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Human Macrophages Can Express the Hodgkin's Cell-Associated Antigen Ki-1 (CD30)

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The normal precursor of the neoplastic cell in Hodgkin's lymphoma is still unknown. Previous reports on the expression of the Hodgkin's cell-associated antigen Ki-1, CD30, on normal cells have been limited to activated lymphocytes. This study demonstrates, however, that cells of the macrophage lineage also are able to express the Ki-1 antigen. The Ki-1 antigen is absent from normal blood monocytes but expressed on up to 85% of macrophage-type cells developed during subsequent in vitro ***differentiation on Teflon membranes. Unlike other maturation-associated antigens, Ki-1 is found only at late stages of the macrophage primary cultures. Its expression can be enhanced by human interferon-gamma in a fashion similar to that of HLA-DR molecules. In addition, freshly explanted tumor cells from three patients with histopathologic and clinical features consistent with the diagnosis of true histiocytic lymphoma or malignant histiocytosis as well as the permanent cell line SU-DHL-1 could be demonstrated to express the Ki-1 antigen. The phenotype of histiocytic malignancy was further evaluated to be HLA-DR$^+$MAX.26$^+$CD25$^+$-EMA$^+$OKT9$^+$Ki-1$^+$. The results could indicate either that Hodgkin's lymphoma may arise not only from the lymphocyte but also from the macrophage lineage or may emphasize a macrophage involvement in the pathogenesis of this disease. (Am J Pathol 1989, 134:187–192)***

Because the nature of the malignant cells in Hodgkin's lymphoma has been under debate ever since their description by Sternberg in 1898, detection of the Hodgkin's cell-associated antigen Ki-1 (CD30) could facilitate definition of the origin of this neoplastic disease.[1] The Ki-1 antigen also was found to be expressed on activation of normal T and B lymphocytes,[2,3] which has led to the hypothesis of the lymphocytic origin of Hodgkin's disease (HD) cells as well as to the description of a new entity of malignant lymphomas, the Ki-1 lymphomas.[3]

This study reports that macrophages are able to express the Ki-1 antigen during terminal differentiation from blood monocytes *in vitro*. Similar to HLA-DR antigens,[4] the expression of Ki-1 on mature macrophages was regulated by interferon-gamma. In addition, tumor cells obtained from three cases of malignant lymphomas with pathologic and immunocytologic features consistent with a histiocytic origin were also of the Ki-1-positive phenotype.

Materials and Methods

Cells

Peripheral blood leukocytes were separated from buffy coat preparations of healthy donors by density gradient centrifugation. Blood monocytes were isolated from other mononuclear cells (MNC) by adherence, cultured overnight in RPMI1640 supplemented with 5×10^{-5} M 2-mercaptoethanol, antibiotics and 5% human AB-group serum, and recovered by vigorous pipetting at 4 C as previously described.[5] Monocytes of more than 90% purity were cultured in suspension on hydrophobic Teflon foils at 4×10^5/ml supplemented RPMI1640 for up to 22 days without feeding. At indicated days cells were harvested from the bags as described.[5] They were more than 95% pure as estimated by morphology, cytochemistry, and the expression of monocytic and macrophage maturation-associated antigens.[5–7] Malignant lymphoma cells were obtained during the diagnostic procedure either by needle aspiration, from minced tissue slices of excised lymph nodes and skin lesions, or, in one patient with central nervous system disease, from the cerebro-spinal fluid (CSF). SU-DHL-1 cells (provided by Dr. M. Scott, Stanford University, Stanford, CA) were kept in RPMI1640 plus 10% fetal calf serum (FCS) and passaged twice weekly.

