JCI The Journal of Clinical Investigation

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J Clin Invest. 1983;72(2):694-706. https://doi.org/10.1172/JCI111019.

Research Article

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Evidence for Genetic Restriction in the Suppression of Erythropoiesis by a Unique Subset of T Lymphocytes in Man

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ABSTRACT The suppression of erythropoiesis by lymphocytes from patients with a T cell lymphoproliferative syndrome and pure erythrocyte aplasia has been previously demonstrated. To study the nature of the suppressor cell and possible genetic restriction of this suppression, we investigated a patient with severe anemia, splenomegaly, lymphocytosis, and erythroid aplasia. A 3-mo course of low-dose daily oral cyclophosphamide achieved a complete remission for over 12 mo. The surface phenotype of his lymphocytes was analyzed by means of antibodies to lineage, differentiation, and activation-specific surface antigens. The cells expressed mature T cell antigens T3, T8, and T11, while lacking T1. Immature T cell, B cell, and the monocyte-specific antigen Mo2 were absent, while Mo1, a monocyte-associated antigen not normally expressed on T cells, was present. T10 and Ia expressed as activation antigens were also present. The cells, cryopreserved at diagnosis, were thawed and co-cultured in plasma clot with patient remission marrow samples at T cell/bone marrow ratios of 1:1 and 2:1. There was nearly 90% suppression of erythroid colony-forming unit expression and 60% suppression of erythroid burst-forming unit expression at 2:1 T cell to bone marrow ratios and somewhat less suppression at 1:1. Granulocyte/macrophage progenitor expression was unaffected. Erythroid progenitor differentiation in the marrows of two HLA identical siblings was similarly suppressed. The cells were co-cultured with the marrows of nine nonrelated donors to investigate the potential genetic restriction of this suppression. Colony suppression equal to that observed in the marrow of the patient and his siblings was found in studies of two partially HLA identical individuals. No suppression was detected in marrow co-cultures of two entirely HLA dissimilar individuals. These results show that suppression of erythropoiesis by a unique subset of T8, Mo1, Ia-positive lymphocytes isolated from a patient with lymphocytosis and erythrocyte aplasia is genetically restricted.

INTRODUCTION

The fine regulation of erythropoiesis is accomplished by both inductive and suppressive mechanisms.

Induction is achieved by a hormone and a less well-characterized factor. The hormone, erythropoietin, stimulates the division of mature erythroid progenitors and their terminal differentiation into proerythroblasts (1, 2), whereas the factor, burst-promoting, or erythroid-potentiating, activity (BPA),[1] appears to influence the capacity of progenitors, particularly immature progenitors, to respond to erythropoietin. Current evidence suggests that an interaction of T lymphocytes and macrophages leads to the elaboration of BPA (3),

This work was presented in part at the 24th Annual Meeting of the American Society of Hematology, Washington, DC, 4–7 December 1982.

Dr. Nadler was supported by a grant from the Medical Foundation of Boston.

Received for publication 13 December 1982 and in revised form 20 April 1983.

[1] *Abbreviations used in this paper:* BFU-E, erythroid burst-forming unit; BM, bone marrow mononuclear cell; BPA, burst-promoting activity; CFU-E, erythroid colony-forming unit; CFU-G/M, granulocyte/macrophage CFU; CTL, cytotoxic T lymphocyte; MC, mononuclear cell preparation; NK, natural killer.

whereas erythropoietin is produced in the kidneys of adults (4).

Suppression of erythropoiesis is regulated by the influence of the level of oxyhemoglobin on erythropoietin production. Recent investigations have additionally suggested the existence of a second suppressive system exerted by T lymphocytes (5, 6). The existence of such a system was initially suggested by clinical examples of anemia or granulocytopenia associated with an expansion of certain T lymphocyte populations (7–13). Furthermore, studies of normal cells have led to the suggestion that T lymphocyte-induced suppression of in vitro erythropoiesis may be genetically restricted (14). To examine the in vivo relevance of the latter possibility, we studied the effects of the T lymphocytes of a patient with the previously described syndrome of T lymphocytosis and erythrocyte aplasia (7–10) upon the in vitro erythropoiesis of the patient, his two HLA identical siblings, and a panel of nine partially or totally HLA dissimilar normal individuals. The results show that these cells specifically and uniquely suppress the differentiation of erythroid progenitors that bear genotypically or phenotypically identical HLA antigens. The unique phenotype of these cells suggests the possibility that they may represent an unregulated expansion of a normal subpopulation of T lymphocytes or a malignant clone, the normal cellular counterpart of which is present at low levels and modulates the production of erythrocytes.

METHODS

Case report. R.McQ. is a 32-yr-old man who first presented with anemia, hepatosplenomegaly, and lymphocytosis. Peripheral lymphadenopathy was absent. His hematocrit was 15% with a mean corpuscular volume of 104, a reticulocyte count of 0.9%, and a lymphocyte count of 21,000/mm^3. The leukocyte differential showed 11% granulocytes, 4% bands, 2% metamyelocytes, 1% basophils, 80% large lymphocytes, and 2% monocytes. The platelet count was 400,000/mm^3. Coombs and heterophile antibody tests were negative. Bone marrow biopsy showed hypercellularity with increased number of myeloid cells and megakaryocytes, but virtually absent erythroid precursors. Occasional cells of an indeterminate type were noted. An exploratory laparotomy was performed and a 580-g spleen was removed. A concomitant liver biopsy was performed. Both the liver and spleen were diffusely infiltrated with large lymphoid cells. After splenectomy, the patient's leukocyte count rose from 20,000 to 35,700/mm^3, with 90% lymphocytes. He was then begun on a regimen consisting of vincristine, cyclophosphamide, bleomycin, and prednisone for 10 mo. His hematocrit rose to between 27 and 30%. The leukocyte count was maintained between 18,000 and 25,000/mm^3, with at least 80% lymphocytes. Treatment was then discontinued for 1 yr, during which time he again developed severe anemia with a hematocrit in the range of 20%. He became transfusion dependent for 8 mo. A repeat bone marrow aspiration showed relative granulocytic hyperplasia, increased megakaryocytes, and minimal lymphoid infiltration. Erythroid precursors were few to absent. At this point, the leukocyte count was in the range of 20,000/mm^3 with 80% lymphocytes, nearly all of which resembled the cells shown in Fig. 1. The patient underwent phlebotomy and $\sim 10^8$ of these cells were isolated by Ficoll-Hypaque centrifugation. These were cryopreserved, and the patient was started on 100 mg of cyclophosphamide by mouth for 2 mo. The dose was decreased to 50 mg/d for 2 additional mo. When the hematocrit was noted to be 45%, a bone marrow aspirate showed normal hematopoiesis with no excess of lymphocytes. No further therapy or transfusions were given for 18 mo. As of December 1982, his leukocyte count was $\sim$10,000/mm^3, with 55% granulocytes, and 31% lymphocytes with no excess of T8 (15) positive cells, 500,000 platelets/mm^3, a hematocrit of 47%, and a mean corpuscular volume of 99.

