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Expression of LFA-1 by a lymphoblastoid cell line from a patient with monosomy 21: effects on intercellular adhesion

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SUMMARY

Monosomy 21 (M21) is a rare aneuploid condition which in certain cases leads to reduced levels of chromosome 21 gene products. We have prepared an Epstein–Barr virus lymphoblastoid cell-line (LCL) from patient with M21 who has immunological abnormalities, and analysed the expression of lymphocyte function-associated antigen-1 (LFA-1). This heterodimeric leucocyte integrin consists of CD11a (α) subunits non-covalently associated with CD18 (β) subunits coded, respectively, by genes on chromosomes 16 and 21. To determine whether monosomy 21 results in decreased expression of LFA-1, monoclonal antibodies were used to compare the expression of CD11a and CD18 on the M21 LCL with LCL from trisomy 21 (Down's syndrome, T21), normal controls and a possible case of leucocyte adhesion deficiency. In addition, phorbol-ester-induced homotypic adhesion, an LFA-1-mediated effect, was compared in these LCLs. The results are consistent with a gene dosage mediated reduction of LFA-1 expression by the M21 LCL.

Keywords monosomy 21 LFA-1 lymphoblastoid cell line gene dosage homotypic adhesion

INTRODUCTION

Monosomy 21 (M21) is an extremely rare condition where only one copy of chromosome 21 is present in each cell (Fryns *et al.*, 1977). Although the existence of true monosomy has been open to doubt (Ackerman *et al.*, 1988), Dalgleish *et al.* (1988) described a patient with immunological abnormalities whose peripheral blood T cells were uniformly monosomic for chromosome 21. We have prepared an Epstein–Barr virus (EBV) lymphoblastoid cell line (LCL) from this patient and analysed the expression of lymphocyte function-associated antigen-1 (LFA-1) in comparison with other LCL.

LFA-1 is one of three leucocyte integrins which have a common 95-kD CD18 (β) subunit non-covalently associated with distinct 150–180-kD CD11a, b or c (α) chains (Springer *et al.*, 1984). These molecules are part of a super-family of integrins which includes the receptors for fibronectin, vitronectin and von Willebrand factor (Hynes, 1987; Ruoslahti & Pierschbacher, 1987) and function as cell substrate and intercellular adhesion structures.

The genes coding CD11a and CD18 are located, respectively, on chromosomes 16 and 21 (Suomalainen *et al.*, 1986; Marlin *et al.*, 1986; Corbi *et al.*, 1988). Studies on leucocyte adhesion deficiency, an inherited defect in the expression of the leucocyte integrins have shown that the expression of CD11a is post-translationally regulated by CD18 (Springer *et al.*, 1984, Kishimoto *et al.*, 1987).

We have shown that LCLs prepared from trisomy 21 (T21) lymphocytes exhibit constitutive overexpression of CD18 (Taylor *et al.*, 1988b) and CD11a (Robson, Taylor & D'Souza, 1989), and that this can result in increased homotypic adhesion when T21 cells are treated with the phorbol ester TPA (Taylor *et al.*, 1988a). If the effect in T21 is gene dosage related, we would expect to find reduced LFA-1 expression, and adhesion in M21. The results presented here, in which an M21 LCL is compared with normal and T21 LCL confirm this conclusion. In addition, we show that an LCL from a previously unreported case with clinical characteristics similar to leucocyte adhesion deficiency lacks LFA-1 and does not form clusters in the presence of TPA.

MATERIALS AND METHODS

Patients

Details of the M21 patient from whom an EBV-immortalized LCL was prepared are described by Dalgleish *et al.* (1988), and are summarized briefly in the Results. The patient diagnosed as possibly leucocyte adhesion deficient (LAD) was initially referred to the Regional Immunology Laboratory at St Mary's Hospital. Brief details of the patient which are relevant to this study are given in the Results.

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Lymphoblastoid cell-lines

The isolation of lymphocytes, and their immortalization as LCLs following deliberate infection *in vitro* with the B95–8 marmoset cell-line strain of EBV has been described previously (Taylor *et al.*, 1988b). In addition to LCL derived from the M21 and LAD patients, LCL derived from T21 and normal lymphocytes were used in the study. All LCL were maintained in conditions of active growth in RPMI-1640 (Northumberland Biological, Cramlington, UK) containing 10% tryptose phosphate broth (Flow Labs., Irvine, UK), 10% fetal bovine serum, and antibiotics (penicillin and streptomycin).