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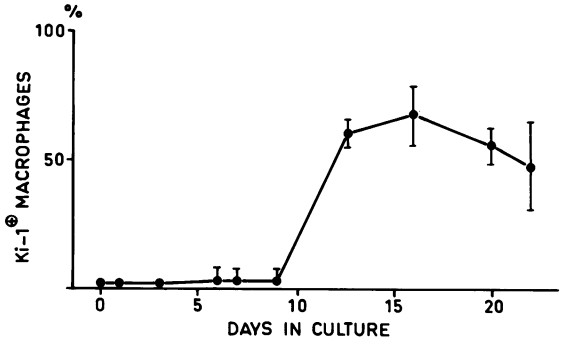

Figure 1. *Expression of the Ki-1 antigen on human macrophages during* in vitro *differentiation from blood monocytes. Adherent monocytes were cultured on Teflon membranes in suppl. RPMI1640 plus 5% AB-serum. At indicated days cells were recovered and stained by an amplified immunoperoxidase technique. Results are expressed as percentage of Ki-1⁺ macrophages. Each point represents the mean of at least three experiments.*

Immunoperoxidase Staining on Adhesion Slides

As described recently,[2] cells were attached to alcian blue-coated slides and either prefixed in 0.05% glutaraldehyde (for surface antigen detection) or air-dried and fixed with aceton/ethanol (for intracellular antigens). They were incubated with the following mouse monoclonal antibodies (MAb): anti-beta2microglobulin (b2M), anti-interleukin (IL)-2 receptor, HLe-1 and Leu3a (Becton & Dickinson, Rödermark, FRG), My4 (CD14) and B1 (both from Coulter Immunology, Hialeah, FL), OKT3, OKT4, and OKT9 (Ortho Diagnostics, Raritan, NY), EMA (Dakopatts, Hamburg, FRG), MAX antibodies,[6] Ki-1 (Behringwerke, Marburg, FRG) as well as rabbit antibodies against human immunoglobulin (Ig), lysozyme, alpha-1-antitrypsin and alpha-1-chymotrypsin (all from Dakopatts). MAb of the IgG1, IgG2a, IgG2b, and IgM isotypes raised against cytoplasmic antigens of Salmonella enteritidis 11RX (gift from Dr. Pilkington, Melbourne) and the IgG3 anti-endothelial MAb BMA120 were used as isotypic controls in concentrations equivalent to their corresponding antibodies. A four-layer peroxidase-antiperoxidase (PAP) technique was applied followed by postfixation with OsO_4. To amplify the staining probes with the Ki-1 and the IgG3 control MAb, we incubated them twice with the third antibody (swine-anti-rabbit) and the PAP complex.

Enzyme-Linked Immunosorbent Assay (ELISA) of Cell Surface Antigens

As described[8] monocytes and macrophages were seeded in RPMI1640 plus 10% FCS in flat-bottom microtiter plates and incubated with or without 200 IU/ml recombinant human interferon-gamma (rhIFN-g, BIOGEN, Geneva, Switzerland), recombinant IFN-alpha-1 (Hofmann-La Roche, Grenzach, FRG), recombinant granulocyte-macrophage-colony-stimulating factor (rhuGM-CSF; Behringwerke, Marburg, FRG) or 10 μg/ml lipopolysaccarides (LPS abortus equi, provided by Dr. Galanos, Max-Planck-Institut, Freiburg, FRG), respectively. Cells were then washed 3 times in PBS, fixed at 4 C with 0.05% glutaraldehyde for 10 minutes and incubated with the MAbs diluted in gelatine-containing medium (0.2%). Immunoperoxidase staining was done using rabbit anti-mouse Ig and peroxidase-conjugated swine anti-rabbit Ig (Dianova, Hamburg, FRG) and phenyl-diamine-dichloride as the substrate. The optical density (OD) was measured at 486 nm.

