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Immunology

Leukemia cell lines can replace monocytes for mitogen-induced T-lymphocyte responses: This accessory function is dependent upon their differentiation stage

(T-cell proliferation/accessory cells/tumor cell lines/interleukin)

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ABSTRACT **Highly purified peripheral T lymphocytes do not proliferate in response to phytohemagglutinin A or concanavalin A, unless adherent HLA-DR$^+$ monocytes are added as accessory cells. The accessory function (AF) of monocytes is mediated through the release of interleukin-1 (IL-1). We here report that cells from three human leukemic lines—K562, HL-60, and U-937—could exert AF and efficiently replace monocytes in a 72-hr mitogen-stimulated proliferation assay. This AF was clearly related to precise maturational stages of these cells, since the hematopoietic precursor K562 cells spontaneously exerted high AF but lost this property when treated with differentiation inducers such as sodium butyrate or phorbol 12-myristate 13-acetate (PMA). On the other hand, untreated HL-60 and U-937 cells exhibited no spontaneous AF, but they acquired this function when induced to differentiate either along the granulocytic pathway (dimethyl sulfoxide-treated HL-60 cells) or along the monocytic pathway (PMA-treated HL-60 and U-937 cells). Supernatants from PMA-triggered K562 or HL-60 cells allowed the proliferative response of murine thymocytes to phytohemagglutinin A and were therefore shown to contain IL-1. Analysis of phenotypical markers showed that AF and IL-1 production were not restricted to cells of the monocytic lineage. No HLA-DR antigen could be detected on K562 and HL-60 cells. Thus, the expression of HLA-DR antigens is not required for AF and IL-1 production in response to mitogens. Human leukemia cell lines could provide useful sources of human IL-1.**

T-cell activation by antigen or mitogen is dependent upon the development of cellular cooperations and the production of regulatory molecules such as interleukins. The T-cell activation pathway, in mice and men, involves a complex cascade of cellular events; thus highly purified T lymphocytes do not exhibit any proliferative response to mitogens such as phytohemagglutinin A (PHA) or concanavalin A (Con A) in the absence of other cells exerting the so-called "accessory activity." Within purified blood mononuclear cells, monocytes are considered to play the major role in this accessory function (AF) (1). Several authors have demonstrated that this function was mediated through the release of interleukin 1 (IL-1), a factor acting as a second signal with antigens or mitogens on T cells (2, 3). Accessory cells (AC) were shown to express Ia antigens (4) in mice and HLA-DR antigens in humans (5). The actual role of these antigens in AF remains controversial (6, 7).

The murine monocytic leukemia cell line P388D1 was shown to exert AF (8) and to produce high amounts of IL-1, allowing its biochemical characterization (9). In humans, IL-1 production by some monocytic leukemia cells has also been reported (10). The study of cell lines capable of exerting AF or producing IL-1 provides a better understanding of the cellular requirements for these functions. Several human leukemic cell lines that express a variety of lineage-specific markers in the presence of differentiation inducers represent valuable models of human hematopoietic differentiation. K562 cells (11) are pluripotent hematopoietic precursors (12), able to enter various differentiation pathways in the presence of hemin (13), sodium butyrate (14) or phorbol 12-myristate 13-acetate (PMA) (15). HL-60 cells (16) are derived from an acute promyelocytic leukemia and can achieve nearly complete differentiation either along the granulocytic pathway in the presence of dimethyl sulfoxide (Me_2SO) (17) or along the macrophage–monocyte pathway in the presence of PMA (18). U-937 (19) cells are derived from a "histiocytic" lymphoma, exhibit macrophagic features, and can differentiate into functional monocytes in the presence of PMA (20). We analyzed these three cell lines for AF and IL-1 production, before and after differentiation induction. The data reported here demonstrate that AF and IL-1 production occur in different cell types and therefore are not restricted to the monocytic lineage. The accessory activities were clearly related to precise maturational stages and could occasionally be exerted by HLA-DR-negative cells.