Monoclonal antibodies (MoAbs)

The CD11a and CD18 MoAbs MHM24 (Hildreth *et al.*, 1983) and 60·3 (Beatty *et al.*, 1983) were kindly provided by Professor A.J. McMichael (Institute of Molecular Medicine, University of Oxford) and Dr Patrick Beatty (Fred Hutchinson Cancer Center, Seattle, WA). The MoAb RR1/1, which reacts with ICAM-1 (CD54) (Rothlein *et al.*, 1986) was a gift from Dr Timothy Springer, Centre for Blood Research, Boston Research, Boston, MA. These MoAbs were used at saturating concentrations in membrane immunofluorescence tests. The hybridoma secreting the HLA class I MoAb W6/32 (Barnstable *et al.*, 1978) was obtained from the American Type Culture Collection (Rockville, MD,), and used as neat supernatant.

Immunofluorescence and flow cytometry

The analysis of antigen expression by the LCL was carried out by indirect membrane immunofluorescence, and quantitative flow cytometry as previously described (Taylor *et al.*, 1988b). Briefly, the cells were incubated with MoAbs, washed and stained with FITC-conjugated goat anti-mouse immunoglobulin (Beckton Dickinson, Mountain View, CA), diluted to 1/20 with phosphate-buffered saline (PBS), washed again, and fixed in 0·5% paraformaldehyde in PBS. Flow cytometry was carried out on a FACS IV (Becton Dickinson) equipped with a Spectra-Physics laser at 220 mW, at a wave length of 488 nm, and PMT of 700 V. In the Results , flow cytometry profiles and mean fluorescence channel (MFC) values are depicted for a fluorecence gain setting of 4·0. Background staining (i.e. cells stained with FITC conjugate alone) was gated out, so that profiles of MoAb-stained cells show specific antigen expression only. Thirty-thousands cells from each sample were analysed, and the profiles plotted on linear axes.

Homotypic adhesion assay

A semi-quantitative homotypic adhesion assay (Taylor *et al.*, 1988a), based on that described by Rothlein & Springer (1986) was used. Briefly, LCLs were washed and resuspended in RPMI 1640 containing 1 mM HEPES buffer (Flow Laboratories) and 5% fetal calf serum (FCS) and checked for viability under phase contrast. Aliquots (90 μl) containing 4×10^5 viable cells were dispensed into 96-well flat-bottomed plates, followed by 10 μl of 12-*o*-tetradecanoyl phorbol-13-acetate (TPA) at various concentrations in duplicate wells. Control wells with no TPA were set up simultaneously. After incubation at 37°C for 60 min– 24 hr, the degree of aggregation was scored on an Olympus inverted microscope thus; 0, no aggregates; 1+, <10%; 2+, 10–50%; 3+, >50% of cells in small clusters; 4+, virtually 100% and 5+, 100% of cells in large compact clusters.

Mixed leucocyte culture

Stimulation of allogeneic lymphocytes by various LCLs was carried out as previously described (Taylor *et al.*, 1984). Lymphocytes were isolated from peripheral blood of healthy donors, and equal volumes (50 μl) containing 10^5 unirradiated responders and irradiated (30 Gy) stimulators were dispensed in quadruplicate combinations into 96-well, round-bottomed microplates. LCL cells used as stimulators were irradiated at 30 Gy, and various dilutions of cells (10, 5, $2{\cdot}5 \times 10^4$ in 50 μl) added to wells containing the responding lymphocytes. The culture plates were incubated at 37°C for 5 days, labelled overnight with 1 μCi/well ^{3}H-thymidine (TdR) (Amersham International), harvested and counted on a liquid scintillation counter. Results are expressed as mean ct/min of ^{3}H-TdR uptake/culture.

RESULTS

Details of M21 and 'LAD' patients

The patient with M21 has been described in detail by Dalgleish *et al.* (1988). He has a history of frequent upper respiratory tract infections, and at 1 year of age was found to have thrombocytopenia and hypo-IgA and IgG gammaglobulinaemia. The immunoglobulin deficiency was treated with human gammaglobulin. During an investigation of mild mental retardation at 3·5 years of age, routine karyotyping revealed 100% M21 in his peripheral blood lymphocytes and 20% M21 in cultured fibroblasts. The peripheral blood M21 was confirmed using chromosome 21-specific DNA probes.