Results

Blood monocytes, when cultured *in vitro,* undergo a terminal differentiation to mature effector cells,[5,6,9] similar to that seen *in vivo* on migration of the cells from the capillary bed into the various tissues.[10] This monocyte-to-macrophage maturation is essential for the functional competence of the macrophage cell system.[6,7] When Teflon cultures of monocyte-derived macrophages were examined for antigen expression on single cells attached to alcian-

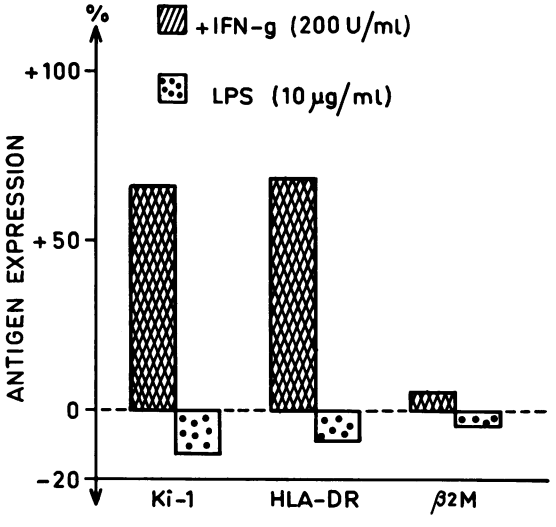

Figure 2. *Enhancement of the expression of HLA-DR and Ki 1 antigens on monocyte-derived macrophages by interferon gamma. Macrophages were cultured from blood monocytes on Teflon for 12 days and then seeded in microtiter plates at 2 × 10⁴/well in suppl. RPMI1640 plus 10% FCS with and without the addition of LPS abortus equi and rhIFN-g, respectively. After 24 hours the antigen expression was evaluated by cell ELISA technique. Data are from a typical experiment and expressed as percentage of controls, mean of triplicate values, SD < 10%. Absolute control values (specific OD) were 2.298 ± 0.21 for b2M, 1.452 ± 0.18 for MAX.21 (HLA-DR), and 0.260 ± 0.03 for Ki-1.*

Table 1. *Phenotype Analysis of Cells from Three Patients with Histiocytic Malignancy and of the Permanent Cell Line DHL-1**

Cells/biopsy	Patient 1 skin and CSF	Patient 2 skin	Patient 3 lymph/n	SU-DHL-1 culture
Membrane antigens				
Common leukocyte	++†	++	++	++
Ki-1 (CD30)	+	++	++	++
HLA-DR	++	++	+	−/+
IL-2 receptor	−/+	++	++	++
Transferrin receptor	+	+	+	++
MAX.26	ND	+/−	+/−	++
EMA	−/+	+/−	+/−	−/(+)
My4	−	−	−	−
B1	−	−	−	−
OKT3	−	−	−	−
OKT4/Leu3a	ND	−/(+)	−	−
MAX.1/.2/.3/.11	−	−	−	−
Cytoplasmic antigens‡				
Lysozyme	ND	+/−	+	−
Alpha-1-chymotrypsin	+/−	+/−	+	−
Alpha-1-antitrypsin	ND	+	+/−	−
Immunoglobulins	ND	−	−	−

* Cells were obtained from cerebrospinal fluids or needle aspirated from lymph nodes or skin tumors and tested for antigen expression after fixation with 0.05% glutaraldehyde by immunoperoxidase staining on adhesion slides.

† Results are expressed as followed: ++, most cells strongly positive; +, most cells positive; +/−, more than 50% of cells positive; −/+, less than 50% of cells positive; −/(+), only few cells weakly positive; −, no cells stained; ND, not done.

‡ Cytoplasmic staining was done on air-dried and ethanol-fixed cells.

blue-coated microspots, 30–80% of macrophages at late culture stages (beyond day 9 in culture) could be shown to react with the MAb Ki-1 (Figure 1). Equivalent concentrations of the anti-endothelial MAb BMA120 of the same isotype did not bind to macrophages, which proved the specificity of the staining. Cells recovered at any culture stage from the Teflon bags proved to be monocyte/macrophages of more than 90% purity by their CD14 expression that was determined in each experiment. Ki-1 antigen expression on macrophage monolayers could also be quantitated using a sensitive cell ELISA that revealed a specific OD_{468} of 0.418 ± 0.2 (N = 7) for macrophages beyond culture stage day 9. The cell ELISA technique was then used to look for possible modulation of macrophage Ki-1 expression by IFN-g and LPS. As demonstrated in Figure 2, when IFN-g was present in the medium for 18 hours, Ki-1 expression increased by about 70% whereas LPS (Figure 2), IFN-alpha, and human recombinant GM-CSF (not shown in detail) had no significant effect. The stimulation of Ki-1 by IFN-g was similar to that of HLA-DR expression whereas the expression of b2M remained unaffected. Similar incubation of blood monocytes with IFN-g did not result in the expression of Ki-1 in these constitutively negative cells (not shown).