MATERIALS AND METHODS

Cell Lines. K562 is a human leukemic cell line derived from the pleural effusion of a patient with chronic myelogenous leukemia in terminal blast crisis (11). HL-60 is a human promyelocytic leukemia cell line (16) and U-937 is a monocytic cell line derived from a patient with histiocytic lymphoma (19). The three lines were kindly given to us by W. Vainchenker (Hôpital Henri-Mondor, Créteil, France).

Preparation of Purified Human Peripheral Blood T Cells: Monocyte Depletion. T cells were purified by a four-step method. Peripheral blood lymphocytes were obtained by Ficoll/Hypaque centrifugation of blood from healthy adult volunteers. The lymphocytes were then depleted of adherent cells by 1-hr incubation on plastic culture flasks followed by elimination of phagocytic cells by carbonyl iron ingestion at 37°C for 1 hr and passage over a magnet. Erythrocyte-rosetting lymphocytes were then obtained by using described methods (20). Finally, residual HLA-DR-positive cells were eliminated by treatment with an anti-HLA-DR monoclonal antibody plus

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Abbreviations: AC, accessory cells; AF, accessory function; Con A, concanavalin A; IL, interleukin; Me_2SO, dimethyl sulfoxide; PHA, phytohemagglutinin A; PMA, phorbol 12-myristate 13-acetate.

complement, using a two-step cytotoxicity assay as described (7). All four steps of purification were necessary for complete elimination of contaminating monocytes as evaluated by the absence of proliferative response in the presence of mitogens.

Monocyte Preparation. Monocytes were prepared by using their adherence property on plastic culture flasks as described by others (21). Erythrocyte-rosetting cells were eliminated. Cell populations obtained usually included more than 90% peroxidase-positive monocytes and were unable to proliferate when cultivated with mitogens as determined by [^{3}H]thymidine incorporation.

Induction of Cell Differentiation. K562 cells were induced to undergo differentiation with sodium butyrate (Sigma, 1 mM for 5 days), with PMA (Sigma, 160 nM for 2 days), or with hemin (Sigma, 0.1 mM for 5 days). HL-60 cells were induced to differentiate either into monocyte-like cells by PMA (160 nM for 2 days) (18) or along the myeloid lineage with Me_2SO [Prolabo (Paris), 1.12% for 5 days] (17). U-937 cells were induced to differentiate along the monocytic lineage with PMA (22), using the same optimal conditions as for HL-60 cells.

Study of Differentiation Markers and HLA-DR Expression. A panel of lineage-specific mouse monoclonal antibodies was used. Briefly, the monoclonal antibody OKM-1 (23) reacts with granulocytes and monocytes, Mo2 (24) reacts with monocytes, and anti-My-1 (25) reacts with granulocytes. J2 (26) recognizes only platelets and activated T lymphocytes. The monoclonal antibody L1CR/LON/R10 (27) is an anti-glycophorin A with specificity for erythroid cells. In addition, three monoclonal antibodies directed against polymorphic determinants of HLA-DR were employed: L 1.1.6 (28) (a gift from J. Kalil, Hôpital Saint-Louis, Paris) and BA206 and BM50 (gifts from D. Charron, Hôpital de la Pitié, Paris). Immunofluorescence assays were performed by both visual examination and cytofluorography using a FACS-IV (Beckton Dickinson) analyzer. Methods for hemoglobin analysis have been described (12).

Ability of Cell Lines to Replace Monocytes in T-Cell Proliferation. Before and after differentiation induction, K562, HL-60, and U-937 were tested for their ability to replace monocytes in mitogen-stimulated T-cell proliferation. Leukemic cells were first treated with mitomycin C (Choay Laboratories, Paris), using 200 μg/ml for 10^7 tumor cells at 37°C for 1 hr. They were then washed and adjusted to 10^6 cells per ml before addition to purified T cells. T-cell proliferation in the presence of PHA (Difco, 10 μg/ml) or Con A (Sigma, 5 μg/ml) was obtained by mixing 100 μl of the highly purified T cell suspension (10^6 cells/ml) with 100 μl of mitomycin-C-treated cell line suspension (10^6 cells per ml) in flat-bottomed 96-well microculture plates. Plates were then incubated at 37°C for either 48 or 72 hr and proliferation was measured by [^{3}H]thymidine uptake during the last 4 hr of the culture.

