulin; LcH = *Lens culinaris* hemagglutinin.

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al. 1981). These molecules are known to play a role in immune response against transplants and also against virally infected and neoplastic cells (Festenstein and Démant 1979, Zinkernagel and Doherty 1975, Gomard et al. 1976). The function of the β_2m molecules is not yet known, although it has been suggested that they have a stabilizing effect on the class I molecules' tertiary structure (Lancet et al. 1979). The two chains are strongly but noncovalently bound. There is evidence that xenogeneic β_2m can be substituted in the dimer for native β_2m. This can be achieved by de novo elaboration of the hybrid dimer, as in the case of man-mouse hybrid cells (Goodfellow et al. 1975, Fellous et al. 1977), or by incorporation of radiolabeled human β_2m into the dimer at the surface of viable mouse spleen cells (Hester et al. 1979). However, there is also evidence that exogeneously added β_2m may bind to a receptor on the cell surface different from H-2 antigens (Sege et al. 1979). In this paper we show that human β_2m can bind to murine H-2D and H-2K alloantigens. This reaction can be achieved on the cell surface or simply by mixing purified H-2 antigens with pure human β_2m in solution, and can be used to study the chemistry and biophysiology of these products in greater depth.

Materials and Methods

Mice. AKR/J and C3H mice as well as B10 congenic mice (both $H\text{-}2^k$) were bred in our colony and used when 6 to 10 weeks old.

Tumors. Tissue culture adapted lines 110, 365, 369, 422, and 424 were derived from spontaneous AKR leukemias and kindly provided by P. Krammer, Heidelberg, FRG. The K-GV leukemia was induced in BALB.K mice by Gross/AKR-murine leukemia virus and was provided by F. Lilly, New York.

Antisera. A monoclonal anti-human β_2m hybridoma line kindly provided by Drs. M. Trucco and R. Ceppellini had been established essentially following the procedure of Köhler and Milstein (1976). Antibody (IgG2 subclass) was isolated from serum following subcutaneous inoculation of tumor into mice and purified on a column of human β_2m coupled to "armed" Sepharose (Cuatrecasas 1972) at a concentration of 1–2 mg β_2m protein per ml of swollen gel; elution was with 0.1 M glycine HCl buffer, pH 3.0, and the eluate was immediately neutralized. This antibody was then coupled at 2–3 mg per ml of swollen gel under the same conditions as for human β_2m and used for isolation of β_2m. BBM-1 monoclonal antibody against human β_2m (Brodsky et al. 1979) was kindly provided by M. Crumpton, London, U. K. Monoclonal antibodies H100-5, H100-27, H100-30, and H116-22 reacting with $H\text{-}2^k$ public specificities (Lemke et al. 1979) were kindly provided by H. Lemke, Cologne, FRG. H-2 alloantisera of restricted specificity (D series) were obtained from the Transplantation and Immunology branch of NIH, Bethesda, Maryland. They include D2, [B10.A(5R) × LP.RIII]F_1 anti-B10; D9, [B10.Da × SJL]F_1 anti-B10.M; D12.b, [SWR × DBA/1]F_1 anti-DA/SN; D18, [B10.A(2R) × C3H.RB]F_1 anti-B10.RIII(71NS); D19, [B10.A × A.CA]F_1 anti-A.SW; D23.b, [B10.D2 × SJL]F_1 anti-B10.A; D30, [B10.A × LP.RIII]F_1 anti-B10.AKM; and D32, [B10.A(2R) × C3H.SW]F_1 anti-C3H. The following hyperimmune sera were raised in our laboratory: anti-H-2.4, (AKR × B10) anti-B10.A; anti-H-2.31, [AKR × B10.A(5R)] anti-B10.D2; and anti-H-2.33, (AKR × B10.A) anti-B10.A(5R).