Normal volunteers. 11 informed normal adults, including the two histocompatible male siblings of the patient, consented to provide the bone marrow samples that made this study possible.

HLA typing. Serotyping of cells for HLA A, B, C and DR antigens on the patient and normal controls (Table I) was kindly performed by Ms. K. Shanley (Dana-Farber Cancer Institute HLA Typing Laboratory), using the standard National Institutes of Health cytotoxicity technique on peripheral blood B cells.

Monoclonal antibodies. The preparation and characterization of monoclonal antibodies used in this study has been previously described in detail (15–25). All antibodies were ascites fluid and used at saturated binding concentrations. Controls were isotype identical nonreactive ascites.

Indirect immunofluorescence and flow cytometry. Analysis of the reactivity of the patient lymphocytes with monoclonal antibodies was performed with a fluorescence-activated cell sorter (FACS IV; Becton-Dickman FACS Systems, Mountain View, CA). In brief, 1–2 $\times$ 10^6 cells were treated with either 0.1 ml of a 1:500 dilution of the specific monoclonal antibody to be tested or 0.1 ml of a 1:500 dilution of an unreactive control antibody, incubated at 4°C for 30 min, and washed three times. These cells were then reacted with 0.1 ml of a 1:40 dilution of fluorescein-conjugated goat anti-mouse Ig G/M (Meloy Laboratories, Inc., Springfield, VA), incubated at 4°C for 30 min, washed three times, and analyzed as previously described (25). Intensity of fluorescence was determined for 10,000 cells in each population on the fluorescence-activated cell sorter and compared with the fluorescence of a control nonreactive ascites. A displacement of the histogram for the test monoclonal antibody compared with the histogram of the control was scored as positive. In addition, for each test sample, a quantitative assessment of the percentage of positive cells was made (number of cells reactive with test monoclonal antibody minus number of cells reactive with the control divided by 10,000 total cells tested).

Cyropreservation. Patient peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density centrifugation as previously described (26). Isolated cells were cryopreserved in 10% dimethyl sulfoxide and 20% fetal calf serum at −196°C in liquid nitrogen until the time of study.

Cell culture. Bone marrow mononuclear cells (BM) prepared by Ficoll-Hypaque density centrifugation (26) were plated at a cell density of 5 $\times$ 10^5/ml in the plasma clot culture system previously described (27) and modified (28, 29). In certain experiments, BM were co-cultured with freshly thawed cryopreserved patient lymphocyutes or control cells at lymphocyte-to-bone marrow ratios that ranged from 1:20 to 2:1. The culture system contained 2 U/ml of erythropoietin (Toyobo Human Urinary Erythropoietin, sp

