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Lymphoproliferation and Heterotransplantation in Nude Mice: Tumor Cells in Hodgkin's Disease*

V. Diehl, H. H. Kirchner, M. Schaadt, C. Fonatsch, and H. Stein

A. Summary

In the last 3 years we were able to establish four long-term cultures from Hodgkin-derived material [pleuraleffusions (2), bone marrow (1), and peripheral blood (1)], consisting of cells which represent morphologic and cytochemical as well as cytogenetic features of their in vivo ancestors. Two of these cell lines are described in this paper.

These two lines share the same features: non-B-T lymphocytes, non-macrophages, non myeloid cells, EBV genome negative, monoclonality, multiple numerical and structural chromosome aberrations, and tumor formation upon intracranial xenotransplantation in nude mice.

The two remaining lines are being characterized at the moment. The common characteristics expressed synonymously in the two described lines suggest that the Hodgkin tumor cell does not seem to share the features of marker-carrying lymphocytes, macrophages, or myeloblasts. The cellular origin of these cells is not clear. The loss of cellular differential markers during the process of possible dedifferentiation is discussed.

B. Introduction

The "Sternberg-Reed" (SR) and "Hodgkin" (H) cells are considered to be the neoplastic cells in Hodgkin's disease. Their cellular origin is still subject of considerable controversy. There are arguments for a histiocytic origin of SR and H cells (Rappaport 1966; Mori and Lennert 1969) as well as for their lymphoid origin (Dorfman et al. 1973; Papadimitriou et al. 1978).

Because of the fragility of freshly isolated H and SR cells and the contamination with numerous reactive cells the precise analysis of their cell type is difficult. These limitations can be overcome by the establishment of in vitro proliferating cell populations with neoplastic properties from patients with Hodgkin's disease. The present report describes the features of two established in vitro cell lines from the pleural effusions of two patients with histologically proven Hodgkin's disease.

C. Patients, Material, and Methods

The pleural effusions were obtained from a 37-year-old woman (E.M.) with histologically proven Hodgkin's disease, which was of the nodular sclerosing type, stage IV B, and was primarily diagnosed in 1972 (L 428), and from a 36-year-old man (HR.) with Hodgkin's disease, nodular sclerosing type, which was diagnosed in 1976 from an inguinal lymph node biopsy (L 439).

I. Establishment of the In Vitro Cultures

The heparinized pleural effusion fluid was centrifuged at 150 g for 10 min and the pellet was resuspended in an 0.84% NH_4Cl solution to desintegrate the remaining erythrocytes. After two washings cells were incubated in RPMI 1640 medium supplemented with 20% fetal calf serum, glutamin, and penicillin/streptomycin at 37° C in a 5% CO_2 air atmosphere.

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II. Cytochemistry

The following enzyme staining methods were performed: Naphthol-AS-D.chloracetate-esterase, peroxidase, acid-α-naphthyl acetate esterase, and alkaline posphatase.

III. Surface Markers

Membrane-bound immunoglobulins were investigated as described earlier. Ia-like antigen and lysozyme was detected by specific antisera in an indirect immunofluorescence procedure.

1. Rosette Assays

The E-rosette technique is described by Jondal et al. (1972). Rosette assays to demonstrate C3b and C3d receptors (EAC rosette assays) and IgG receptors were performed as described by Stein (1978). Intracellular antigens were demonstrated by immunostaining for k, λ, and lysozyme by means of the PAP technique (Stein and Kaiserling 1974).

2. Immune Phagocytosis

From the L 428 and L 439 line 1×10^6 cells were mixed with either IgG-E_{ox}A or EAC 3b, pelleted by centrifugation at 200 g for 5 min, and incubated at 37° C for 2 h. The pellet was resuspended by gentle shaking and centrifuged onto glass slides. The slides were stained with Grünwald Giemsa and the proportion of cells with ingested indicator cells was counted.

IV. EBV-Specific Antigens (EBNA, EA, and VCA)

The detection of EBV-associated nuclear antigen (EBNA) was performed according to Reedman and Klein (1973). For the demonstration of the viral capsid antigen (VCA) and the early antigen (EA) the method described by Henle and Henle (1966) was applied.

V. Cytogenetic Studies

The cytogenetic techniques are described elsewhere (Hellriegel et al. 1977). For a detailed analysis of metaphases the Giemsa-banding method was performed (Sumner et al. 1971).

VI. Heterotransplantation in Nude Mice

Details of breeding and colony maintenance of the Balb C nu/nu mice have been described previously (Krause et al. 1975). Cell suspension of 1×10^6/0.01 ml were injected intracranially or subcutaneously into 4-week-old mice. Transplantation of cells embedded in a plasma clot was performed according to the technique described by Lozzio et al. 1976.