Purifications. Human β_2m was purified from the urine of patients with Wilson's disease or renal failure (Berggard and Bearne 1968). Purity was established by SDS gel electrophoresis and analytical isoelectric focusing of the final product, which indicated essentially no contamination with other proteins. H-2 antigens were partially purified from C3H lymphocytes by NP40 lysis and affinity chromatography on *Lens culinaris* hemagglutinin (LcH) Sepharose (Hayman and Crumpton 1972). Complexes on the surface of AKR/J lymphocytes containing ^{125}I-labeled human β_2m were solubilized with 0.5% NP40 in 0.01 M Tris, 0.15 M sodium chloride, 0.0015 M magnesium chloride buffer (pH 7.4) and partially purified by chromatography on Sephacryl S200 and LcH-Sepharose.

Binding studies with human β_2m. Binding of human β_2m to murine lymphocytes was measured by mixing ^{125}I-labeled β_2m with 5×10^6 AKR/J lymphocytes in 0.2 ml of RPMI 1640 medium containing 10% fetal calf serum and 50 µg/ml Puromycin. After incubation at 37° C for 2 hours the cells were washed three times and counted for uptake of radioactivity in an LKB Mini-Gamma counter.

Binding of ^{125}I-labeled human β_2m to partially purified H-2 antigens isolated from C3H lymphocytes was measured by mixing the components in 0.01 M Tris buffer containing 0.15 M NaCl and 0,25% NP40 at 37° C for 2 h and fractionation on Sephacryl S200 in buffer containing the detergent.

Quantitative absorption and indirect immunoprecipitation using *Staphylococcus aureus* was carried out as described previously (Schmidt et al. 1979).

Results

Association of human β_2m with mouse alloantigens after partial purification. H-2 antigens were isolated from C3H (*H-2^k*) lymphocytes and partially purified by affinity chromatography on LcH-Sepharose. These mouse alloantigens were incubated with ^{125}I-labeled human β_2m as described above. The mixture was separated on a Sephacryl S200 column. Twenty-nine percent of the radiolabeled human β_2m was found complexed to the H-2 antigens (Fig. 1), while the unbound radioactive β_2m eluted as a retarded peak at fraction 105.

The fractions containing the β_2m complex (fractions 49–58) were pooled and used for indirect immunoprecipitation. Alloantisera, reacting with the H-2K^k and H-2D^k antigens (anti-H-2.23 and H-2.32, respectively) precipitated bound ^{125}I-β_2m (Table 1). ^{125}I-β_2m was also precipitated by a rabbit anti-H-2 serum and by monoclonal antibodies directed against H-2^k specificities. Maximum precipitation was obtained when several monoclonal H-2^k-specific antibodies were combined. No precipitation occurred with antisera against Ia antigens or surface immunoglobulin, nor with antisera directed against H-2 antigens of irrelevant haplotype (*b, s, f, q*), which rules out the theoretical possibility that the β_2m is associating nonspecifically with other cell-surface molecules.

Comparison of the amount of ^{125}I-β_2m precipitated by monoclonal antibodies against human β_2m with that precipitated by monoclonal H-2^k-specific antibodies,

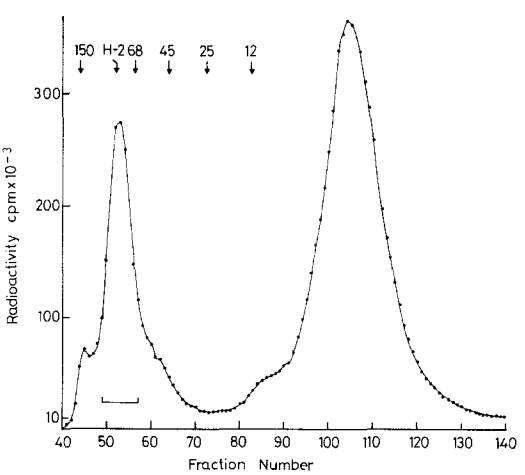

Fig. 1. Sepacryl S200 chromatography of ^{125}I-labeled human β_2m after incubation in vitro with partially purified H-2 antigens from C3H lymphocytes. Elution profile shows two peaks of radioactivity – one containing human β_2m bound to H-2 and the other free human β_2m. The position of the molecular-weight markers (mol. wt. $\times 10^{-3}$) immunoglobulin G (150), bovine serum albumin (68), ovalbumin (45), chymotrypsinogen (25) and lysozyme (12), and of H-2 antigens were determined in similar separations under identical conditions. The elution profile of H-2 antigens was obtained in related separations of purified H-2^d glycoprotein which was used to inhibit the precipitation of radiolabeled H-2D^d antigens.

