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Aberrant Expression of T Cell and B Cell Markers in Myelocyte/Monocyte/Histiocyte-Derived Lymphoma and Leukemia Cells

Is the Infrequent Expression of T/B Cell Markers Sufficient to Establish a Lymphoid Origin for Hodgkin's Reed–Sternberg Cells?

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Most Hodgkin's mononuclear cells and Reed–Sternberg (H-RS) cells are characterized by the expression of the antigen CD30, but not of T or B cell markers. A few H-RS cells, however, may express a limited number of T or B cell markers. Whether this expression is sufficient to allow the conclusion that H-RS cells are derived from T and/or B cells has been debated vigorously. The present study examined whether CD30 and aberrant T and B cell markers are expressed in cell lines that are well documented as being derived from the granulocyte/monocyte/histiocyte lineage. These cells included HL-60, KG-1, ML-1, THP-1, and U-937. Four other cell lines derived from patients with leukemias/lymphomas of monocytic or granulocytic origins also were studied. These cells included BV173, CML-Brown, CTV-2, and SU-DHL-1. If aberrant expression is detected, by analogy one may expect that rare T or B cell marker expression may occur in H-RS cells, because abundant evidence has indicated that H-RS cells may be related to cells in histiocyte lineage. In all nine of the cell lines studied, it was confirmed that numerous monocyte/granulocyte markers were expressed. The marker expression was enhanced after cells were induced to differentiate with phorbol ester (TPA) and tumor necrosis factor (TNF). It was noted that several T and B cell markers also were expressed by these cells. Unlike the expression of monocyte/granulocyte markers, the expression of T or B cell markers was not affected, or only minimally affected, by treatment of the cells with TPA or TNF. Five of the cell lines (BV173, CML-Brown, CTV-2, SU-DHL-1, and THP-1) were shown to be CD30-positive. In CTV-2 and BV173, the expression of CD30 was greatly increased after induction with phorbol ester or TNF. Based on these studies, the following conclusions were reached: 1) The expression of aberrant B or T cell markers is not an uncommon finding in granulocyte/monocyte/histiocyte-related neoplastic cells. 2) The expression of granulocyte/monocyte markers in these cells is related to the state of cell differentiation, whereas the expression of T or B cell markers is not. 3) CD30 is not necessarily a proliferation-related antigen, and its expression is not a sole property of T or B cells, but can be present in granulocyte/monocyte/histiocyte-related cells. Therefore, the expression of a few T or B cell markers in rare Hodgkin's Reed–Sternberg cells, and of CD30 in a few T or B lymphoma cells, cannot be used as the sole basis for a conclusion that Reed–Sternberg cells are derived from T or B cells. (Am J Pathol 1989, 134:203–212)

The use of monoclonal antibodies (MAbs) has facilitated the diagnosis of lymphomas and leukemias, however, the specificity of MAbs in identifying the lineage of neoplastic cells is not exclusive.[1] Examples of broad reactivity include that of CD10 (CALLA) in B cells, T cells, and granulocytes; Leu M3 (CD14) in T cells and monocytes; CD5 (Leu 1) in T and B cells; CD4 (Leu 3a) in T cells and histiocytes; and many others.[1–5] In Hodgkin's disease (HD), the antigen CD30, which is recognized by MAbs Ki-1/HeFi-1, was initially thought to be specific for Reed–Sternberg (H-

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RS) cells.[6,7] Later, CD30 was detected in some mitogen-activated normal T and B cells, in cytokine-activated macrophages/monocytes, and in rare T and B cell lymphomas and anaplastic large cell lymphoma.[8–12] These findings have added confusion to the already complicated subject of the lineage of H-RS cells. In particular, in rare forms of Hodgkin's Disease (HD), H-RS cells were also shown to express T or B cell markers.[13–15] Some researchers have used this information to conclude that H-RS cells are derived from either T or B cells.

It is inappropriate, however, to use the expression of one or a few B or T cell markers in a few H-RS cells as the sole basis for identifying the cellular lineage of H-RS cells. Most H-RS cells do not express T or B cell markers; instead, they frequently exhibit markers commonly associated with interdigitating reticulum (IR) cells or histiocytes.[16–19] This latter finding has prompted us to consider the possibility of a relationship between H-RS cells and IR cells; such a hypothesis is based not only on phenotypic similarity, but also on information obtained in extensive biochemical studies (involving cytokines and arachidonic-acid metabolism), functional studies, and studies on the induction of cell differentiation.[16–27] In the present report, to show the lack of significance of the occasional expression of T or B cell markers in H-RS cells, we provide evidence that aberrant expression of T and B cell markers is not an uncommon finding in myelocyte/monocyte/histiocyte (macrophage) (MMM)-related neoplastic cells.