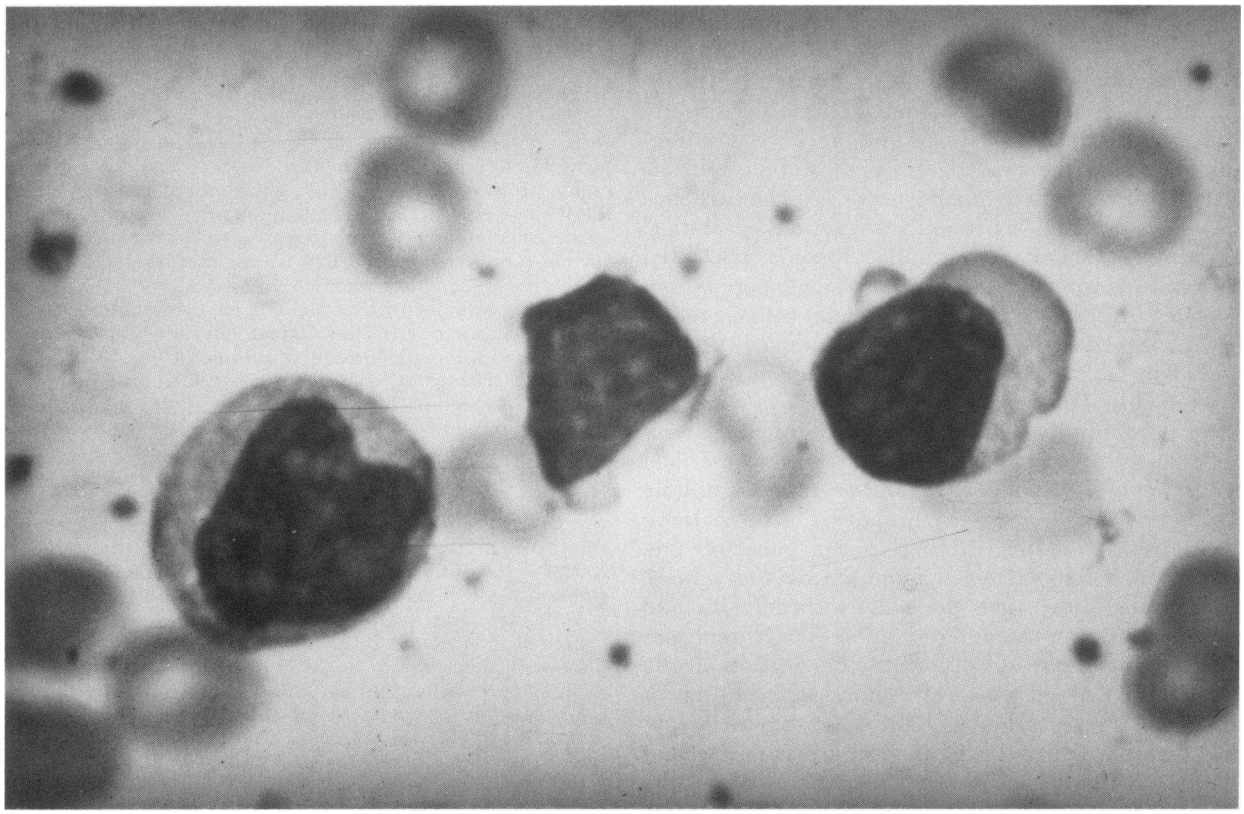

FIGURE 1 Light microscopic photomicrograph of the patient peripheral blood mononuclear cells used in this study. The lymphocytes are moderately large with slightly indented nuclei. Nucleoli are not apparent. With Wright-Giemsa staining, the abundant cytoplasm is slightly basophilic and is dotted with small azurophilic granules.

act 49 U/mg protein, Toyobo Co., Ltd., Osaka, Japan). In some experiments, BPA, the supernatant of the Mo cell line (30) kindly provided by Dr. David Golde, was substituted for NCTC-109 (Microbiological Associates, Bethesda, MD) as 5% of the plasma clot culture.

Clotting was initiated by the addition of 0.1 ml of NCTC-

TABLE I

HLA Phenotypes of a Panel of 12 Bone Marrow Donors, including Patient R.Mc.Q., Siblings J.McQ. and L.McQ., and Normal Controls

Marrow donor	HLA locus						
R.Mc.Q. (Patient)	A-2	*	B-15	B-18	Cw3	DR2	DR4
J.Mc.Q. (Sibling)	A-2	*	B-15	B-18	Cw3	DR2	DR4
L.Mc.Q. (Sibling)	A-2	*	B-15	B-18	Cw3	DR2	DR4
R.D.	A-2	A-28	Bw-39	B-18	Cw3	DR2	DR4
J.T.	A-2	A-1	B-7	B-40	Cw3	DR2	DR4
J.G.	A-28	*	Bw-35	Bw-44	Cw4	*	DR4
N.B.	A-2	*	B-13	Bw-51	*	DR7	DR4
L.C.	A-3	Aw-30	Bw-35	Bw-42	Cw4	DR3	DR4
L.K.	A-3	A-26	Bw-41	Bw-44	Cw5	DR2	DR2‡
J.F.	A-2	A-28	B-27	Bw-51	Cw1	DR5	DR7
M.P.	A-11	Aw-32	B-27	Bw-51	Cw2	DR7	*
D.M.	A-3	A-29	Bw-44	*	Cw6	DR3	DR7

* Untypeable locus or homozygosity.

‡ Proof of homozygosity established by family study.

109 containing 1 U of grade 1 bovine thrombin (Sigma Chemical Co., St. Louis, MO). The 1.0 ml of clotting mixture was dispensed in 0.1-ml aliquots into 0.2-ml microtiter culture wells (Linbro plates; Linbro Chemical Co., New Haven, CT) and incubated under 5% CO_2 in high humidity.

The plasma clots were incubated for up to 14 d. The clots were fixed and stained as described by McLeod et al. (27). Erythroid colonies in three to six clots were counted and the results expressed as the mean and standard error of the mean of the number of erythroid colonies per 10^5 cells plated. Erythroid colony-forming units (CFU-E) were enumerated on day 7 and erythroid burst-forming units (BFU-E) on days 11–14, as previously described (29).

For evaluation of the granulocyte/macrophage colony-forming unit (CFU-G/M), 0.1 ml of NCTC-109 was substituted for NCTC-109 containing erythropoietin. No cell-conditioned medium as a source of colony-stimulating activity was substituted for an additional 0.05 ml of NCTC-109. Colonies were fixed and stained as for erythroid colony evaluation. GFU G/M colonies were enumerated on days 7 and 11–14.

Cytolytic activity: natural killer (NK) and cytotoxic T lymphocyte (CTL) assay. An assay for NK and CTL activity using K562 or JY cells as targets and T8ES cells as effectors at effector (T8ES)-to-target (K562 or JY) ratios of 100, 25, and 4 to 1 were performed as previously described (31). Activity was measured as percentage of specific release of ^{51}Cr from labeled targets. CTL activity was measured in lectin (concanavalin A)-dependent and independent systems (32). Assays were performed in triplicate.

RESULTS

Flow cytometric analysis of patient peripheral blood mononuclear cells. Greater than 90% of the patient cells that were harvested and cryopreserved before cyclophosphamide therapy were viable, as assessed by dye exclusion after thawing.

Fig. 2 shows representative flow cytometric histograms of these cryopreserved and thawed peripheral blood mononuclear cells.

The histograms in Fig. 2 revealed that >95% of the cells co-expressed T3, T8, and T11 antigens. Contaminating T1- and T4-positive cells represented <5% of the population. Since expression of the immature T cell antigens T6 and T9 was lacking (data not shown), the T cell phenotype described here appears to define a mature suppressor cell. A peculiar feature of these cells was the anomalous absence of detectable T1 antigen. T1 is expressed on all normal peripheral blood and thymic-derived T lymphocytes. Its absence additionally suggests that these cells represent a unique subpopulation of T lymphocytes.