Table 1. Incorporation of ^{125}I human β_2m into class I H-2 antigens either on the cell surface followed by solubilization, or directly in solution

Antisera	Antigen recognized	AKR/J Intact lymphocytes (NP40 lysate)*		C3H glycoprotein (S200-filtrate)†	
		cpm	Percent total	cpm	Percent total
Conventional antisera					
Normal mouse serum		41	0.4	254	0.9
Anti-H-2.23	K^k	4056	42.1	10655	39.4
Anti-H-2.32	D^k	1767	18.4	3903	14.4
Anti-H-2I	Ia^k	n.d.	n.d.	206	0.8
Anti-S-IgI	IgI	n.d.	n.d.	171	0.6
Anti-H-2.2	D^b	85	0.9	n.d.	n.d.
Anti-H-2.33	K^b	70	0.7	n.d.	n.d.
Anti-H-2.18	K^r	69	0.7	n.d.	n.d.
Anti-H-2.30	D^q	79	0.8	n.d.	n.d.
Normal rabbit serum		322	3.3	687	2.5
Rabbit anti-H-2	K, D	6741	70.1	13141	48.5
Monoclonal anti-bodies					
Anti-H-2^k (100/5 + 100/27 + 100/30)	K^k, D^k	6237	62.8	17894	66.1
Anti-human β_2m	M8 β_2m(hu)	n.d.	n.d.	9424	34.8
	BBMI β_2m(hu)	7209	74.9	11901	44.0

* The antigen was the NP40 lysate of AKR/J lymphocytes after exchange with ^{125}I-labeled β_2m. Immunoprecipitation using as antigen the pooled fractions after Sepharcryl S200 or LcH-Sepharose chormatography (see Fig. 2) gave similar results.

† Fractions 49 to 58 (Fig. 1) were pooled and used as antigen.

by H-2 xenoantiserum and by the alloantisera, suggests that most, if not all, human β_2m is associated with H-2 antigens.

Association of human β_2m with murine H-2 alloantigens on the cell surface. Lymphocytes from AKR (*H-2^k*) mice incubated with ^{125}I-labeled human β_2m in vitro bound 8.4 percent of radioactivity (Table 2). Binding of radiolabeled β_2m could be inhibited by an excess of unlabeled native human β_2m. The complex on the cell surface containing human β_2m was solubilized in 0.5% NP40 and purified by gel filtration on Sephacryl S200 and affinity chromatography on LcH-Sepharose (Fig. 2). During separation the complex showed chromatographic behavior identical to that of histocompatibility antigens. Indirect immunoprecipitation with H-2 alloantisera and monoclonal antibodies from the solubilized complex or after partial purification showed that the exogenously added ^{125}I-labeled human β_2m was associated with H-2 antigens (Table 1).

Radioimmunoprecipitation assay for H-2 antigens on cells following solubilization but without further purification. β_2m exchange experiments were carried out with intact normal and tumor cells. The normal lymphoid cells were taken from mice of various B10 congenic strains. Lymph-node suspensions were first incubated with

Table 2. Binding of human β_2-microglobulin to lymph-node cells from AKR mice

	Lymphocytes	Incubation	Unlabeled β_2m (hu)	^{125}I β_2m bound to cells	
				cpm	Percent of total
Exp. 1	20×10^6 AKR	37° C	–	260449	8.39
		37° C	+	5327	0.17
		0° C	–	3596	0.12
Exp. 2	5×10^6 AKR	37° C	–	58297	2.40
		37° C	+	2840	0.12
		0° C	–	3213	0.13