Materials and Methods

Cell Lines and Cultures

Cells of cell lines including BV173, CML-Brown, CTV-2, HL-60, KG-1, ML-1, U-937, SU-DHL-1, and THP-1 were cultured in RPMI 1640 medium as described previously.[19–21] The nature and phenotypes of these cells, except for CML-Brown, have been reported on previously.[28–38] Previously, five of the cell lines (HL-60, KG-1, ML-1, THP-1, and U-937) have been used extensively as models for the study of monocytes/granulocytes. CML-Brown was established from a patient with chronic myelogenic leukemia; the cells had been cultured continuously for more than 18 months. CML-Brown is characterized by the presence of bands or segmented nuclei in cultured cells. The CML-Brown cells appear morphologically to be more highly differentiated than are those of KG-1 and HL-60.

HL-60, KG-1, U-937, and THP-1 were obtained from the American Type Culture Collection (ATCC, Rockville, MD). BV173, CTV-2, ML-1, and SU-DHL-1 were provided by Drs. H. P. Koeffler, P. M. Chen, M. S. Lok, and A. L. Epstein, respectively.

Marker Determination

We used the avidin-biotin-peroxidase method and an indirect immunofluorescence method to determine the phenotypes of the above cells.[39–40] The following MAbs were employed: T11 (CD2), T3 (CD3), anti-Leu 1 (CD5), anti-Leu 9 (CD7), T8 (CD8), T4 (CD4), anti-Leu 14 (CD22), B2 (CD21), BA-1 (CD24), anti-Leu M1 (CD15), anti-Leu M5 (CD11c), Mo1 (CD11b), Mo2 (CD14w), MY4 (CD14w), MY7 (CD13), MY9 (CD33), 1E9, 2H9, Ki-1/HeFi-1 (CD30), βF1, and δTCS1. These MAbs were obtained from Becton-Dickinson (Sunnyvale, CA), Coulter Immunology (Hialeah, FL), Dako (Santa Barbara, CA), and T Cell Sciences, Inc. (Cambridge, MA). MAbs 1E9 and 2H9 were prepared in our laboratory.[19] MAbs βF1 and δTCS1 are specific for the β chain of the $\alpha\beta$ T cell antigen receptor (TcR) and the δ chain of the $\gamma\delta$ TcR, respectively.[41,42] The reactivities of these MAbs in normal lymphoid tissues have been reported previously.[2–4,41,43] In a previous experiment, MAb δTCS1 reacted with approximately 5% of T cells randomly scattered in normal lymphoid tissues (unpublished data).

Induction of Differentiation

We examined the relationship between marker expression and cell proliferation or differentiation. The cells used were induced to differentiate by administration of TPA, tumor necrosis factor (TNF), interferon-γ (IFN-γ), or TNF +IFN-γ for 1–3 days. The marker expression on induced cells was compared with that on control cells. Recombinant TNF and IFN-γ were obtained from Amgen (Thousand Oaks, CA) and were used at 100 U/ml. The induction protocol was similar to that reported previously.[30–32] Furthermore, we examined the DNA cycles of control and induced cells to determine the effect of TPA or cytokines on cell proliferation.[22,44]

Results

Granulocyte/Monocyte and T/B Cell Marker Expression

The marker expression of the cells studied is summarized in Tables 1 and 2. The results for granulocyte/monocyte (GM) marker expression in these cells were similar to those reported by others.[33] Leu M5 and Mo1 were the most common markers and were found to be associated with all nine cell lines tested; they were followed by 1E9 and MY4, each of which reacted in seven cell lines, and by 2H9 in six. Numerous T and B cell markers also were detected in virtually all of the MMM cells tested. Among them, CD3 and CD7 were the two most common markers found. The staining of T or B cell markers in these cells