D. Results

From the pleural effusions both cell lines (428, L 439) could be established after a lag phase of 4 to 6 weeks. Morphologically the L 428 cultures varied greatly in size and structure (Fig. 1). Beside large (50–80μm) multinucleated cells with smooth membrane surfaces, medium sized (30–50 μm) mono- or binucleated cells with "hairy" membrane protrusions became obvious. A third variant consisted of rather small cells (15–30 μm).

The second culture (L 439) contained round mono- or multinucleated cells with prominent nucleoli and few villi on the surface membrane.

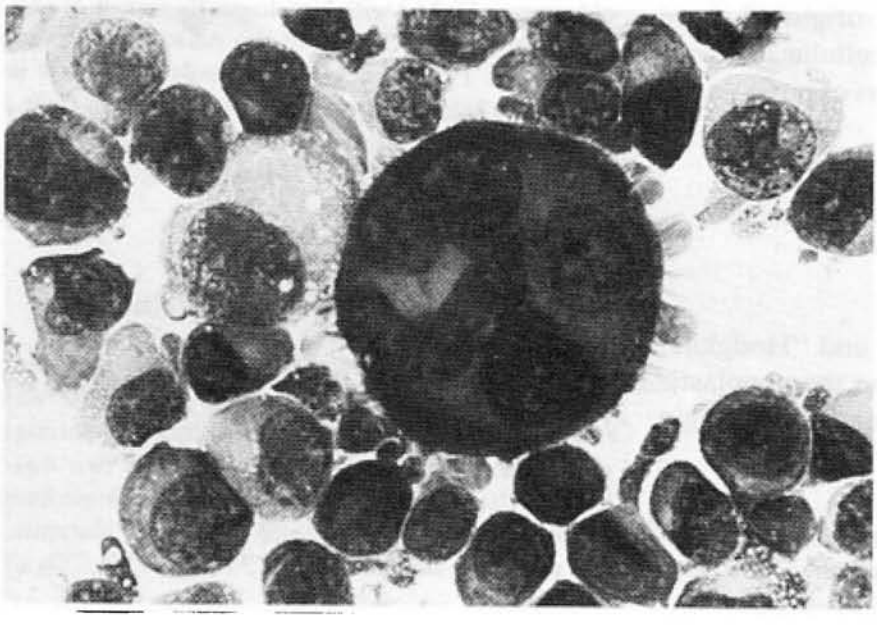

Fig. 1. L 428 cultures using Pappenheim. ×613

The findings of the evaluation of surface membrane and cytoplasmic constitutents are summarized in Table 1. The only structurally defined surface antigen demonstrable in both lines was the Ia-like antigen. Membrane receptors capable of binding T-lymphocytes were formed in more than 90% of the L 428 and L 439 cells. By enzyme cytochemistry the reactions for acid phophatase and acid esterase were found positive in two cell lines.

The L 428 and L 439 cells did not exhibit EBV-specific antigens (EBNA, VCA, EA). EBV-receptors could be demonstrated on the L 428 cells.

Cytogenetic evaluation revealed various structural and numerical chromosome aberra-

Table 1. Characteristics of the Hodgkin cell lines L 428 and L 439

Properties/reagents	L 428 cells	L 439 cells
Surface staining for:		
Polyvalent Ig	Neg.	Neg.
IgM	Neg.	Neg.
IgA	Neg.	Neg.
IgG	Neg.	Neg.
K	Neg.	Neg.
λ	Neg.	Neg.
Ia-like antigen	Pos. (60%–80%)	Pos. (70%–95%)
Lysozyme	Neg.	Neg.
Binding of aggr. IgG	Neg.	Neg.
Rosette assays with:		
EAC3b	Neg.	Neg.
EAC3d	Neg.	Neg.
IgG-EA	Neg.	Neg.
Sheep E[a]	Neg.	Neg.
Human T – cells	Pos. (90%)	Pos. (90%)
Cytoplasmic Staining for:		
IgG	Neg.	Neg.
K	Neg.	Neg.
λ	Neg.	Neg.
Lysozyme	Neg.	Neg.
Immunophagocytosis of:		
C3b-coated E	Neg.	Neg.
IgG-coated E	Neg.	Neg.
Enzyme cytochemical staining		
Naphthol chloracetate esterase	Neg.	Neg.
Peroxidase	Neg.	Neg.
Acid α-naphthyl acetate esterase	Pos.	Pos.
Alkaline phosphatase	Neg.	Neg.
Epstein-Barr virus specific antigens		
EBNA	Neg.	Neg.
EA	Neg.	Neg.
VCA	Neg.	Neg.
EBV receptors	Pos.	Not tested
Heterotransplantation in nude mice		
Primary material		
Intracranial	2/2	Not tested
Cultured cells		
Intracranial	5/6	4/4
Subcutaneous (suspension)	0/1	0/3
Subcutaneous (plasma clot)	2/2	Not tested