Lymphocytes from AKR mice were incubated with 10 µl ^{125}I-labeled human β_2m (50 ng; 160 mCi/mg) for 2 h at 37° C (or on ice/0° C). In controls 40 µg of unlabeled human β_2m was included. After repeated washing of the cells, bound radioactivity was measured.

radiolabeled human β_2m as described above. The cells were washed, lysed with 0.5% NP40 and immunoprecipitated with rabbit H-2 xenoantiserum, H-2 alloantisera and monoclonal antibodies. The results are shown in Table 3. Four haplotypes were tested, i.e., *H-2*^{b}, *H-2*^{d}, *H-2*^{k} and *H-2*^{s}, with anti-H-2 sera against private specificities. Specific precipitations according to the *H-2* haplotype were found with these reagents, except for B10.D2, which gave very weak precipitation with anti-H-2.31 and no precipitation with anti-H-2.4. However, a rabbit anti-H-2 xenoserum precipitated 62.7 percent of the radioactivity incorporated on the cell surface, which approximates well with the results obtained with the other haplotypes and with the anti-β_2m serum.

The tumor cells were all Gross-virus related and all except K-GV (of BALB.K origin) derived from AKR mice. They differed in their H-2-antigen expression as calculated by their capacity to absorb specific antibodies (Table 4). In these experiments the intact tumor cells were incubated with radiolabeled human β_2m as in the case of normal cells, and the uptake of radioactivity was measured and

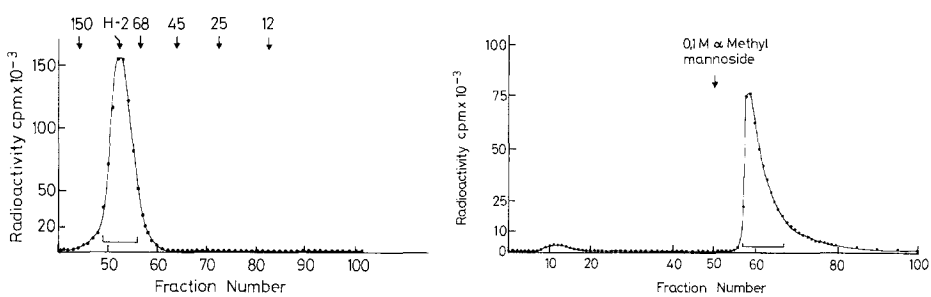

Fig. 2A and B. Fractionation of human β_2m-receptor complex from AKR/J lymphocytes. Five million AKR/J splenic lymphocytes were incubated with ^{125}I-labeled human β_2m (120 mCi/mg) for 2 h at 37° C washed three times, and lysed with 0.5% NP40. The lysate was fractionated by gel filtration on Sephacryl S200 (**A**). Material eluting between fractions 49 and 58 was pooled and further separated by affinity chromatography on LcH-Sepharose (**B**). Glycoproteins were eluted with 0.1 M α-methylmannoside as indicated. Molecular-weight markers as in Figure 1.