Table 1. *Properties and Phenotypes of MMM Cells*

Cell lines	Origin/Lineage	Gene rearrangements*	Markers on control cells†	Changes after induction†	Inducer
BV173	CML-blast	TcR-β and IgH	Leu M5 (30%), MY4 (50%), 2H9 (5%)	Increase in MY4 (70%), 2H9 (30%)-positive cells, and become 1E9-positve	TPA
CML-Brown	CML	unknown	Leu M5, Mo1, MY4, 1E9, 2H9	Slight increase in staining intensity	TPA
CTV-2	AMol	TcR-β	Leu M1 (30%), Leu M5 (5%), Mo1 (5%), 2H9 (30%)	Slight increase in staining intensity and no. of cells stained	TPA
HL-60	Promyelocytic leukemia	germ line	Mo1 (5%), MY4 (5%), Leu M5 (30%, weak), 1E9	Increase in no. of Leu M5-, Mo1-, and MY4-positive cells (>90%)	TPA, TNF
KG-1	CML	germ line	Leu M5 (30%), Mo1 (10%), MY4 (70%), MY9 (40%), 1E9 (5%), 2H9	Increase in no. of the Leu M5 (50%) and Mo1 (20%)-positive cells	TPA
ML-1	Monomyelocytic leukemia	IgH	Leu M5 (15%), Mo1 (25%), MY4 (5%), 1E9	Increase in no. of Leu M5-, Mo1-, and MY4-positive cells (Fig. 1)	TPA, TNF
SU-DHL-1	THL	TcR-β	Leu M1, Leu M5 (25%), Mo1 (5%), 1E9, 2H9	Slightly increase in no. of cell stained	TPA
THP-1	AMoL	germ line	Leu M1, Leu M5 (35%), Mo1 (5%), Mo2 (5%), MY4 (10%), MY7, MY9, 1E9, 2H9	Increase in no. of MY4-, Leu M5-, and Mo1-positive cells (Fig. 1), decrease in no. of MY9-positive cells	TPA, TNF
U-937	MH	germ line	Leu M1, Leu M5 (25%), Mo1 (20%), Mo2, MY7, MY9 (5%), 1E9	Decrease in MY7 staining intensity, increase in Leu M5-, Mo1-, MY4, and 2H9-positive cells (Fig. 1)	TPA, TNF + IFN-γ

CML, chronic myelogenic leukemia; AMoL, acute monoblastic leukemia; THL, true histiocytic lymphoma; MH, malignant histiocytosis.
* TcR-β and IgH genes were examined.
† The numbers in parentheses indicate the percentages of cells stained. If not specified, most (>90%) cells were stained. GM markers not mentioned in the table were absent.

generally was noted in the periphery, which might indicate a reaction on the cell surface or within the cytoplasm. These markers, when detected, were usually observed in most of the cells (Figure 1). A few, such as CD2 in ML-1 and U-937, were present only in subpopulations of cells.

TcRδ was absent in all cell lines. TcRβ, however, was detected in CTV-2, THP-1, U-937, and KG-1 cells (Figure 2). In CTV-2 and THP-1 cells, the TcRβ protein was observed in the cytoplasm and on the cell membrane; in other two, it was localized in the cytoplasm.

Table 2. *Expression of CD30 and of T or B Cell Markers in MMM Cell Lines*

Cell lines	CD30	CD2	CD3	CD4	CD5	CD7	CD8	βF1 (TcR-β)	B cell markers	Marker changes after induction
BV173	++ (5%)*	−	+++	−	++	++	weak +	−	B2, Leu 14	Increase in no. of CD30-positive cells (30%)
CML-Brown	weak +	weak +	++	weak +	−	+	weak +	−	B2, Leu 14	No significant changes
CTV-2	+ (10%)	−	+	−	−	++	−	−	−	Increase in no. of CD30-positive cells (70%) Slightly increase in CD3 staining intensity
HL-60†	−	−	−	+	−	−	−	ND	−	No significant changes
KG-1	−	−	+ (c)	+ (5%)	−	−	−	+ (weak, c)	BA1 (5%)	No significant changes
ML-1	−	+ (20%)	+	−	++	+	weak +	−	−	Slightly increase in CD7 staining intensity
SU-DHL-1	+++	−	−	−	+++	+	−	−	BA1‡	unchanged
THP-1	±	−	++	+ (80%)	++	++	+	+	B2 (70%)	Slightly increased staining intensity in CD3, decrease in CD7 and CD4
U937§	−	+ (<2%)	++	+	−	+ (50%)	+ (20%)	+ (c)	−	No significant changes

* The staining intensity is classified as strongly positive (+++) to positive (+). The numbers in parentheses indicate the percentages of cells stained. If not specified, most (>90%) cells were stained. Abbreviations: c, cytoplasmic staining; ND, not done.
† The phenotype of HL60 cells is determined with the immunofluorescence method. The CD4 molecules on HL60 cells have been reported to serve as receptors for human immunodeficiency virus.
‡ BA-1 was reported by Winter et al to be positive, however, the SU-DHL-1 cells cultured in our laboratory are BA-1-negative.
§ U937 cells were reported by Winter et al to express CD1 and CD4.