Fig. 2 also shows indirect immunofluorescence histograms using antibodies to Mol, Mo2, and Ia. Mol is an antigen expressed on granulocytes, monocytes, and some null cells including NK cells (20, 21). It has never been found on T cells (20, 21), but was readily detectable on this patient's T cells. The myeloid antigen, Mo2 (20), was not present. In addition, the Ia antigen was present at an antigen density similar to that found on activated T cells (19). This finding together with the presence of T10, another activation antigen (15, 33), indicate also that these cells have a

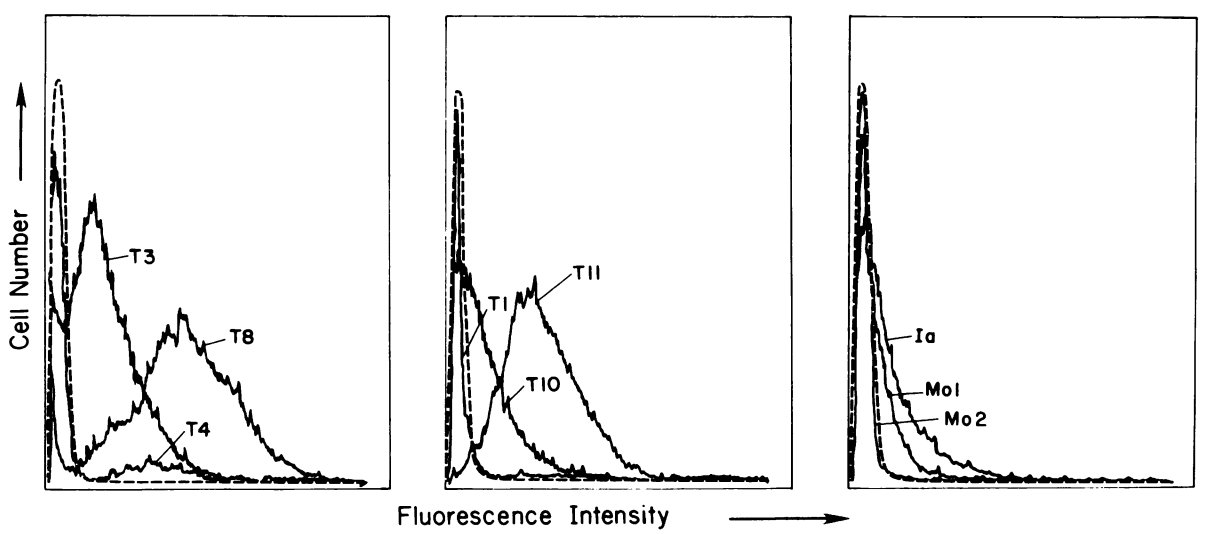

FIGURE 2 Flow cytometric analysis of patient peripheral blood mononuclear cells. Analysis was done with antibodies to T3, T4, and T8 (*left* panel); T1, T10, and T11 (*middle* panel); and Mol, Mo2, and Ia (*right* panel) with a fluorescein conjugated anti-mouse IgG/M. The histograms (*left*, *middle* panels) show >95% of the cells to co-express T3, T8, and T11 with contaminating T1- and T4-positive cells representing <5% of the cells analyzed. Further analysis (*right* panel) shows the cells to express, at low fluorescent intensity, Mol and Ia but not Mo2.

surface phenotype similar to that of activated T lymphocytes.

Finally, the patient's cells lacked any of the presently known B cell antigens B1, B2, common acute lymphoblastic leukemia antigen. Nor did their surfaces contain kappa or lambda light chains or intact IgG, IgM, or IgD (data not shown).

Effect of the co-culture of patient-derived peripheral T8-positive mononuclear cells and autologous bone marrow upon hematopoietic progenitor (BFU-E and CFU-E, CFU-G/M) expression in plasma clot. To evaluate the in vitro suppressive effect of patient-derived lymphocytes on autologous progenitors, the cryopreserved mononuclear cells described above were thawed and co-cultured with fresh autologous and other normal bone marrow cells.

Fig. 3 shows the effect of patient and other mononuclear cells preparations (MC) co-cultured with patient BM in ratios that ranged from 1:20 to 2:1, MC/BM and at constant BM concentration (5×10^5/ml). The other MC included samples of cryopreserved T4-positive T cell chronic lymphocytic leukemia and B1-positive B cell chronic lymphocytic leukemia. The figure represents the percentage of maximum colony growth achieved as a function of the ratio of MC to BM. The results demonstrate that co-culture of the patient's cells with autologous BM at a ratio of 1:1 resulted in only 30% CFU-E-derived colony expression and that complete suppression of CFU-E-derived colony expression occured at MC/BM ratio of 2:1. Co-culture with similarly prepared B cell or T4 CLL cells at identical ratios caused no suppression of CFU-E-derived colony expression. BFU-E colony expression was also inhibited by the patient cells but to a somewhat lesser extent. Significant suppression of in vitro BFU-E colony expression occured at a ratio of 1:1, and colony expression was only 20% of control at a ratio of 2:1. There was an absence of any effect of the patient cells on CFU-G/M colony expression. To determine whether the cells interfered with the production of a necessary co-inducer, such as BPA, rather than directly inhibiting progenitor differentiation, these studies were repeated in the presence of Mo conditioned medium, a potent source of BPA. The results were identical (data not shown). The erythroid specificity of the suppression induced by these T8-positive cells therefore prompted the use of the term T8ES (erythroid suppressor) to describe these cells.

Effect of T8ES cells upon normal marrow hematopoietic progenitor colony expression. To evaluate