Analysis of normal and inflammatory, *in situ* matured macrophages from the alveolar lavage, peritoneal, and pleural effusion fluids did not demonstrate any significant staining with the Ki-1 MAb (not shown in detail). Also, monocytic or myelomonocytic leukemias (N = 5) were found to be Ki-1 negative (not shown in detail). However, the majority of tumor cells obtained from biopsies of lymph nodes or skin lesions and from the cerebrospinal fluid, respectively, of three patients with malignant lymphoma of histiocytic origin showed a strong Ki-1 expression (Table 1 and Figure 3). The diagnosis of a histiocytic malignancy was based on the combination of morphologic characteristics,[11,12] the absence of lymphoid antigens and intracellular Ig, and the presence of lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin. The tumor cell content in the cell suspensions exceeded by far the number of macrophages and, furthermore, the two cell types could be distinguished by their reactivity with the My4 MAb. Histiocytic tumor cells did not bear common monocyte/macrophage surface antigens nor did they express restricted macrophage maturation-associated antigens[6,8,13] but coexpressed HLA-DR molecules as well as receptors for IL-2 (CD25) and TF. They reacted with the MAX.26 MAb (raised against monocyte-derived macrophages)[8,13] and the EMA MAb directed against an epithelial antigen but also reactive with some lymphocytic lymphomas and activated lymphoid cells.[11,14] Much the same phenotype ($HLA\text{-}DR^+Ki\text{-}1^+CD25^+OKT9^+MA\text{-}X26^+EMA^+$) was found for the permanent cell line SU-DHL-1, which, however, was found to be negative for lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin (Table 1). The HD-derived cell line L428[15] differed from the SU-DHL-1 only in its negative reaction with both the MAX.26 and the EMA MAb (not shown in detail).

Discussion

The HD-associated antigen Ki-1,CD30 is a 120 kd glycoprotein, probably a phosphokinase.[16,17] It is present on