The patient with possible LAD is previously unreported. He had recurrent bacterial infections from birth, with accummulations of 'pus' requiring surgical drainage and parenteral antibiotic treatment. He had pyogenic abscesses, delayed umbilical cord separation and post-immunization abscesses. Bacterial infections included catalase positive staphylococci, *Streptococcus faecialis*, *Str. pneumoniae*, *Escherichia coli*, *Pseudomonas aerogenes coliforms*, and *Proteus* spp (R. Pumphrey, personal communication). The patient also had persistent splenomegaly. *Clostridium difficile* colitis at 4 years of age, failed to respond to antibiotics and leucocyte transfusions, and *Cl. septicum* was later isolated from the stools. A duodeno-sigmoid fistula was diagnosed, but septicaemia, thrombocytopenia, jaundice, and abnormal clotting prevented surgical intervention. The patient died aged 4·3 years, following gastrointestinal haemorrhage. At no time did he have gingivitis.

The patient's neutrophils stained normally with the CD11b MoAb OKM1 at 1 year of age but at 4 years, no CD18 or CD11a expression could be detected with seven CD18 and one CD11a MoAb. CD11b was 70% normal with OKM1, but only 30% with another CD11b MoAb. Other leucocyte phenotype and functional studies of this patient will be reported elsewhere.

CD18 and CD11a expression by M21 and LAD LCL

Lymphocytes from the M21 and LAD patients were separated from peripheral blood, and immortalized as LCLs. The M21 LCL was coded CV143, and the LAD LCL coded CV166. These two LCLs showed striking differences. CV166 LAD grew as a single cell suspension, with little evidence of spontaneous aggregation; CV143 (M21) grew in small clusters in which a significant proportion of the cells appeared to undergo spontaneous disintegration, although this occurred over a period of

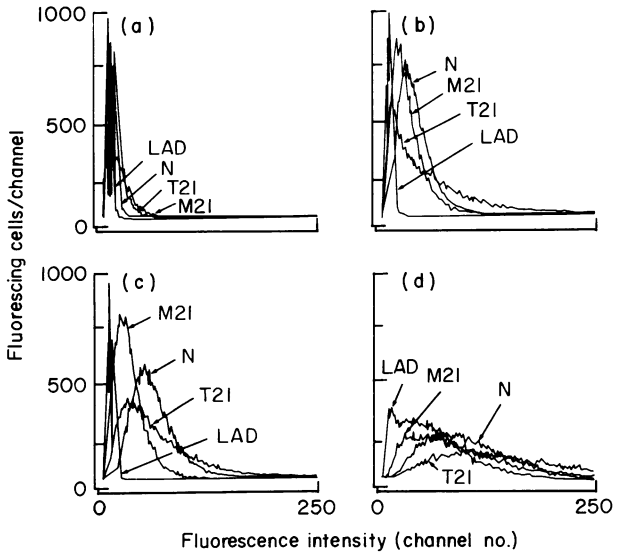

Fig. 1. Flow cytometry profiles comparing normal (N), monosomy 21 (M21), trisomy 21 (T21) and 'leucocyte adhesion deficient' (LAD) lymphoblastoid cell lines (LCLs), stained indirectly with the MoAbs: (b) MHM24 (CD11a); (c) 60.3 (CD18); and (d) W6/32 (HLA class I), followed by goat anti-mouse immunoglobulin FITC. Control profiles (a) show LCL stained with FITC conjugate alone. Cell lines shown are: M21 CV143; LAD, CV166; N, SV28; and T21, CV79.

weeks. The two LCLs showed extremes of growth; CV166 grew more rapidly than normal LCL, whereas CV143 grew extremely slowly. Attempts to increase the growth rate of CV143 using interleukin-2 (IL-2) were not successful, although the cells looked healthier in a crude 5% B cell growth factor preparation.