IL-1 Production by Cell Lines. HL-60, K562, or U-937 cells (3×10^6 cells per ml) were triggered with PMA at 1 μg/ml for various periods, and IL-1 production was tested in the cofactor assay on thymocytes from C3H/OL mice according to published methods (29).

RESULTS

Differentiation Markers and HLA-DR Expression on Leukemic Cells. The expression of various markers in cells cultured in the presence of differentiation inducers was studied and the previously published data (11–19) were confirmed. After PMA induction, U-937 acquired mature monocyte phenotype (plastic adherence, reactivity with OKM1 and Mo2 monoclonal antibodies). HL-60 cells achieved granulocytic differentiation after Me_2SO treatment. On the other hand, culture of HL-60 cells in the presence of PMA resulted in the appearance of specific monocytic features (data not shown). Sodium butyrate and PMA acted on K562 cells by reducing the expression of glycophorin A and the myeloid antigen My-1, but they increased the reactivity of K562 cells with the antiplatelet monoclonal antibody J2. These inducers thus appear to commit K562 cells along the megakaryocytic lineage. Hemin had a different effect; it enhanced hemoglobin accumulation without a significant modification of the expression of other erythroid markers and slightly increased myeloid markers without modification of megakaryocytic ones. Thus, hemin appeared to cause K562 cells to commit to hemoglobin synthesis rather than to exert an actual differentiating role. Neither before nor after treatment with any differentiation inducer did K562 exhibit monocytic markers such as reactivity with OKM1 or Mo2 monoclonal antibody.

K562 cells did not react with any of the anti-HLA-DR monoclonal antibody tested, either before or after treatment with differentiation inducers. Similarly, no HLA-DR could be found on HL-60 cells. These results confirmed previously reported data (22). HLA-DR antigens were expressed on about 10% of untreated and on about 50% of PMA-treated U-937 cells.

Accessory Activity of U-937 Cells. The capacity of the histiocytic U-937 cells to replace monocytes for mitogen-induced T-cell proliferation was first investigated. It can be seen in Table 1 that T cells purified by the four-step protocol described above were nearly unresponsive to both PHA and Con A, whereas monocyte addition elicited a marked T cell proliferative response in the presence of these lectins. U-937 cells appeared to be practically devoid of monocyte-replacing properties in this assay (Table 1). However, PMA treatment induced the appearance of clear accessory properties in U-937 cells, which became as efficient as monocytes in our proliferative assay. These data show that the AF of U-937 is strongly increased by cell differentiation along the monocytic lineage.

Accessory Activity of HL-60 Cells. It can be seen in Table 2 that noninduced promyelocytic HL-60 cells were devoid of any monocyte-replacing activity in the T-cell proliferative assay. Strikingly, both Me_2SO and PMA treatments, though committing HL-60 in distinct pathways, elicited the appearance of a strong accessory activity, because Me_2SO-treated as well as PMA-treated HL-60 cells induced strong T-cell proliferative responses after mitogen stimulations (Table 2). Thus whereas the AF of HL-60 cells was clearly dependent upon their differentiation stage, their cellular commitment to either the granulocytic or the monocytic lineage did not play any apparent role in this function.