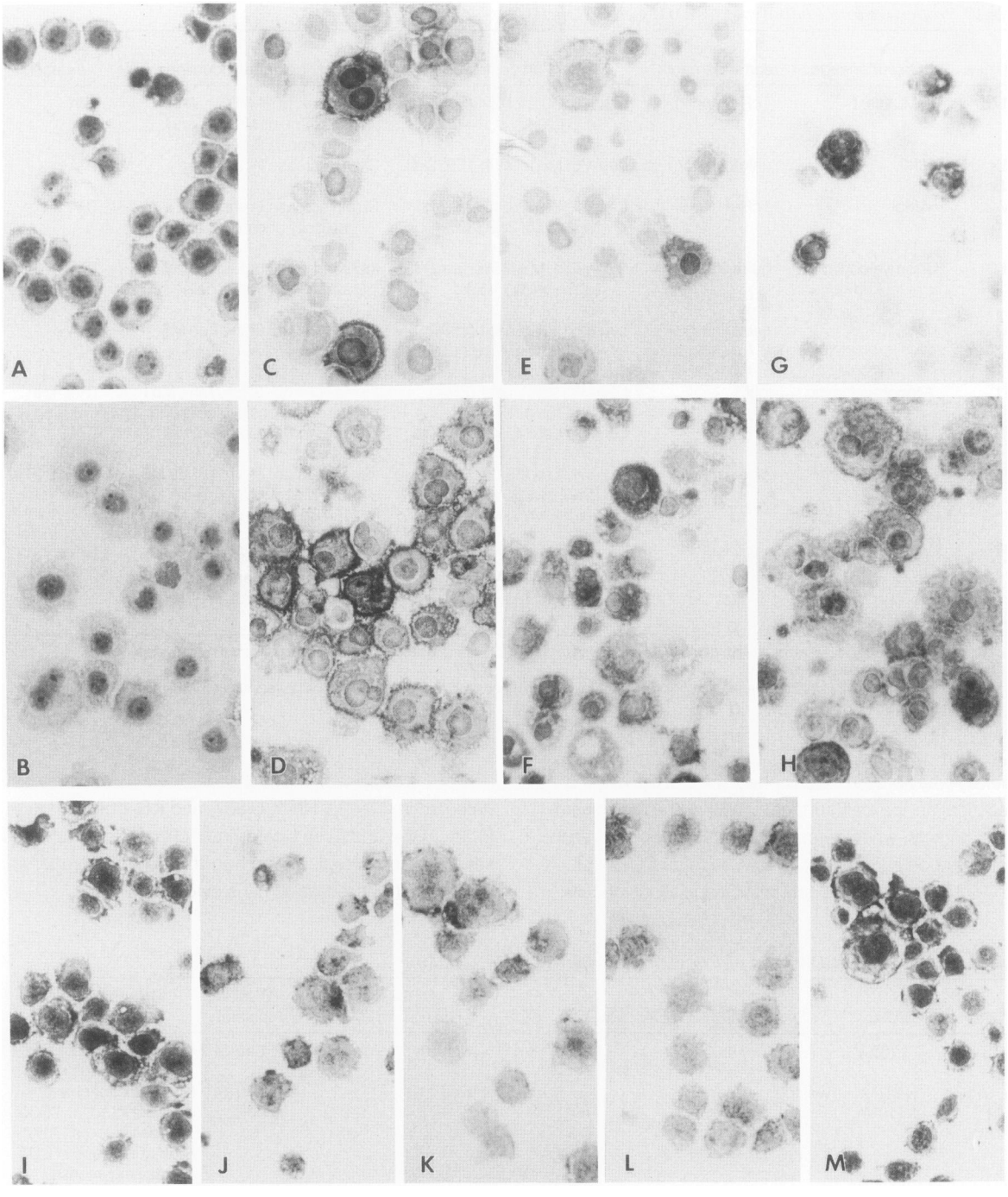

Figure 1. *Staining patterns of THP-1 cells with granulocyte/monocyte and T cell MAbs.* **A:** *THP-1 (control), stained with Dif-Quick.* **B:** *THP-1 cells after TNF treatment. Note the decreased nuclear/cytoplasmic ratio and the morphologic differentiation toward histiocytoid cells. Control (***C, E,** *and* **G***) and TNF-treated (***D, F,** *and* **H***) cells were stained with anti-Leu M5 (C and D), My4 (E and F), and Mo1 (G and H). There was an increase in staining intensity and in the numbers of cells stained after TNF treatment. The THP-1 cells were also positively stained by anti-CD3 (I), CD4 (J), CD5 (K), CD7 (L), and CD8 (M).*

Effect of TPA, TNF, and IFN-γ on Marker Expression and Cell Differentiation

TNF was effective in inducing differentiation in seven of the nine cell lines tested; the exceptions were U-937 and CML-Brown, however, the degree of change varied greatly from one cell line to another, with TNF strongly effective in ML-1 and THP-1 (Figure 3), modestly effective in HL-60, BV173, and SU-DHL-1, and only minimally effective in CTV-2 and KG-1 (Table 1). The degree of cellular differentiation was evaluated based on a decreased nuclear/cytoplasmic ratio, phenotypic changes, or a de-