The expression of CD18 and CD11a by the M21 and LAD LCLs CV143 and CV166, respectively, was analysed in comparison with T21 (Down's syndrome), and normal LCLs by flow cytometry. As shown in Fig. 1, CD11a expression detected with MHM24 is virtually absent from the LAD line (MFC = 1) and much reduced on the M21 LCL (MFC = 22) compared with the normal and T21 LCL (MFC = 35 and 48, respectively). Similar results were obtained for CD18 expression using MoAb 60·3. The LAD LCL lacks CD18 (MFC = 0·3) and the M21 LCL shows reduced expression (MFC = 22), compared with the normal and T21 LCL (MFC = 53 and 54, respectively). In contrast, expression of the HLA class I heavy chain is stronger on the M21 than the LAD LCL (MFC = 98 and 61, respectively) although less than on the normal and T21 LCL (MFC = 118 and 116, respectively)

Experiments were carried out using the MoAb RR1 to detect ICAM-1 which is one of the ligands of LFA-1 on the M21 and LAD LCLs, CV143 and CV166. On the LAD LCL, ICAM-1 is expressed at a lower level (MFC = 34) than on a normal and T21 (MFC = 69 and 54, respectively). In contrast, the M21 LCL (MFC = 53) expresses more ICAM-1 than three normal or T21 LCLs (mean MFC = 36 and 47, respectively).

Homotypic adhesion

It has been shown that LFA-1 is involved in homotypic adhesion by EBV-immortalized LCLs using CD11a and CD18 MoAbs to block cluster formation (Patarroyo *et al.*, 1986; Rothlein & Springer, 1986). To discover whether the reduction

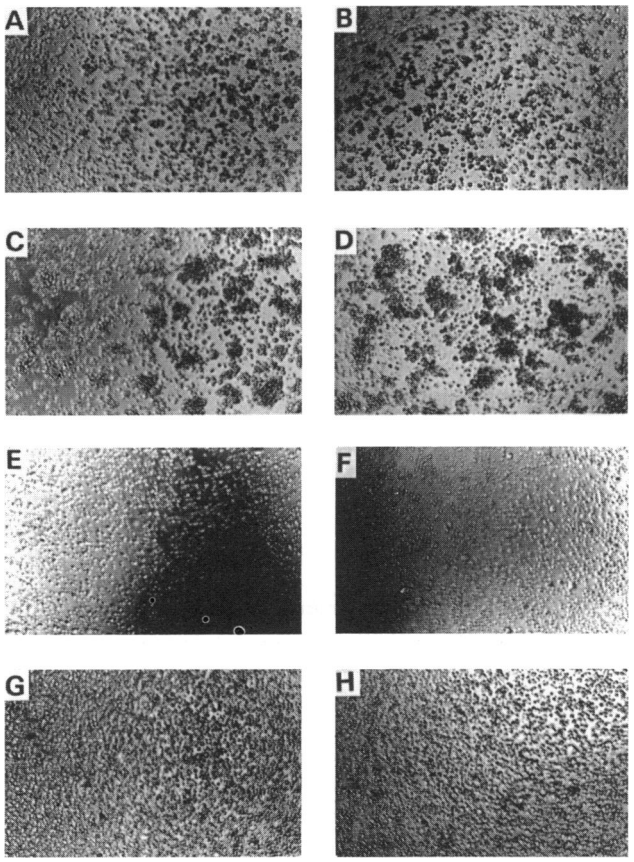

Fig. 2. Low-power photomicrograph comparing the aggregation of M21, T21, LAD, and N-LCL incubated for 45 min with or without 1 ng/ml phorbol ester (TPA). A, B, CV86 (T21); C, D, SV20 (N); E, F, CV143 (M21); G, H, CV166 (LAD).

in CD18 and CD11a expression on the CV143 LCL resulted in altered homotypic adhesion, we incubated the M21 and LAD LCL, in comparison with a normal and T21 LCL in the presence of 1 ng/ml of TPA.

The results in Fig. 2 show that the LAD LCL completely failed to aggregate after 45-min incubation in TPA; aggregation by the M21 LCL was less than expected, considering the relatively small reduction in LFA-1 expression compared with the normal and T21 LCL. There was no evidence that this was due to a lack of viability of the M21 or LAD LCLs. All LCLs were viable at the start of the adhesion assay, and none, including the M21 LCL showed evidence that the assay was cytotoxic. Incubation of these two LCLs for 24 h revealed no evidence of a substantial delayed effect on adhesion, neither was there a TPA concentration-dependent increase in cluster formation (Table 1). The very small amount of aggregation by the LAD LCL after 24 h incubation was probably not due to LFA-1, since TPA-treated CV166 showed no evidence of CD11a or CD18 expression.