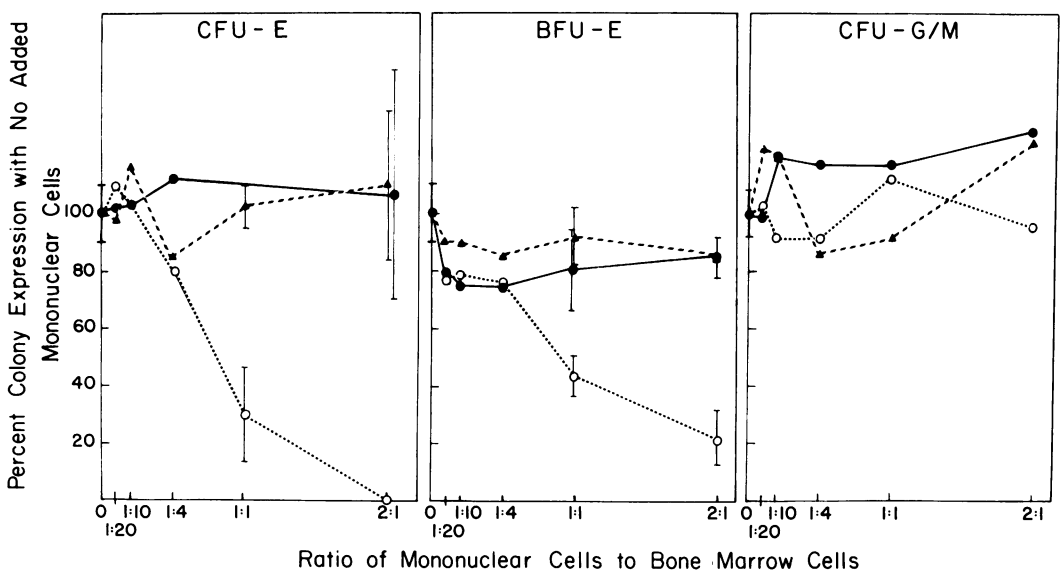

FIGURE 3 Effect of co-culture of patient-derived T8 positive mononuclear cells and autologous bone marrow upon hematopoietic progenitors. The percentage of progenitor colony expression compared with that with no added autologous or control cells is shown on the verticle axis vs. the ratio of autologous T8 cells (○), control T4 CLL cells (●), and B CLL cells (▲) to BM on the horizontal axis. Suppression of CFU-E colony expression (*left* panel) is first seen at a T8ES:BM ratio of 1:1 and is maximal at 2:1. Control T4 and B cells have no suppressive effect. The SEM is shown for the relevant points. A similar analysis (*middle* panel) is shown for BFU-E colony expression. Again suppression is first noted at 1:1 and is maximal at 2:1. Analysis of bone marrow-derived CFU-G/M (*right* panel) shows no suppressive effect of patient T8-positive or control cells upon colony expression. Maximal colony expression was 298±50, 63±6, and 124±7 for CFU-E, BFU-E, and CFU-G/M/10^5 cells plated, respectively.

TABLE II

Erythroid Colony Frequencies in Patient, Siblings, and Normal Control Bone Marrow Cultures

Marrow donor* Experiment 2	CFU-E/10^5 cells plated‡						BFU-E/10^5 cells plated‡					
	1	2	3	4	5	6	1	2	3	4	5	6
R.McQ. (patient)	298±50	189±32	107±10	133±26	87±25	—	63±6	26±5	37±6	24±7	26±5	—
J.McQ. (sibling)	—	—	376±20	—	195±31	—	—	—	56±8	—	33±10	—
L.McQ. (sibling)	—	—	—	195±58	—	—	—	—	—	43±12	—	—
R.D.	—	—	—	—	383±67	302±59	—	—	—	—	46±6	40±6
J.T.	—	—	260±16	—	—	—	—	—	61±8	—	—	—
J.G.	—	—	—	—	—	492±53	—	—	—	—	—	47±5
N.B.	236±12	174±13	—	—	—	—	33±5	20±5	—	—	—	—
L.C.	—	—	—	—	541±116	—	—	—	—	—	32±5	—
L.K.	—	—	—	—	—	177±49	—	—	—	—	—	29±6
J.F.	—	—	—	—	—	621±86	—	—	—	—	—	77±9
M.P.	—	—	—	—	203±15	501±65	—	—	—	—	37±11	74±4
D.M.	—	—	—	262±26	—	—	—	—	—	77±6	—	—

* The marrow donors participated in at least one of six experiments.

‡ CFU-E and BFU-E colony frequencies are the mean and SE of the mean erythroid colony frequencies observed in these marrows in 3–6 replicate plasma clots in the absence of added T8ES or control cells. In subsequent studies of T8ES cells shown in Table III, the percentage of suppression was calculated from the change in these frequencies.

TABLE III

Suppression of Erythroid (CFU-E and BFU-E) Colony Formation in a Panel of 12 Bone Marrow Cultures by the T8ES Cells of Patient R.Mc.Q.

Marrow donor	HLA antigens shared by bone marrow donor and T8ES cells*						Suppression of CFU-E‡							
							1		2		3		4	
	A	C	B		D		2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1
R.McQ.	A-2	Cw3	B-15,	B-18	DR2,	DR4	100	70	92	64	90	38	100	89
J.McQ.	+	+	+	+	+	+	—	—	—	—	89	68	—	—
L.McQ.	+	+	+	+	+	+	—	—	—	—	—	—	87	63
R.D.	+	—	—	+	+	+	—	—	—	—	—	—	—	—
J.T.	+	+	—	—	+	+	—	—	—	—	68	52	—	—
J.G.	—	—	—	—	—	+	—	—	—	—	—	—	—	—
N.B.	+	—	—	—	—	+	38	10	24	(+1)	—	—	—	—
L.C.	—	—	—	—	—	+	—	—	—	—	—	—	—	—
L.K.	—	—	—	—	+	—	—	—	—	—	—	—	—	—
J.F.	+	—	—	—	—	—	—	—	—	—	—	—	—	—
M.P.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
D.M.	—	—	—	—	—	—	—	—	—	—	—	—	8	2

* The HLA antigens shared by the marrow donor with R.Mc.Q. T8ES cells are indicated by the symbol +.