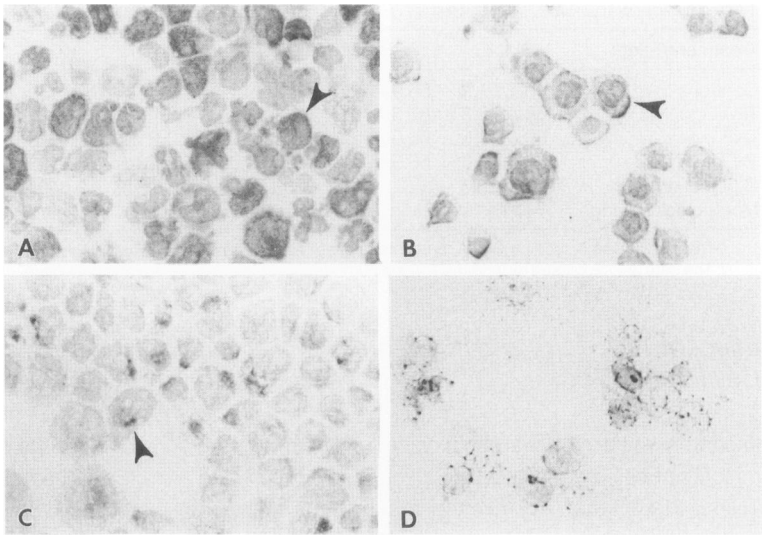

Figure 2. *Expression of TcRβ (βF1) in CTV-2 (A), THP-1 (B), KG-1 (C), and U-937 (D) cells. The protein appears to be localized in the cytoplasm, except in CTV-2 and THP-1 which also exhibit membrane staining. The result indicates that TcRβ or TcRβ-like substances are expressed in these cells. It has been reported that unrearranged TcRγ or Igμ can be expressed in transformed myeloid, pre-B, and pre-T cells*[50]

crease in numbers of cells in S and G_2/M phase (see below). The changes in these three parameters were not necessarily completely in parallel; for example, in SU-DHL-1 and BV173 cells, TNF induced a G_0/G_1 block that resulted in a decreased numbers of cells in S and G_2/M phase, but there were no significant phenotypic changes in these cells.

IFN-γ was effective in inducing the differentiation of only THP-1 and U-937 cells. A synergic effect of both IFN-γ and TNF was observed clearly in U-937 cells, but was not noticeable in the others. TPA was effective in the majority of cell lines, but not in SU-DHL-1, CTV-2, or CML-Brown, in all three of which the changes were minimal. When TNF, IFN-γ, or TPA did induce phenotypic changes (ie, in HL-60, ML-1, THP-1, and U-937 cells), almost without exception the number of cells stained positively by GM markers was greatly enhanced (Table 1).

With all types of induction, the expression of T or B cell markers, if present, remained unchanged (Table 2). A slightly increased staining intensity was noted with CD3 in CTV-2 and THP-1, and with CD7 in ML-1, whereas a slight decrease in staining intensity was noted with CD4 and CD7 in THP-1 cells. The numbers of cells stained were the same with and without induction, however.

Expression of CD30

SU-DHL-1 cells were strongly CD30-positive, and the expression was not affected by treatment with cytokines or TPA. In BV173 and CTV-2, only approximately 5–10% of the cells were CD30-positive; after induction with TPA or TNF, however, a considerable number of cells became CD30-positive (Table 2). In CML-Brown cells, CD30 appeared to stain the cytoplasm, in contrast to eliciting a typical membrane reaction in other cell types. CD30 was weakly positive in THP-1 cells. CD30 staining remained negative in HL-60, KG-1, ML-1, and U-937, regardless of whether or not the cells were treated with TPA or cytokines.

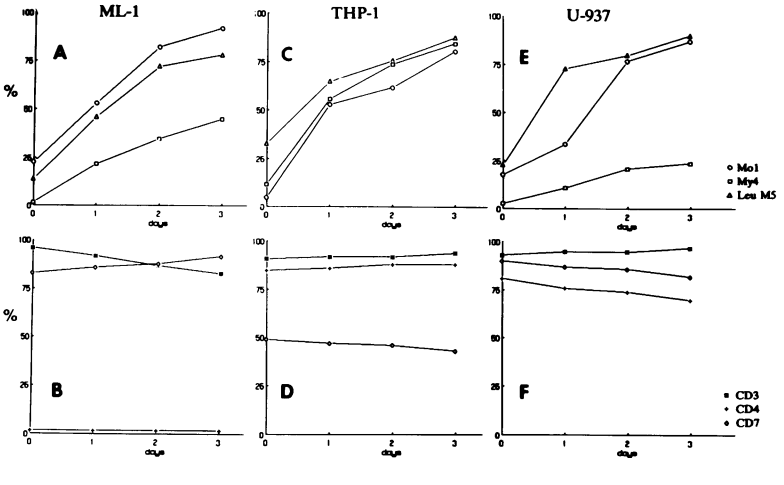

Figure 3. *Phenotypic changes in ML-1 (A, B), THP-1 (C, D), and U937 (E, F) cells. The changes were induced by treatment of THP-1 and ML-1 cells with TNF, and of U-937 cells with TPA. The expression of T cell markers remained unchanged in the TNF- and TPA-treated cells (B, D, F). In contrast, the number of cells positively stained with granulocyte/monocyte markers was greatly enhanced in all three cell types (A, C, E).*