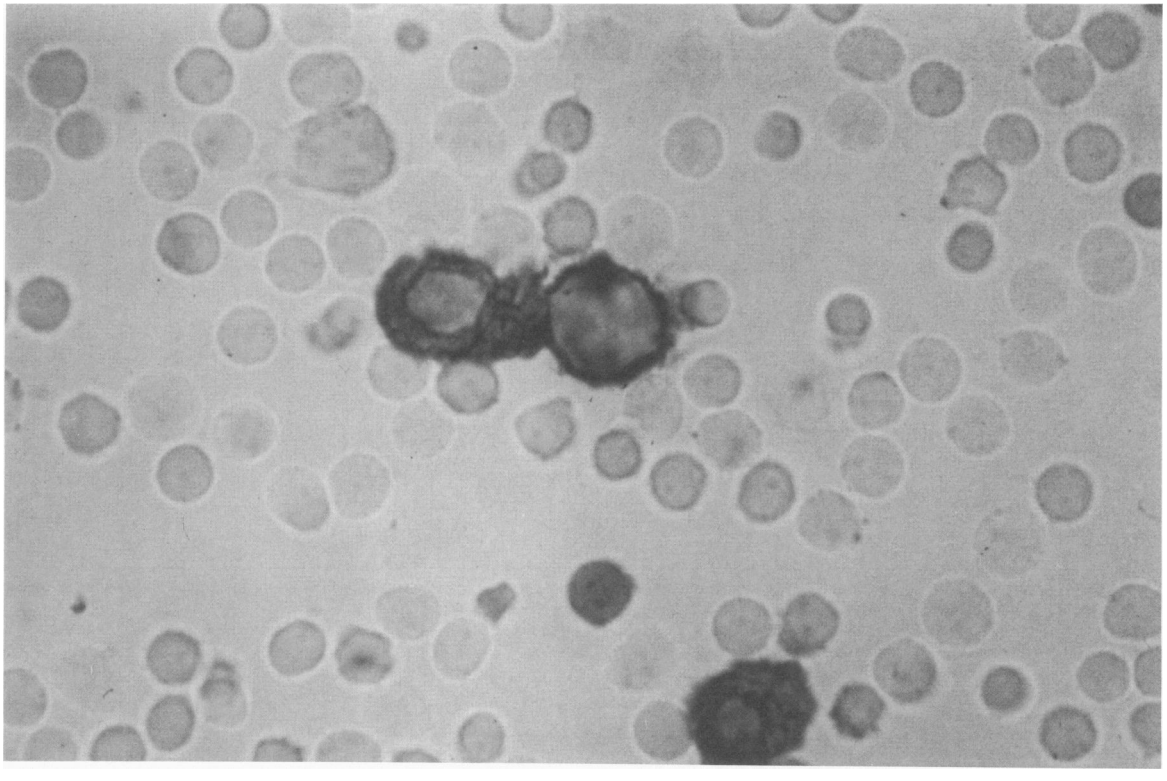

Figure 3. *Immunoperoxidase staining of histiocytic lymphoma cells with the MAB Ki-1 (anti-CD30). Cells were needle-aspirated from a cervical lymph node of patient 3 (see Table 1) and stained on alcian blue-coated slides as described in the Material and Methods section. (Original magnification, ×600)*

some tumor cell lines like the chronic myelogenous leukemia K562 and on a minor percentage of promyelocytic HL60 cells but not on the promonocytic U937 cells.[2] In normal cells, this molecule is expressed on activated and virally transformed lymphocytes[2,3,18] as well as on macrophages derived *in vitro* from blood monocytes, as shown here. Its biologic function is not known but is likely to be associated with the functional activity of cells either as the result of cellular activation or of cellular maturation, as in the case of monocyte-derived macrophages, or of both as seen from its increased expression on macrophages after activation (Figure 2). It might also be of interest that cultured Ki-1$^+$ macrophages show a tendency to fuse to multinucleated, sometimes giant cells.[5] This tendency is increased, similar to the expression of Ki-1, by IFN-g.[19] Our observation that human macrophages can express the Ki-1 molecule is based on the detection of the antigen on *in vitro* differentiated, monocyte-derived macrophages and on malignant lymphoma cells considered to be of histiocytic origin. Whereas the cellular identity of Teflon-cultured, monocyte-derived macrophages is beyond doubt, the histiocytic nature of the lymphomas may be proven by their lysozyme positivity.[20] Although possible, a false identification of concomitant reactive histiocytes as tumor cells seems unlikely because the latter were CD14$^-$ and outnumbered the CD14$^+$ histiocytes, which could be easily distinguished on the multiple spots stained in parallel. In addition, morphology and other criteria (lack of lymphocytic marker, presence of alpha-1-antitrypsin, alpha-1-antichymotrypsin), which by themselves are not specific for macrophages,[20,21] are strongly suggestive of the histiocytic origin when seen in combination with one another and with the lysozyme results. As the SU-DHL-1 cell line lacks lysozyme, its histiocytic nature cannot be proven with certainty,[22] but Delsol et al described three cases of lymphoma of probably histiocytic origin that also failed to express lysozyme.[11] It is of interest that another lymphoma cell line (Karpas 299) is Ki-1$^+$EMA$^+$HLA-DR$^+$.[23] These cells, besides expressing alpha-1-antitrypsin, nonspecific esterase, and the monocytic antigen Mac387, have a chromosomal breakpoint at band 5q35.1, close to the c-*fms* oncogene coding for the macrophage-CSF receptor. The last finding is strongly suggestive of a histiocytic origin of the cell line.