Table 3. Incorporation of ^{125}I human β_2m into class I H-2 antigens

Antisera	Antigen recognized	Immunoprecipitation of ^{125}I β_2m after exchange from (percent of total incorporated)			
		B10 K^bD^b	B10.D2 K^dD^d	B10.BR K^kD^k	B10.S K^sD^s
Conventional antisera					
Normal mouse serum	–	0.5	0.9	0.7	0.8
Anti-H-2.2	D^b	38.3	1.1	1.0	1.1
Anti-H-2.33	K^b	7.4	0.8	0.9	0.8
Anti-H-2.4	D^d	0.8	1.4	0.7	1.1
Anti-H-2.31	K^d	0.6	4.8	1.5	1.0
Anti-H-2.32	D^k	1.6	2.1	16.5	1.5
Anti-H-2.23	K^k	1.0	0.9	33.3	1.1
Anti-H-2.12	D^s	1.3	5.6	7.8	53.1
Anti-H-2.19	K^s	1.0	1.2	0.8	17.1
Anti-H-2.30	D^q	1.2	0.7	0.7	0.9
Anti-H-2.18	K^r	0.9	0.9	0.8	2.1
Anti-H-2.9	D^fK^f	0.7	0.9	1.2	1.2
Normal rabbit serum	–	2.0	5.0	2.7	4.9
Rabbit anti-H-2	K,D	57.2	62.7	64.6	60.4
Monoclonal antibodies					
Anti-H-2^k (100/5 + 100/27 + 100/30)	K^kD^k	5.9	3.2	67.2	7.7
Anti-human β_2m M8	β_2m(hu)	56.5	53.7	51.6	51.4
Anti-human β_2m BBMI	β_2m(hu)	72.5	72.0	78.5	75.7

Table 4. Radioimmune precipitation assay for analysis of quantitative H-2 antigen expression on tumor cells

Tumor	Origin	H-2 antigen* expression		β_2m exchange†	Immunoprecipitation after β_2m exchange‡			
		H-2K^k	H-2D^k	(Uptake/10^7 cells)	AntiH-2.23	Anti-H-2.32	Rabbit anti-H-2	Monoclonal anti β_2m (BBMI)
110	AKR	1.00	1.00	62812 (1.00)	1233 (1.00)	606 (1.00)	1891 (1.00)	1817 (1.00)
365	AKR	3.30	1.65	143524 (2.28)	3360 (2.72)	742 (1.22)	4170 (2.21)	4250 (2.34)
369	AKR	25	13.9	503688 (8.01)	8908 (7.22)	7986 (13.18)	14521 (7.68)	13877 (7.64)
422	AKR	0.25	0.42	43276 (0.69)	41 (0.03)	557 (0.92)	884 (0.45)	808 (0.44)
424	AKR	1.21	1.88	143604 (2.28)	2508 (2.03)	1616 (2.67)	4256 (2.25)	4575 (2.52)
K36	AKR	0.25	4.94	182976 (2.91)	35 (0.03)	3778 (6.23)	3980 (2.10)	4511 (2.48)
k-GV	BALB.K	0.25	1.14	52657 (0.84)	21 (0.02)	611 (1.01)	603 (0.32)	857 (0.47)

* Absorption indices relative to normal lymphocytes from the strain of origin were normalized to make tumor 110 values (H-2K^k = 0.58; H-2D^k = 0.85) = 1.00 in each case.

† 10^7 tumor cells in 0.5 ml of medium containing 50 µg Puromycin were incubated with 100 µg of ^{125}I-labeled β_2m (specific activity 160 µCi/µg) for 1 h at 37° C and washed three times with cold phosphate-buffered saline. Normalized values (see *) are given in brackets.

‡ NMS controls (range 42–114) and NRS controls (57–241) substracted. Normalized values are given in brackets.

compared. Despite the large variation in uptake of radioactive β_2m there is a clear correlation with the amount of H-2 antigens on the cell surface (Table 4). The cells were then lysed with NP40 and immunoprecipitated with anti-H-2.23, anti-H-2.32, rabbit anti-H-2 serum and mouse anti-human β_2m monoclonal antibody. The amount of radioactive β_2m precipitated with anti-H-2.23 and anti-H-2.32 again correlated with the amount of H-2K^k and D^k known to be present on these cells by absorption analysis (and by immunoprecipitation of biosynthetically radiolabelled material – not shown). Furthermore, where a small amount of H-2 is indicated by absorption analysis as in tumors 422, K36, and k-GV for H-2K^k antigen, the same low value is given in the quite independent assessment by our radiolabeled β_2m assay. A single exception appears to be the H-2K^k antigen on tumor 369, which is determined in the β_2m assay to be present in a substantially smaller amount (anti-H-2.23).