Table 3. *Effects of TPA, TNF, and TNF + IFN-γ on the Cell Cycle and Morphology of MMM Cells**

Treatment/Effect	BV173	CML-Brown	CTV-2	HL-60	KG-1	ML-1	SU-DHL-1	THP-1	U937
Control	45.1/51.2	56.9/41.9	69.6/29.7	65.2/33.6	67.5/30.1	44.7/53.2	56.6/40.7	49.1/43.2	73.0/25.6
TPA	68.4/28.2	57.3/41.6	72.3/26.5	79.3/19.6	72.3/25.5	76.7/21.8	65.6/31.7	72.5/20.4	92.1/6.7
TNF	66.0/31.5	56.0/41.4	76.3/22.6	77.0/21.0	73.1/23.7	74.2/23.6	71.5/25.6	66.6/25.2	74.2/24.2
TNF + IFN-γ	64.6/32.6	56.5/39.2	75.7/24.0	74.1/23.1	70.3/25.2	67.1/26.8	72.4/25.1	59.4/31.2	89.9/9.4
G_0/G_1 changes†	19.5–23.3	0–0.4	2.7–6.7	8.9–14.1	2.8–5.8	22.4–32	9–15.8	10.3–23.4	1.2–19.1
Morphologic changes	Yes	Minimal	Minimal	Yes	Minimal	Yes	Minimal	Yes	Yes

* The data are expressed as A/B; A, percentage of cells in G_0/G_1 phase; B, percentage of cells in S + G_2/M.
† The data are expressed as increment in the number of cells in G_0/G_1 phase after induction.

Effect of TPA, TNF, and IFN-γ on Cell Proliferation

Interferon-γ had a minimal effect on cell proliferation in the majority of cell lines tested; exceptions were U-937 and THP-1. In contrast, TNF exerted a significant effect on most of the cells tested (exception: U-937, Table 3 and Figure 4). TNF blocked the exit of cells from G_0/G_1 phase and their entry into S phase, which resulted in a decreased rate of cell proliferation. A similar finding was noted with TPA, but U-937 cells were also affected.

The extent of blocking of cell proliferation was generally well correlated with the degree of differentiation induced by TPA and cytokines.

Discussion

Positive staining with T and B cell MAbs in MMM cells provides evidence that one cannot simply use one or a few MAbs to deduce the lineage of lymphoma or leukemia cells. Staining does not necessarily indicate the presence in these cells of antigens identical to those in T or B cells, however, but may result from the presence of a similar epitope in otherwise different molecules. Nevertheless, our findings clearly show that none of the MAbs is absolutely lineage-specific when used for routine lymphoma/leukemia phenotyping.[1]

The MMM lineage of the cell lines studied was established by the expression of numerous GM markers. The expression was enhanced when cells were treated with TPA, TNF, or TNF+IFN-γ. The cytokines IFN-γ and TNF are known to induce phenotypic and morphologic differentiation in MMM cells synergically.[31,32] In our study, TNF alone appeared to be as effective as TNF+IFN-γ in inducing the differentiation of most of the cells, although the degree of differentiation varied from one cell line to another. These two cytokines, as well as TPA, inhibited cellular proliferation, as evidenced by the increased number of cells in G_0/G_1 and the decreased number in S and G_2/M phase. The degree of enrichment in GM marker expression was correlated with the morphologic differentiation and the degree of G_0/G_1 block imposed by TPA and by