Accessory Activity of K562 Cells. In contrast with U-937 and HL-60 cells, which did not have AF before differentiation in-

Table 1. Accessory activity of U-937 cells before and after differentiation induction

Accessory cells	Mitogen	[^{3}H]Thymidine uptake, cpm		
		Exp. 1	Exp. 2	Exp. 3
—	—	290	280	640
—	PHA	670	400	860
—	Con A	480	370	700
Monocytes	—	360	280	610
Monocytes	PHA	88,770	211,130	99,640
Monocytes	Con A	50,980	98,640	62,430
U-937	—	370	470	290
U-937	PHA	5,450	2,230	1,920
U-937	Con A	6,300	3,350	710
PMA-U-937	—	4,670	2,290	1,050
PMA-U-937	PHA	60,050	269,670	86,580
PMA-U-937	Con A	42,670	192,770	58,690

Table 2. Accessory activity of HL-60 cells before and after differentiation induction

Accessory cells	Mitogen	[^{3}H]Thymidine uptake, cpm		
		Exp. 1	Exp. 2	Exp. 3
—	—	360	460	510
—	PHA	680	750	850
—	Con A	650	650	760
Monocytes	—	470	470	480
Monocytes	PHA	161,120	116,040	123,460
Monocytes	Con A	115,630	83,150	89,630
HL-60	—	350	270	400
HL-60	PHA	5,330	860	1,380
HL-60	Con A	2,110	820	2,300
PMA-HL-60	—	940	1,430	400
PMA-HL-60	PHA	128,330	113,460	111,080
PMA-HL-60	Con A	85,440	66,180	81,180
Me_2SO-HL-60	—	360	380	290
Me_2SO-HL-60	PHA	119,180	108,000	81,720
Me_2SO-HL-60	Con A	72,650	67,420	79,790

duction, K562 cells exhibited strikingly high accessory activity before any treatment (Table 3). Untreated K562 cells were as efficient as peripheral blood monocytes in eliciting purified T-cell response to mitogens. This capacity was not modified by hemin induction. By contrast, both sodium butyrate and PMA, which exerted clear differentiating action on K562 cells, drastically reduced their accessory activity.

IL-1 Production by Cell Lines. It can be seen in Fig. 1 that after triggering of the cells with PMA, HL-60 and K562 cells could both produce IL-1, whereas no significant amount of the interleukin could be detected in U-937 cell supernatants.

DISCUSSION

Understanding of the mechanisms of T-cell activation by antigen or mitogen has made considerable progress in the past few years. T-cell proliferation is dependent upon the presence of interleukin 2 (IL-2) (30) produced by some T-cell subset (31, 32). Two distinct signals are required for IL-2 production: (*i*) mitogen or antigen processed by some antigen-presenting cells and (*ii*) IL-1. The essential role of macrophages in both the antigen-processing event and IL-1 production has been emphasized (33).

Table 3. Accessory activity of K562 cells before and after differentiation induction

Accessory cells	Mitogen	[^{3}H]Thymidine uptake, cpm		
		Exp. 1	Exp. 2	Exp. 3
—	—	220	230	370
—	PHA	230	470	390
—	Con A	370	420	410
Monocytes	—	210	350	410
Monocytes	PHA	88,190	154,390	105,460
Monocytes	Con A	64,580	110,100	92,450
K562	—	250	410	220
K562	PHA	128,550	161,930	139,770
K562	Con A	58,150	86,450	101,430
PMA-K562	—	1,240	1,610	1,070
PMA-K562	PHA	14,160	55,840	53,020
PMA-K562	Con A	8,340	13,220	9,100
Butyrate-K562	—	290	460	320
Butyrate-K562	PHA	48,370	27,740	41,110
Butyrate-K562	Con A	9,010	8,610	12,000
Hemin-K562	—	390	260	250
Hemin-K562	PHA	135,980	184,960	165,970
Hemin-K562	Con A	71,820	92,090	112,650