‡ The percentage of suppression (or stimulation) of individual donor erythroid colonies by T8ES cells at 2:1 and 1:1 T8ES/BM ratios. The percentage was calculated from the change in the colony frequencies enumerated in Table II.

the possibility of HLA restriction of erythroid suppression by T8ES cells, a panel of BM was developed consisting of two HLA identical siblings and nine nonrelated normal individuals with similar and dissimilar HLA loci (Table I). The erythroid colony frequencies observed in the marrow cultures of these donors are shown in Table II. T8ES cells were co-cultured with the BM of all the members of this panel at ratios of 2:1 and 1:1 MC/BM and the percentage of suppression of CFU-E and BFU-E colony formation was determined. Four of six studies were accompanied by a control consisting of BM alone and BM to which T4-positive CLL or B-CLL cells were added at 2:1 and 1:1. In all studies, a previously suppressed individual was studied in addition to individuals from the panel. Thus, the absence of suppression in a particular control was always seen in a study in which suppression was demonstrated. Percentage of suppression in all studies was calculated from the ratio of colonies observed in the presence of T8ES cells to colonies observed in the absence of added cells. The results are set out in Table III. The results show that the patient's BM erythroid colony formation was consistently suppressed by T8ES cells in five studies and that the BM of his HLA identical siblings were similarly affected. In contrast, the erythroid colony expression in BM cultures of non-related donors, M.P. and D.M., who were entirely nonidentical with respect to HLA, was not affected. However, erythroid colony formation was markedly suppressed by T8ES cells in BM cultures of two other nonrelated individuals with complete phenotypic DR identity and partial HLA A, B and HLA A, C identity (R.D. and J.T.). An individual with A locus identity only (J.F.) exhibited no evidence of suppression, suggesting that DR region homology could be related to the above observed suppression. To evaluate this further, we studied the effect of T8ES cells on the BM of two nonrelated individuals with DR4 identity; DR4/DR− (J.G.), DR3/DR4 (L.C.) and one with A-2 and DR4/DR7 (N.B.). In two of these individuals (J.G. and N.B.) there was slight suppression of CFU-E but not BFU-E colony formation, and in one such individual (L.C.) there was no suppression. A DR2/DR2 homozygote (L.K.) also with no HLA A identity exhibited no evidence of suppression. It is of interest to note that in those cultures in which no erythroid colony suppression was observed, stimulation of erythropoiesis was found rather frequently. This effect may be due either to improved growth conditions caused by a more favorable cell density, or, more likely, to lymphokines provided by T cells when they are nonspecifically stimulated in such cultures (29). Fig. 4 summarizes the percentage of CFU-E and BFU-E colony suppression as a function of DR type shared between R.McQ. T8ES cells and donor marrow at T8ES:BM ratios of 2:1. Homology between T8ES and BM at HLA A, either A-2 or crossreacting A-28, is indicated by an asterisk. Maximal suppression was noted in those individuals with DR 2/4 and perhaps HLA A homology between T8ES and BM.

NK and CTL activity of T8ES cells. Since the T8 antigen defines a subset of T cells with a cytotoxic/

TABLE III (*Continued*)

Suppression of BFU-E‡															
Experiment															
5		6		1		2		3		4		5		6	
2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1
81	26	—	—	77	56	54	12	78	28	33	26	7	(+42)	—	—
84	34	—	—	—	—	—	—	43	24	—	—	27	9	—	—
—	—	—	—	—	—	—	—	—	—	35	26	—	—	—	—
81	39	89	51	—	—	—	—	—	—	—	—	43	13	57	40
—	—	—	—	—	—	—	—	36	20	—	—	—	—	—	—
—	—	18	23	—	—	—	—	—	—	—	—	—	—	(+2)	(+2)
—	—	—	—	(+14)	(+16)	(+18)	(+20)	—	—	—	—	—	—	—	—
(+6)	(+1)	—	—	—	—	—	—	—	—	—	—	(+97)	(+81)	—	—
—	—	(+10)	(+36)	—	—	—	—	—	—	—	—	—	—	(+31)	(+37)
—	—	6	6	—	—	—	—	—	—	—	—	—	—	(+1)	5
(+35)	(+34)	(+2)	6	—	—	—	—	—	—	—	—	(+62)	(+42)	2	2
—	—	—	—	—	—	—	—	—	—	(3)	3	—	—	—	—

suppressor phenotype (17), further functional analysis of T8ES cells was undertaken to determine the presence of cytolytic capability. By the use of a cytotoxicity assay, T8ES cells were screened for NK and CTL activity. NK activity was determined by the capability of these cells to spontaneously lyse the NK-sensitive cell line K562 (34). CTL activity was determined by the ability of T8ES cells to lyse JY cells, a lymphoblastoid B cell line (35) in a lectin-dependent system. The addition of lectin allows for HLA nonrestricted antigen-nonspecific lysis of targets if the effector cells have CTL capability (32). These results in Table IV show that T8ES cells have neither NK nor CTL activity.

DISCUSSION

In six incidences of aregenerative anemia and two cases of neutropenia, some evidence of lymphocyte-mediated erythroid or granulocyte suppression has been demonstrated in vitro (7–11, 13, 36).

In the study reported here, we describe a patient with lymphocytosis and aregenerative anemia whose T cells were clearly capable of in vitro suppression of erythroid progenitor differentiation, but had no effect on granulocyte/macrophage progenitor development. In addition, the erythroid suppression identified here was genetically restricted, and the suppressor cells possessed a unique cell surface phenotype (T3, T8, T11, Mol, Ia). We refer to cells that bear this phenotype as T8ES to signify that these are T8-positive erythroid but not granulocyte suppressor cells.

The specific function of T8ES cells was demonstrated by co-culture of these cells with the autologous marrow cells of the patient when he was in cyclophosphamide-induced remission, with the marrow cells of two histocompatible siblings and with the cells of nine additional unrelated individuals. The results clearly establish that T8ES cells uniquely suppressed erythroid but not granulocyte progenitors with a greater effect on the terminal differentiation of CFU-E than BFU-E. Furthermore, as previously observed in T cell-B cell-macrophage interactions (37), the suppression was observed only when the target marrow and T8ES cell population shared HLA antigens. For example, there was virtually no suppression of erythroid colony formation when T8ES cells were incubated with the marrow cells of either of two totally HLA dissimilar individuals. In contrast, erythroid colony formation was equally suppressed by T8ES cells cultured with the marrow cells of the patient, his HLA identical siblings and two unrelated donors with incomplete phenotypic identity at HLA AB and AC, respectively, but with complete identity at DR (DR2/DR4).