Allogeneic lymphocyte stimulation

To determine whether differences in LFA-1 expression and homotypic adhesion could affect the ability of CV143 and CV166 to stimulate allogeneic T cell proliferation, lymphocytes from two normal blood donors were stimulated with three different concentrations (10, 5, 2·5 × 10^6/well) of CV166, CV143

Table 1. Aggregation of LCL after incubation for 24 h

LCL	Code	TPA concentration (ng/ml)* —	1	50	100
Normal	SV20	3+	3+	3+	2+
Monosomy 21	CV143	0	0	0	0
LAD	CV166	+	+	+	+
Trisomy 21	CV86	5+	5+	5+	4+
Trisomy 21	CV77	3+	3+	3+	3+

* Score: 0, no aggregates; 1+, <10%; 2+, 10–50%; 3+, >50% of cells in small clusters; 4+, virtually 100% and 5+, 100% of cells in large compact cluster.

LCL, lymphoblastoid cell line; LAD, leucocyte adhesion deficient.

Table 2. Stimulation of normal lymphocytes by LCL

Stimulator	Responder* 10^5 A	10^5 B	5×10^4 A	5×10^4 B	$2{\cdot}5\times10^4$ A	$2{\cdot}5\times10^4$ B
Autologous lymphocytes	1·3	1·8	0·5	3·7	0·5	2·7
N-LCL (SV28)	100	100	100	64	61	59
N-LCL (SV20)	66	56	50	44	42	49
T21 (CV77)	140	107	102	97	77	56
T21 (CV79)	75	36	74	39	52	41
T21 (CV108)	91	99	68	50	41	50
M21 (CV143)	69	62	26	39	11	13
LAD (CV166)	85	58	90	47	46	35

* Responding lymphocytes at 10^5/well, stimulating cells at 10^4, 5×10^3 and $2{\cdot}5\times10^3$/well; maximum uptake of ^{3}H-TdR (100%) by responders A and B was to 10^5 SV28 (N-LCL)/well; response by A = 45149 ± 4459 ct/min and B = 55093 ± 5391 ct/min. Other results are expressed as percentages of these values.

N-LCL, normal; T21, trisomy 21; M21, monosomy 21; and LAD, leucocyte adhesion deficient.

and with T21, and normal LCL (N-LCL). The results shown in Table 2 are calculated by taking stimulation by one of the N-LCL as 100%, and relating the other LCL to this value. It can be seen from these calculations that all three T21 LCLs stimulated at levels similar to the normal LCL, and that CV166 also stimulated normally. In contrast, CV143 showed a considerably reduced stimulatory capacity compared with the other LCLs. These results show that the level of LFA-1 expression by the LCL stimulators did not affect their ability to stimulate allogeneic T cells.

DISCUSSION

The main findings in this paper concern the expression of CD11a and CD18 by an LCL-derived from a child with M21. When compared with normal and T21 LCLs, our results show that the M21 LCL exhibits reduced CD11a and CD18 expression. To our knowledge, this is the first report of reduced LFA-1 expression due to M21. Although other gene dosage effects have been reported in M21, notably decreased Cu/Zn superoxide dismutase (Yoshemitsu *et al.*, 1983; Philip *et al.*, 1984; Ackerman *et al.*, 1988), in other cases normal levels of this enzyme have been detected (Yamamoto *et al.*, 1979; Wulfsberg *et al.*, 1983).

M21 is an extremely rare condition, although several reports have suggested that it may be a distinct syndrome (Fryns *et al.*, 1977). In some cases, such as that described by Ackerman *et al.* (1988), the monosomy seems to originate as an unbalanced translocation, whereas complete M21 may be incompatible with post-natal viability. The patient in the present study showed complete M21 in his T lymphocytes, but only 20% of his fibroblasts were monosomic, the remainder containing a ring 21 (Dalgleish *et al.*, 1988). Cytogenetic analysis of CV143, the M21 LCL, showed this to be completely monosomic for chromosome 21. It is not clear whether loss of chromosome 21 has occurred in the patient's other haemopoietic cell lineages.