[a] Untreated and neuraminidase-treated sheep erythrocytes

	L 428	L 439
Cell size (mean diameter)	15–80 μm	40–60 μm
Growth pattern	Single cells	Single cells with small clumps
Doubling time	42–46 H	38–72 H
Max. concentration (cells/ml)	1.56×10^6	0.72×10^6

Table 2. Growth characteristics of cell lines L 428 and L 439 in vitro

tions in both lines. In the L 428 line the total number of chromosomes per cell amounted to 48–50. The monoclonal origin was ascertained by identifying several identical marker chromosomes: 1 p+, 2 p+, 6 q+, 7 q+, 9 p+, 11 q-, 13 p+, and 21 q-. Each metaphase evaluated showed an extra chromosome No. 12 and lacked one chromosome 13. Some cells exhibited a 14 q+ marker.

In the L 439 line likewise a variety of identical marker chromosomes could be shown which indicated its monoclonal origin: 1 p+, 1 q-, 1 p-, 2 q+, 10 p-, 15 p+, 18 q+, and 21 q-. In addition an extra chromosome No. 5 and No. 12 was constantly detected.

Intracranial heterotransplantation of L 428 and L 439 cells into nude mice resulted in tumor formation. Intracerebral tumor growth could be histologically confined (Fig. 2).

After subcutaneous inoculation both lines failed to induce tumors. When the L 428 cells (1×10^6) were embedded in a fibrin clot prior to transplantation, infiltrative tumor growth could be induced subcutaneously (Fig. 3).

Detailed growth characteristics are shown in Table 2.

E. Discussion

The L 428 line as well as the L 439 line have been obtained from the pleural effusion of two patients with histologically proven Hodgkin's disease. The monoclonal origin of the culture cells was demonstrated by identical marker chromosomes in each metaphase, despite the morphologic variations among individual cells. An additional argument for the malignant nature of both lines is the lack of EBV specific antigens, since hitherto EBV-negative lymphoid cell lines of a nonneoplastic nature have not been described.

The tumorigenicity of L 428 and L 439 cells after intracranial heterotransplantation in nude mice does not provide proof of the neoplastic nature, since this is a common feature of LCL and lymphoma lines (Schaadt et al. 1979). Tumor formation after subcutane-