However, there is in every case a good correlation of β_2m uptake and β_2m complex immunoprecipitated by rabbit anti-H-2 and monoclonal anti-β_2m. This again suggests that the main β_2m cell-surface receptor is H-2.

Discussion

It is clear from these results that the β_2m subunit of natural H-2 antigens can be efficiently substituted by its human analogue. Furthermore, although the use of ^{125}I-labeled human β_2m can serve as a convenient marker for the process, the exchange is not dependent on any artifactual modifications introduced as a result of iodination. Thus native human β_2m achieves similarly effective exchange and monitoring in this case is achieved by use of intrinsically labeled ^{3}H- or ^{14}C-labeled H-2 alloantigen glycoprotein chains (W. Schmidt, A. R. Sanderson, and H. Festenstein, manuscript in preparation). The kinetic and thermodynamic parameters of the exchange reaction have been established and these results will be published elsewhere. This exchange can take place on the surface of viable cells or in solution with the purified native H-2 antigen. Since most of the bound β_2m can be precipitated with monoclonal H-2-specific antibodies, it indicates that association occurs only with MHC antigens.

Human β_2m/H-2 association was measured by radioactivity (β_2m-derived) in H-2 immunoprecipitates. H-2 xeno- and alloantisera produced radioactive precipitates (^{125}I-human β_2m associated with H-2) with cells of all the haplotypes tested except for *H-2^d*. In this case, anti-H-2D^d sera produced no radioactive precipitate and anti-H-2K^d sera a precipitate with little radioactivity. The inability to precipitate β_2m bound to cell-surface products from B10.D2 with H-2 alloantisera is in agreement with the results of Sege and co-workers (1979) who studied only this haplotype and made the generalization that human β_2m did not associate with H-2. From our data we can see that the B10.D2 strain is an exception and that in all the other haplotypes studied (i. e., all those in Table 3 plus *H-2^q*, *H-2^f* and *H-2^r*; not shown) human β_2m was indeed associated with the H-2 heavy chain. This view was further strengthened by the results with monoclonal antibody. We have also found that the human β_2m bound to lymphocyte surfaces of B10.D2 mice appears to dissociate much more rapidly than when bound to lymphocyte surfaces

from B10.BR mice (not shown). This suggests that the binding to B10.D2 is probably also due to the H-2 antigens (as in B10.BR), but that the rapid dissociation of the complex does not permit the precipitation of the human β_2m together with the H-2 product using anti-H-2 alloantisera. Nathenson (personal communication) has also found that B10.D2 ($H\text{-}2^d$) haplotype has a uniquely weak association with β_2m.

In this communication we have shown, using a limited analytical technique, that H-2 can associate with human β_2m. The exploitation of this phenomenon on a large scale will have obvious value in purification of H-2 molecules. Indeed analogous protocols may apply for purification of many other species' MHC molecules where human β_2m can be efficiently associated with the respective alloantigen dimers. Experiments along these lines as well as others designed to show if the association involves an exchange between mouse and human β_2m are in progress. Elution from monoclonal anti-human β_2m immunosorbents by displacement with readily available human β_2m represents a particularly gentle preparative method for alloantigens as has already been shown using a polyclonal xenospecific antiserum (Robb et al. 1976).

The availability of large amounts of biologically active, purified class I products will now allow experiments to investigate mechanisms and functions of many immune reactions. In particular the immune response to allo- and other foreign antigens with special reference to altered self, MHC restriction and the study of the T-cell receptor can be undertaken.

Another application of this finding includes the development of quantitative binding or radioimmunoassays for class I MHC antigens of man (Hyafil and Strominger 1979), mouse, and other species. The potential applications of our findings also extend beyond the primary association with MHC products in themselves to differentiation molecules, e. g., TL and Qa, the former of which is found only on thymic cells and leukemic cells. This approach would thus allow detailed study with simplified quantitative assays, purification and bioassay of the products with the consequent elaboration of the physiological role in cell differentiation and the development of leukemia.

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