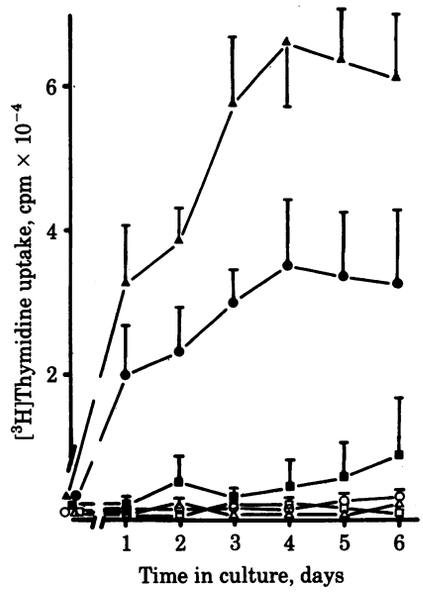

FIG. 1. Presence of IL-1 in various cell line supernatants originating from U-937 (□), U-937 triggered with PMA (■), HL-60 (○), HL-60 triggered with PMA (●), K562 (△), and K562 triggered with PMA (▲). When cells have been triggered with PMA, the background due to PMA alone has been subtracted from cpm values. Time in culture indicates the day of supernatant collection after addition of PMA.

Our experiments indicate that several human leukemia cell lines can replace macrophages in a 72-hr mitogen-induced proliferation assay. AF for T-cell proliferation was clearly not the exclusive property of cells from the monocytic series. Indeed, the undifferentiated hematopoietic precursor K562 cells and the myeloid HL-60 cells driven along granulocytic lineage by Me_2SO were excellent AC in this assay, being as efficient as either normal monocytes from peripheral blood or PMA-treated HL-60 and U-937 cells that exhibited monocytic markers. Furthermore, our data showed that in various hematopoietic cells, AF is restricted to a precise differentiation stage: U-937 and HL-60 cells acquired their AF after treatment with differentiation inducers. On the other hand, K562 cells, which were highly efficient as AC, lost this property when treated with either sodium butyrate or PMA, but not with hemin.

The mechanism of the AF exerted by these human leukemia cell lines was probably related to their ability to produce IL-1. The supernatants of PMA-triggered K562 and HL-60 cells demonstrated co-stimulating activity with PHA towards murine thymocytes. This assay is commonly considered as specific for IL-1 (9). No IL-2 could be found in the same supernatants, added to cultures of the IL-2-dependent murine CTLL-2 cells (34) (data not shown). Furthermore, these supernatants were submitted to filtration through Ultrogel AcA 54 (LKB). The fraction containing the thymocyte-stimulating activity was identical to that obtained with supernatants of muramyl-dipeptide-triggered monocytes (35). The corresponding molecular weight was around 20,000, as reported for IL-1 (36) (data not shown, to be reported elsewhere). In mice, the production of IL-1 by cultured epidermal cells (37) and keratinocyte cell lines (38) has been reported. It therefore appears that a variety of cells other than macrophages can actually produce IL-1.

The murine Ia and human HLA-DR molecules are known to be involved in cell–cell cooperation. Both antigen- and mitogen-induced activation of T cells has been shown to require the presence of HLA-DR$^+$ (Ia$^+$) AC in human and mouse systems (39, 40). It was reported that IL-2 production by purified T cells could be achieved only when HLA-DR$^+$ cells, such as mono-

cytes (41, 42) or B-lymphoma Daudi cells (43), were added to the culture. However, we here present the evidence that some HLA-DR$^-$ cells, such as K562 and HL-60, can exert AF and produce IL-1. These results suggest that, if HLA-DR (Ia) molecules are actually required for antigen presentation to responding T cells by AC, they are not directly concerned with the induction of IL-1 production by these cells. It was previously reported that anti-HLA-DR antibodies inhibited antigen-induced T-cell responses (44) but did not affect T-cell proliferation induced by Con A (45). Our results thus confirmed that HLA-DR antigens on AC are not directly involved in the process of T-cell activation by mitogens.

The finding that human leukemia cell lines can exert AF and produce IL-1 at some stage of their differentiation can lead to new information in the understanding of these functions. Such leukemic lines could be useful sources of human IL-1 for biochemical characterization and gene cloning.

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