Nevertheless, while we agree with Hsu and coworkers,[24] our report contrasts the results of Pileri et al[12] and Stein et al,[3] who did not find Ki-1 reactivity on true histiocytic, lysozyme positive lymphomas. In the latter report, 3 of 45 cases of Ki-1$^+$ lymphomas (designated as "null-cell" Ki-1 lymphoma) appeared to be similar to our cases with the exception of the negativity for lysozyme.[3] It seems noteworthy that Delsol et al, who described a sim-

ilar co-expression of EMA, Ki-1, IL-2 receptor, and HLA-DR in 9 of 63 cases, did not find lysozyme in the 3 cases of true histiocytic malignancy labeling with anti-macrophage MAbs,[11] as mentioned above. Furthermore, there is one report on the detection of lysosomal lysozyme in HD cells by electron immunomicroscopy only,[25] and recently, by the same technique, HD cells were reported to react with the anti-histiocyte antibody Ki-M7.[26]

Based on the observation of the Ki-1,CD30 expression on activated lymphocytes[2,3,18] but not on normal blood monocytes or tissue macrophages, it was assumed that Hodgkin's disease results solely from the malignant transformation of activated lymphoid cells. In addition, genotypic analysis of most of the HD lymph node biopsies, as well as of all the permanent cell lines yet established from freshly explanted HD cells, revealed rearrangement of the T cell receptor and Ig genes.[27-29] However, our results on the expression of Ki-1 on macrophage-type cells derived from blood monocytes in culture as well as on malignant histiocytes *in situ* leaves it open to the possibility that HD cells in some cases may be derived from cells of the monocyte/macrophage lineage.

Another speculative explanation may be derived from the observation that many systemic immunopathologic abnormalities are associated with Hodgkin's lymphoma.[30] *In situ,* the actual lymph node tumor is composed mainly of reactive cells whereas the malignant cell population often is scarce. This may be either due to a desparate but apparently ineffective immune response against the neoplastic cells or, more likely, these tumor cells release potent mediator molecules that continuously recruit and activate immune effector cells leading to polyclonal proliferation of normal cells. Macrophage T lymphocyte cooperation is a prerequisite for this immune cell activation to occur, and involves HLA-DR molecules and close physical contact between the two cells. It has been suggested that during this multipoint attachment membrane permeability may be transiently disturbed.[31] Because, apart from the cited macrophage characteristics of HD cells, experimental data also favor a lymphocytic origin of HD cells and because the Ki-1 antigen seem to comodulate with HLA-DR molecules (Figure 2), it is tempting to hypothesize a possible hybrid nature of HD cells.[29] Multinucleation, bilobated giant cell features, the appearance of huge hyperchromatic nuclei, and polyploid karyotypes[32-34] may be in line with a syncytial origin of the HD cells that arises from the T cell/macrophage functional unit and may be reflected by the lymphocyte/HD cell rosette formation[35] as an aberrant attempt for cell cooperation. Retroviruses may be involved as causative agents in this pathologic fusion.[36] A similar hypothesis had been put forward about the somatic hybridization of macrophages and tumor cells.[37] The phenotype of such lymphocyte/macrophage hybrids may vary among individual cases. In most instances the genotype and the antigenic phenotype of the lymphocyte fusion partner seem to dominate, and it could well be that only lymphocytic HD cells are adaptable to *in vitro* growth.[29] All of the established HD cell lines present lymphocytic features; however, accessory cell function and IL-1 production may be attributes of the monocytic fusion partner.[38,39]

Not only HD cells but also activated lymphocytes coexpress the Ki-1 antigen with HLA-DR molecules.[2,3] Thus, both antigens may be closely associated; however, whether, unlike HLA-DR molecules, the Ki-1 expression on activated lymphocytes and HD cells can be also modulated by IFN-g must be studied.

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