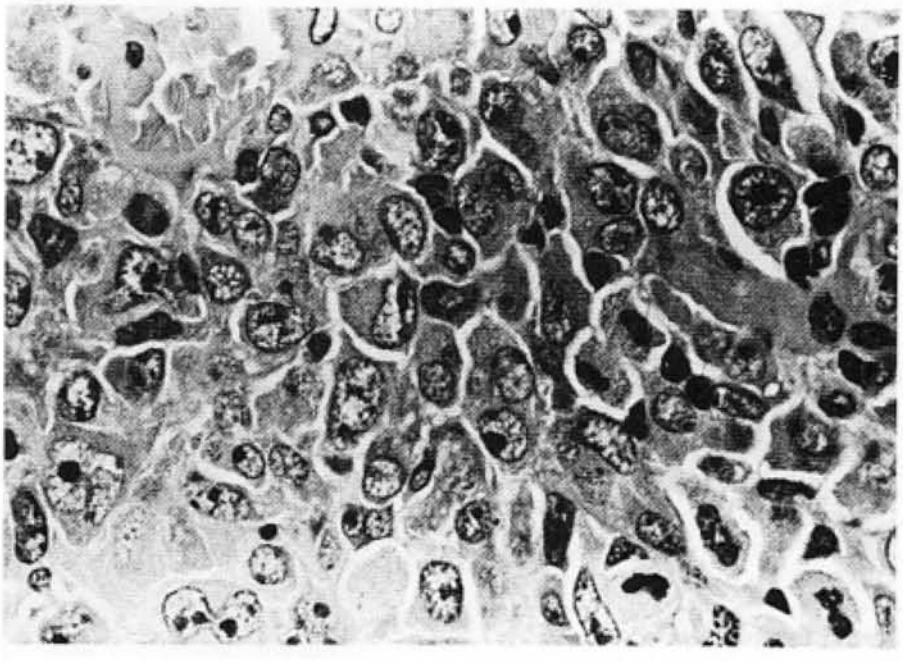

Fig. 2. Intracerebral tumorgrowth of the cell line L 428. Giemsa. ×244

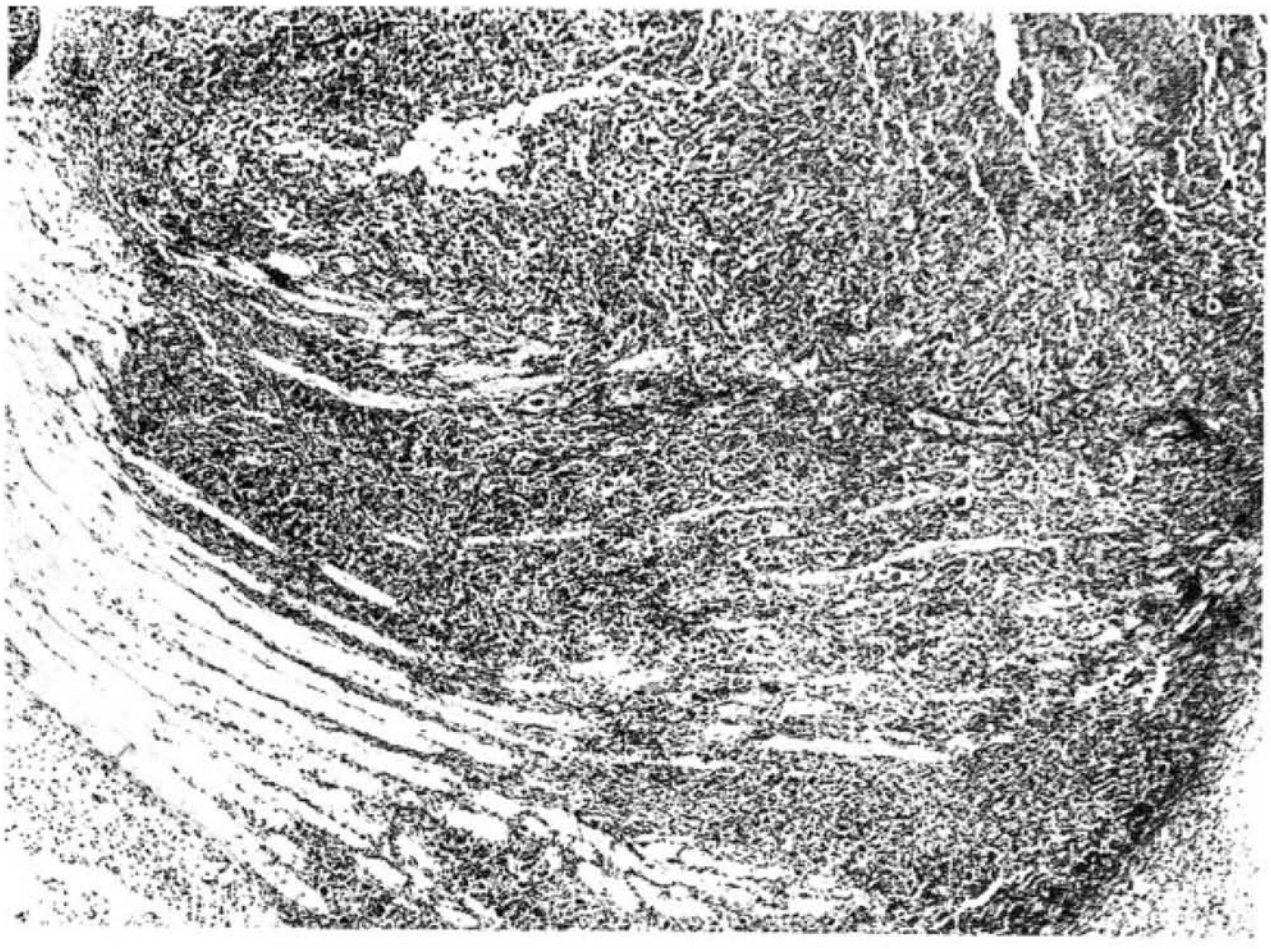

Fig. 3. Tumor growth of L 428 cells after transplantation in a fibrin clot into the subcutaneous tissue. Giemsa ×32

ous transplantation, which seems to be more strongly correlated to other markers of malignancy (Diehl et al. 1977), did not occur after inoculation of a cell suspension from the cell lines but did occur after embedding of L 428 cells in a fibrin clot before subcutaneous transplantation.

The histologic sections of the mouse tumors derived from the L 428 and the L 439 lines showed cell individuals representing a morphology very similar to that of "Sternberg-Reed" and "Hodgkin" cells. The immunologic and biologic characterization of both Hodgkin lines revealed the identical pattern observed in freshly obtained H and SR cells (Papadimitriou et al. 1978): they lack SIgG, HTLA, and receptors for C3b, C3d, IgFc, mouse E, and sheep E; are devoid of lysozyme, peroxidase, and chloracetate esterase; and they express Ia-like antigens and receptors for T cells and contain acid phosphatase and acid esterase. The origin of the cultured cells thus far is still obscure. These cells, however resemble very closely features of cultured Hodgkin cells described by Kaplan et al. (personal communication).

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