To examine whether the suppression was uniquely governed by recognition of class I or class II antigens, we evaluated three individuals phenotypically haploidentical for DR (DR4/DR−), (DR3/DR4), and (DR4/DR7) and found some suppression of CFU-E differentiation and no BFU-E suppression in two of

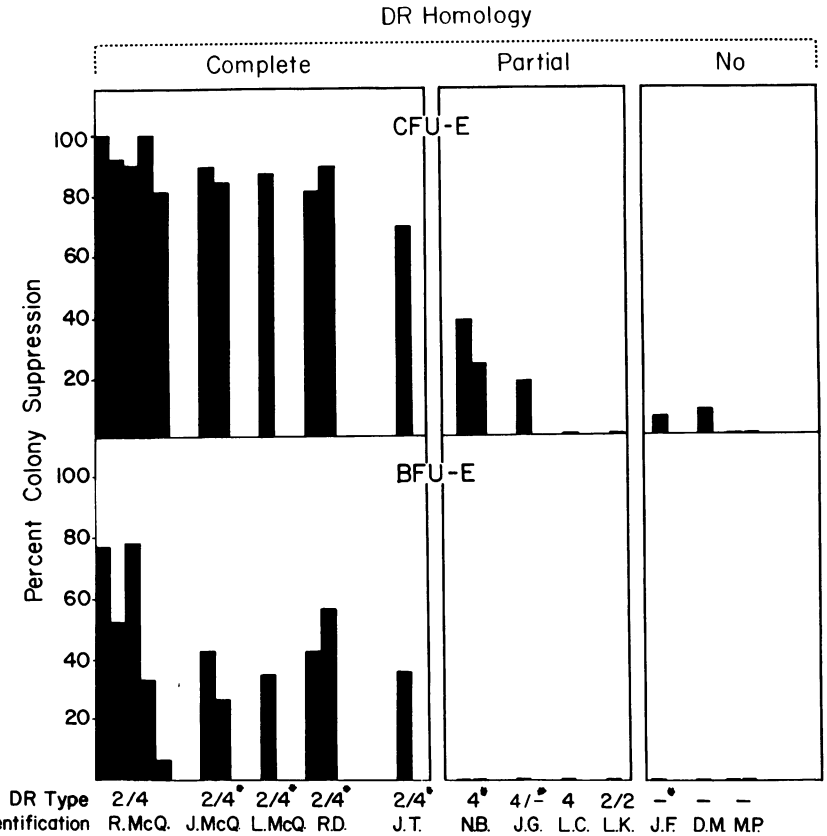

FIGURE 4 Summary of percentage of erythroid colony suppression as a function of shared HLA DR antigens between the T8ES cells from R.McQ. and bone marrow cells in culture at T8ES/BM ratios of 2:1. Donors with complete DR homology are grouped in the box on the left, those with partial DR homology in the middle, and those with no DR homology on the right. CFU-E colony expression (*top* panel) is highly suppressed by T8ES cells from the DR2/DR4 patient only when there is complete DR2/DR4 homology. There is minimal suppression in two of three individual (N.B. and J.G.) with DR4 homology only (DR4/− signifies that only DR4 is identified with current typing sera and no family study was done to identify potential DR4 homozygosity), and no suppression with identity at DR2 only (homozygosity of DR2 was determined by family study), or when no homology exists. BFU-E suppression (*bottom* panel) at T8ES:BM ratios of 2:1 is less pronounced than for CFU-E suppression and is only noted with DR2/DR4 homology. Homology between T8ES and BM at HLA A, either A-2 or cross-reacting A-28, is indicated by an asterisk. Maximal erythroid colony expression for all studies are shown in Table II.

three. The two individuals with CFU-E suppression (N.B. and J.G.) were HLA A-28 and A-2. Thus, these two individuals share either A-2 or an antigen closely related to A-2, A-28, with R.McQ. The differentiation of erythroid progenitor cells of one individual homozygous for DR2 and noncompatible at HLA A was not suppressed, leading to the conclusion that either identity at both DR loci was required for full expression of T8ES function or that HLA A region identity was required. A requirement for full DR homology suggests that DR locus complementation (38) might be necessary for suppression or perhaps that the known polymorphisms of the DR4 locus may be operative. The DR4 locus in the unsuppressed individuals who expressed DR4 may be insufficiently homologous with the DR 4 of T8ES cells to permit suppression (DR4 polymorphism has been detected by Dr. Bernard Carpenter, Brigham and Women's Hospital [unpublished observation]). The nonrelated DR-compatible individuals who were suppressed to an extent equal to that of the patient and his HLA identical siblings were also HLA A-2 identical, and, as stated, the individuals in whom only CFU-E were suppressed (N.B. and J.G.) were positive for A-2 and A-28 (an antigen cross-reactive with A-2 [39]). The lack of suppression in the marrow of one individual with identity at HLA A (J.F.) could be explained by the polymorphism of this very common allele A-2 (39). Therefore, although we sug-

TABLE IV
Assays of NK and CTL Activity of T8ES Cells

Target	Effector	Effector-to-target ratio	Specific release*		
			K562	JY	JY(Con A)‡
				%	
	T8ES	100:1	12	0	17
		25:1	9	2	4
		4:1	3	0	3
	Control	100:1	91	81	72
		25:1	43	67	70
		4:1	49	44	26

* The percentage of specific release = $100 \times [(E - C)/(T - C)]$, in which E is counts per minute released by incubating ^{51}Cr-labeled targets with immune cells (T8ES or control), C is counts per minute released from targets by medium alone, and T is total counts per minute releasable from targets with 100 μl 5% Triton X-100. Assays were performed in triplicate. Percentage of release < 20 is considered negative.
‡ Concanavalin A (Con A) when added activates CTL and removes HLA and antigen specificity. Cells capable of lectin (Con A)-dependent killing are defined as CTL (32).

gest that class II antigen recognition is involved in the mechanism of erythroid suppression by T8ES cells, we cannot rule out class I antigen recognition in this system.