We included in this study an LCL which was completely deficient in LFA-1 expression, as shown by flow cytometric analysis with subunit-specific MoAb. This LCL was derived from lymphocytes obtained from a previously unreported patient who died from recurrent infections, aged 4·3 years. Although the lack of LFA-1 on this LCL is consistent with LAD, a number of the clinical findings in this patient leave the question open. For instance, the formation of pus and the absence of gingivitis are difficult to reconcile with LAD, as is the occurrence of OKM1 reactivity in the absence of other CD11b epitopes. It is likely that the nature of the defect in this patient will only be resolved by analysis of subunit-specific translation products and mRNA levels.

In view of the role of LFA-1 as an accessory adhesion molecule (Patarroyo *et al.*, 1986; Rothlein & Springer, 1986), we investigated the capacity of the M21 LCL to form homotypic aggregates in the presence of TPA. We found that the M21 cells were only weakly adherent, irrespective of the concentration of TPA used, or the time of incubation. This is unlikely to be due to cell death, since this LCL like the others in this study was viable at the start of the assay, and no cytotoxic effect was noted when the cells were examined by microscopy at the end of the assay. The lack of growth in this LCL occurred over a period of weeks and was a chronic rather than immediate effect. Since CD11a and CD18 are expressed by the M21 cells, the poor adhesion suggests that small changes in LFA-1 expression can have a large functional effect. It can be envisaged that changes in LFA-1-mediated adhesion could be regulated by different levels of CD18 mRNA. Such a mechanism could influence the interaction of normal leucocytes during recirculation and immune responses.

Our results lend support to the important role of CD18 in determining leucocyte integrin expression and function (Springer *et al.*, 1984). Human–mouse hybrids made by fusing the LAD LCL CV166 with mouse BW5147 cells were found to express human CD11a$^+$/CD18$^-$ (Taylor, Robson & d'Souza, 1989) presumably due to the formation of complementary human CD11a/murine CD18 complexes as reported by Marlin *et al.* (1986) and Tetteroo *et al.* (1987).

Whether reduced LFA-1 expression by CV143 is the cause of

the frequent upper respiratory tract infections in the patient with M21 is not known. It is interesting that the expression of ICAM-1, the ligand of LFA-1 (Rothlein *et al.*, 1986; Makgoba *et al.*, 1988), is increased above normal levels on the M21 LCL, since this is a receptor for rhinovirus (Greve *et al.*, 1989; Staunton *et al.*, 1989). The stikingly slow growth and apparently shortened life span of the M21 LCL suggests that genes other than CD18 on chromosome 21 can affect leucocyte growth and differentiation.

Stimulation of normal T cells in allogeneic mixed leuycocyte culture (MLC) is dependent on adhesion mediated by LFA-1 expressed by the responding cells (Martz, 1987). In the present study only the M21 LCL, and not the LAD LCL was an ineffective stimulator in MLC. This contrasts with results showing that other LAD cells are poor stimulators of T cells (Krensky *et al.*, 1985), further emphasizing the diversity of this condition (Fischer *et al.*, 1988). The weak stimulatory capacity of the M21 LCL is unlikely to be the result of reduced HLA class II expression, since CV143 and CV166 showed similar levels of staining with the HLA-DR MoAb L243 (unpublished observations). Clayberger *et al.* (1987) have suggested that reduced LFA-1 expression by lymphoma cells could explain their escape from immunosurveillance. Gregory *et al.* (1988) have proposed a similar explanation for the inability of HLA-restricted cytotoxic T cells to destroy certain Burkitt's lymphomas. Such a mechanism could be a consequence rather than a cause of neoplasia, since T21, which has markedly increased risk of leukaemia (Fong & Brodeur, 1987) exhibits increased rather than decreased LFA-1 expression.

Constitutive alterations in the expression of leucocyte antigens as a result of aneuploidy have not been widely reported, probably because most affected fetuses other than T21 do not reach full term. The use of EBV to prepare immortalized LCLs should be useful in studying the effects of aneuploidy, particularly in relation to leucocyte differentiation.

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