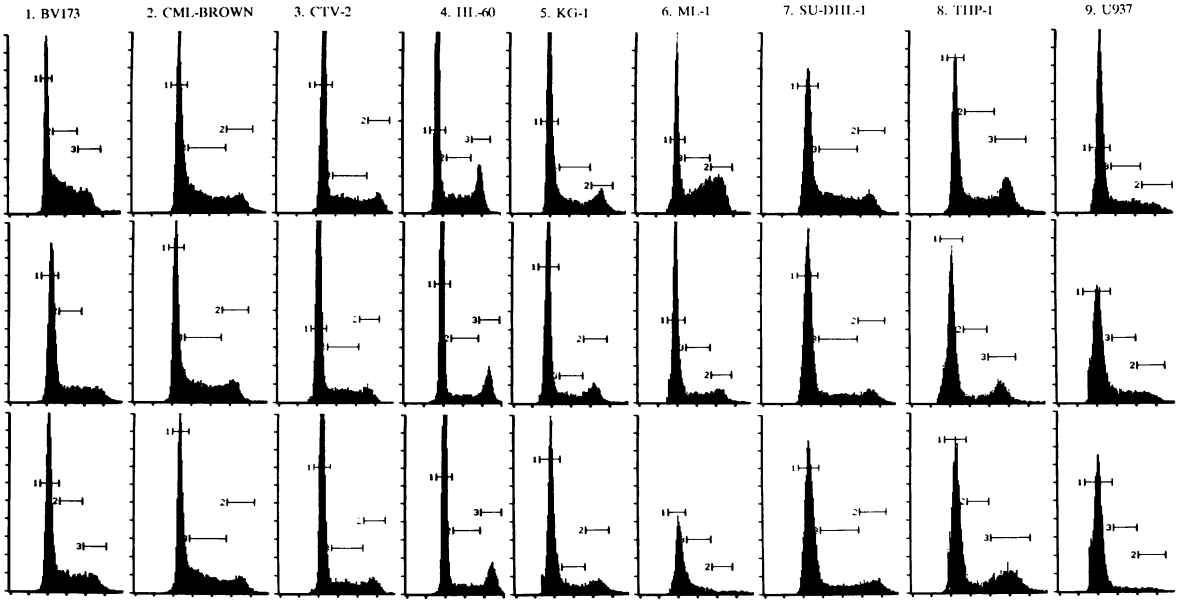

Figure 4. *Effects of TNF and TNF+IFN-γ on proliferation of cells. Row 1, control cells without treatment; row 2, cells treated with TNF; and row 3, cells treated with TNF+IFN-γ. A synergic effect of TNF and IFN-γ was detected in ML-1 and U-937 cells. TNF was effective in inducing G_0/G_1 block in all except CML-Brown and U-937 cells. Region 1 includes cells in G_0/G_1 phase; region 2, S phase; and region 3, G_2/M phase. The x axis represents the fluorescence staining intensity of the nucleus, and y indicates the number of cells stained.*

cytokines in these cells. In contrast, the expression of T and B cell markers was not affected, or only minimally affected, by treatment with TPA, IFN-γ, and TNF. This indicates that the GM marker expression in these cells is related to the status of their differentiation, whereas the expression of T and B cell markers is not.

The reason for the expression of T and B cell markers in MMM cell lines is not known. It is difficult to attribute this expression to a mixed lineage in all of these cells.[45] Our study was designed to provide evidence that aberrant T or B cell marker expression could be detected in cells of MMM lineage; we did not intend to engage in a dispute on theories of lineage infidelity, promiscuity, or multipotentiality.[45–47] Two factors may explain the expression of T or B cell markers in MMM cells.

First, the antibodies used are not absolutely specific for T or B cells. Most MAbs, when first reported on, had not been screened extensively. For example, CD10 was reported to be a common acute lymphoblastic leukemia antigen (CALLA); later, however, this antigen was found in granulocytes, germinal center B cells, thymocytes, and many other nonlymphoid cells.[1–4] CD5 (Leu 1/OKT1) was originally reported to be a T cell marker. The finding that CD5 can be detected in some B cell lymphomas/leukemias, however, has prompted researchers to look for rare normal B cells that are CD5-positive.[5,48] It is possible that many of these so-called T or B cell antibodies react with rare monocytes, histiocytes, or granulocytes.

Second, even though the reactivity of MAbs with normal lymphoid cells was well defined, their distribution in tumor cells had not been studied in detail. In particular, screening of MAbs on MMM cell lines has been limited to those MAbs thought to be related to granulocytes or monocytes. It is likely that the expression of T or B cell markers in MMM cells is due to as yet undefined and lesser known reactivities of these antibodies. Alternatively, their expression may be a simple result of the complicated genetic alterations that occur during neoplastic transformation; that is tumor cells are not expected to behave like their normal counterparts.

The expression of T or B cell markers is not restricted to cultured MMM cells, but can also be detected in monocytic or myelogenic leukemic cells obtained directly from patients.[45–47] This expression may be a property related to immature precursors rather than to mature, differentiated cells. Monocytes, free or fixed histiocytes, and IR cells are derived from similar, if not identical, precursors, and they have a similar immunophenotype, ie, Leu M1, Leu M5, Ki-M1, 2H9, and 1E9.[16,17,19,43,49] Because fixed histiocytes and IR cells in lymphoid tissues are end-stage-differentiated cells, aberrant marker expression in fixed-histiocyte- and IR-cell-related lymphomas, if it occurs, would be expected at a lower rate than that in immature leukemia cells. As a matter of fact, an unusually high incidence of aberrant gene rearrangements (ie, Igμ in T cells and TcRγ in B cells) has been reported in immature murine lymphocytes transformed with Abelson virus (A-MuLV).[50,51]