Given the fact that the erythroid suppression induced by T8ES cells demands identity at either the DR or HLA A locus, the mechanism of that suppressor function remains obscure. The lack of CFU-G/M colony suppression, the presence of autologous erythroid colony suppression, and the absence of erythroid or myeloid colony suppression in HLA dissimilar individuals indicates that transfusion sensitization is not a likely mechanism of suppression in this case. The inhibitory effect of T8ES cells was not abrogated by the addition of BPA to the culture, suggesting that suppression was not mediated via an effect of an erythroid inducer cell that produces BPA. The data are compatible with the notion that T8ES cells recognize erythroid progenitors themselves in the context of HLA antigens present on the surface. However, BPA could act by exerting an effect on another bone marrow-derived inducer cell. It is therefore possible that the T8ES cell could inhibit these inducers. In addition, these data are compatible with the hypothesis that T8ES cells act by stimulating a secondary suppressor of erythropoiesis. The recent studies of Robinson et al. (40) demonstrate that Ia antigens are strongly detectable on most BFU-E, and less so on CFU-E.[2] Since T8ES cells regularly suppressed CFU-E expression to a greater extent than that of BFU-E and always failed to suppress CFU G/M differentiation (the majority of the latter being strongly Ia as well as HLA A and B positive [41]), it is clear that if an HLA antigen is recognized on the progenitors, it must be done so in the context of an erythroid antigen. The putative erythroid antigen may increase in availability on the surface of erythroid progenitors as they mature from BFU-E to CFU-E; this could account for the increased sensitivity of CFU-E to the T8ES cells. If suppression is induced by the effect of T8ES cells on a specific bone marrow-derived effector cell, such a putative T8ES-induced suppressor cell must in turn specifically suppress only erythroid cells.

The discovery of a unique surface antigen phenotype of the activated (Ia^+, $T10^+$) T8ES cells ($T1^-$, $T3^+$, $T4^-$, $T6^-$, $T8^+$, $T9^-$, $T11^+$, and $Mo1^+$) is particularly intriguing. The cell may represent either an unregulated proliferation of a clone of naturally occuring erythroid suppressor cells or a neoplastic clonal expansion of such cells. We believe that the former is the more likely case. Both possibilities are significant, however, since the great majority of malignant lymphoid and myeloid cells do have a normal cellular counterpart (43). Cells with similar morphology have

[2] The measurement of expression of Ia antigen on progenitors with monoclonal antibodies represents a technical problem. Winchester et al. (42), who used heterosera, demonstrated the presence of Ia antigen on CFU-E, as well as BFU-E and CFU-G/M. Our recent unpublished experience with a monoclonal antibody confirms the findings of Winchester et al. (42).

been described in patients with T cell lymphocytosis associated with anemia (7, 9, 10), neutropenia (12, 13), anemia with hypogammaglobulinemia (8), thrombocytopenia (44), hypogammaglobulinemia (45), or hypergammaglobulinemia, as well as cases with no obvious functional abnormality (44). In a recent review of 25 cases, the lymphocytes in T lymphocytosis were found most frequently to be $T8^+$, $T3^+$, and $T1^-$ (44). Antibody-dependent cytotoxicity by the T cells from patients with T lymphocytosis and cytopenias has been reported (12, 44). Phenotypically and morphologically similar cells have been demonstrated to have NK as well as antibody-dependent cytotoxicity activity. However, NK activity has not been a feature of the T cells present in T lymphocytosis associated with cytopenias (44). The T8ES cells in this study likewise lack NK activity. In contrast, several cases of E rosette-positive, $T3^-$, $T8^-$ proliferations have been reported with significant NK activity (44). In addition, the lack of cytolytic activity demonstrated by T8ES cells indicates that these cells are neither NK cells nor CTL, but are suppressor cells capable of genetically restricted suppression of erythroid differentiation. Evidence, both morphologic, and phenotypic, is beginning to accumulate to suggest that this hybrid T lymphocyte/myelomonocyte (45) has a normal cellular counterpart and is not the conseqence of aberrant gene expression in a transformed cell. Ferrarini et al. (46) have identified a subset of normal Tγ cells (47) with identical electron and light microscopic and cytochemical features. Lohmeyer et al. (48) and Fast et al. (49) have isolated a subset of normal peripheral blood mononuclear cells that simultaneously express thymic and myelomonocytic markers. Abo et al. (50) have identified an array of cells from peripheral blood that share an NK cell surface marker (HNK-1) (51), Mol, and a variety of T cell antigens, excluding those expressed on immature T cells, T6 and T9. In addition, although Mol has not been found on normal resting T cells, Ortaldo and Timonen (52) have been able to induce T cell antigen expression on morphologically similar cells possessing Mol, using T cell growth factors. Thus, a subset of Tγ and hybrid thymic-myelomocytic cells may be one and the same. The Ia^+ erythroid suppressor T cells demonstrated by Torok-Storb and Hansen (14) may belong to this family.

A complex T cell network has been found to regulate the immune response in man (53) and considerable data have implicated what is believed to be a T lymphocyte as an inducer of erythroid differentiation (5, 6, 29, 54, 55). In addition, normal Tγ cells have been shown to suppress in vitro erythroid (6, 36) and granulocyte (56) colony expression. These observations are strengthened by clinical examples, such as the one presented in this report, where anemia or neutropenia is correlated with in vitro inhibition of erythroid (7–10) or myeloid (11, 13) differentiation by such suppressor cells. These in vitro studies and clinical examples support the growing notion that a complex, genetically regulated network of T cells, some with a hybrid phenotype are involved in the regulation of hematopoietic differentiation.

ACKNOWLEDGMENTS

We wish to thank Dr. Stuart F. Schlossman and Dr. Steven J. Burakoff for their support of this study and Dr. Norman Letvin for his excellent care of this patient. Drs. Fred S. Rosen, Robertson Parkman, and David Linch provided stimulating discussion and critical review of the manuscript. We also thank the nurses and facilitators in the Dana-Farber Cancer Institute pediatric clinic for their assistance in obtaining marrow samples. We owe a particular debt of gratitude to Karen Shanley and her staff in the HLA laboratory at the Dana-Farber Cancer Institute, without whose assistance this study would have been impossible.

This work was supported by U. S. Public Health Service grants HL 27375-01, CA18662, HL 19646, AI 12069, CA 19589, CA 25369, and CA 028740 and a grant from the Dyson Foundation.

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