We detected CD30 in five of the nine MMM cell lines tested. The degree of expression of this marker in monomyelogenic leukemia cells isolated directly from patients is not known; however, we and others have shown that CD30 is also a marker for activated histiocytes and neoplastic cells in true histiocytic lymphoma.[12,19,52,53] Because there may be no marker that is exclusively specific for the determination of lineage, the expression of CD30 in rare normal B or T cells or in neoplastic B or T cell lymphomas is not totally unexpected. It is not logical, however, to regard this rare expression as indicating that H-RS cells are derived from either T or B cells. Recently, CD30 has been detected in a group of so-called anaplastic large cell lymphomas. Some of these lymphoma cells also express T or B cell markers, but the expression is usually limited to one or a few markers.[8] It is noteworthy that cells in several anaplastic lymphomas also exhibited characteristics of monocytic/histiocytic or H-RS cells.[8,54] The fact that aberrant T or B cell marker expression is not an uncommon phenomenon in MMM cell lines should not lead to the conclusion that all CD30-positive anaplastic lymphomas are related to B or T cells, unless further biochemical, genetic, and functional data are provided.

The mechanism of the CD30 expression in neoplastic or transformed cells is still unknown. It cannot be related simply to the activation or proliferation of cells because, in BV173 and CTV-2 cells, the expression of CD30 increased markedly after TPA treatment, and because the treated cells had a decreased rate of cell proliferation (Figure 4). Furthermore, the expression of CD30 cannot be attributed to a particular viral infection or a particular transformation. Both HTLV-1- and EBV-transformed T and B cells are known to express CD30, but the two viruses are unrelated.[6]

Three of the five CD30-positive cell lines (CTV-2, SU-DHL-1, and BV173) were noted to have rearrangements of the T cell receptor (TcR) gene or TcR+immunoglobulin heavy-chain (IgH) gene.[37,55] The nature of these three cell lines is subject to controversy. However, the presence of rearranged TcR or Ig genes cannot be used alone for concluding that these cells are T or B cells. It was noteworthy that aberrant Ig or TcR rearrangements could be detected in 5–30% of lymphoma/leukemia cells, including those of myelocytic and monocytic origin.[56] Many of these cells may not show expression of TcR and/or Ig transcripts (mRNA) or proteins. Perhaps the presence of gene transcripts and protein products is more specific than is gene rearrangement by itself for the delineation of cell origin. Nevertheless, we demonstrated the expression of TcRβ protein in cells without apparent gene rearrangements. These results seem to indicate an aberrant and unpredictable pattern of TcR gene rearrangements and expression in neoplastic cells of non-T cell origin.

Moreover, we recently showed that TcRβ rearrangements can be detected in most CD30-positive EBV-transformed B cell lines, but not in CD30-negative cell lines[55,57] (also unpublished data). We also observed the expression of TcRβ in CD30-positive B lymphoma cells from patients with human immunodeficiency virus infections. Therefore, one should carefully evaluate the significance of TcR gene rearrangements, especially in CD30-positive cells. The possibility of aberrant Ig gene rearrangements in CD30-positive T cell lines has not been studied, because these cells are rare.

Because positivity for CD30 is not an absolute property of T and B cells, and because aberrant T or B cell marker expression is not an uncommon finding in tumor cells related to monocytes/histiocytes, one should not use the expression of a few T or B cell markers in rare cases of HD, and of Ki-1 in a few T or B cell lymphomas, to conclude that H-RS cells are derived from T or B cells. Instead, we believe that H-RS cells have functional similarities with IR cells/histiocytes. This belief is based on several factors: 1) the expression of Leu M1, IRac, 1E9, and 2H9 in most H-RS cells as well as IR cells;[16–19] 2) an antigen-presenting function of H-RS cells;[58,59] 3) production of cytokines (interleukin-1, granulocyte colony-stimulating factor, and TNF-α) in H-RS cells;[20,24,60] 4) the capacity to convert arachidonic acid to prostaglandin E_2 and the presence of cyclooxygenase in H-RS cells and IR cells;[26,27,61] 5) the expression of macrophage colony-stimulating factor receptor (c-*fms*,[62,63] unpublished data); and 6) failure of differentiated H-RS cells induced by TPA, retinoic acid, and extracellular matrix to show increased expression of T and B cell markers.[21,22]

In conclusion, a certain degree of aberrant (nonspecific) marker expression by lymphoma cells is always possible. One should not use one or a few markers to establish the lineage of tumor cells. The expression of aberrant T or B cell markers in lymphomas of histiocyte lineage could pose significant problems for a precise diagnosis, especially in fixed-histiocyte-related lymphomas, because fixed histiocytes are normally devoid of markers known to be associated with monocytes.[43] Furthermore, the tumor cells may express a limited number of GM markers, and this expression may become evident only after induction with TPA or cytokines. Our study strongly suggests that MAbs (and gene rearrangements as well) are not absolutely specific when used to define the lineage of histiocyte-related neoplasms. Further studies are needed before we can understand the mechanism of aberrant expression in lymphoma/leukemia cells and the association of CD30 with aberrant gene rearrangements.

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