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Hodgkin's Disease Cell Lines: Characteristics and Biological Activities

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A. Summary

In the last 4 years we have established five long-term cultures from tumor material of Hodgkin's disease. The in vitro cells have malignant characteristics and represent the in vivo Hodgkin- and Sternberg-Reed-cells as shown by the identity of multiple properties.

Common immunological, functional, and morphological assays did not characterize the in vitro cells as a known cell type of lymphoid, myeloid, or monocytoid tissue. The in vitro Hodgkin's disease cells are biologically active by producing factors involved in regulation and promotion of immunological response and granulopoiesis.

The relevance of the findings for pathogenesis and clinical appearance of Hodgkin's disease is discussed.

B. Introduction

Hodgkin's disease is still one of the most challenging entities in hematooncology. Of patients with this histopathological diagnosis, 60%–70% can possibly be cured [3, 11] and 30%–40% do not respond with complete remission upon first treatment; of this group 15%–20% will achieve remission upon secondary treatment, but 15%–20% will die within 4–18 months in spite of intensive treatment strategies (Fig. 1). Secondary neoplasias are the most hazardous consequence of intensive combined treatment modalities (radiochemotherapy) at the moment and amount to 5%–10% of all initially diagnosed Hodgkin's disease patients [1, 2, 16]. The incidence of acute myeloid or myelomonocytoid leukemia is 130 times higher in Hodgkin's disease patients than in normal individuals [9].

Attempts to investigate the nature of the pathognomonic Hodgkin- (H) and Sternberg-Reed (SR)-cells have been hampered by the fact that these cells constitute only a small minority in the primary biopsy and seem to be utterly growth restricted in the hitherto available culture systems [6].

Since 1978 we have established five cell lines from tumor-involved specimens derived from HD patients [5, 6, 12]. All histologies were confirmed by four independent hematopathologists.

C. Patients, Material, and Methods

All five lines were grown from HD specimens of four patients with nodular sclerosing-type histologies, clinical stage IVB. The patients had been submitted to intensive combination chemoradiotherapy. The sources for the culture material were pleural effusions in three cases and bone marrow and peripheral blood in one case. Two identical cultures were established from the two different sources of this particular patient. Four cell lines continously proliferate in vitro, while on line stopped growing for unknown reasons after 7 months in culture (L 439).

D. Results

The cell lines share an identical (L 428) or partially identical phenotype (L 538/540,

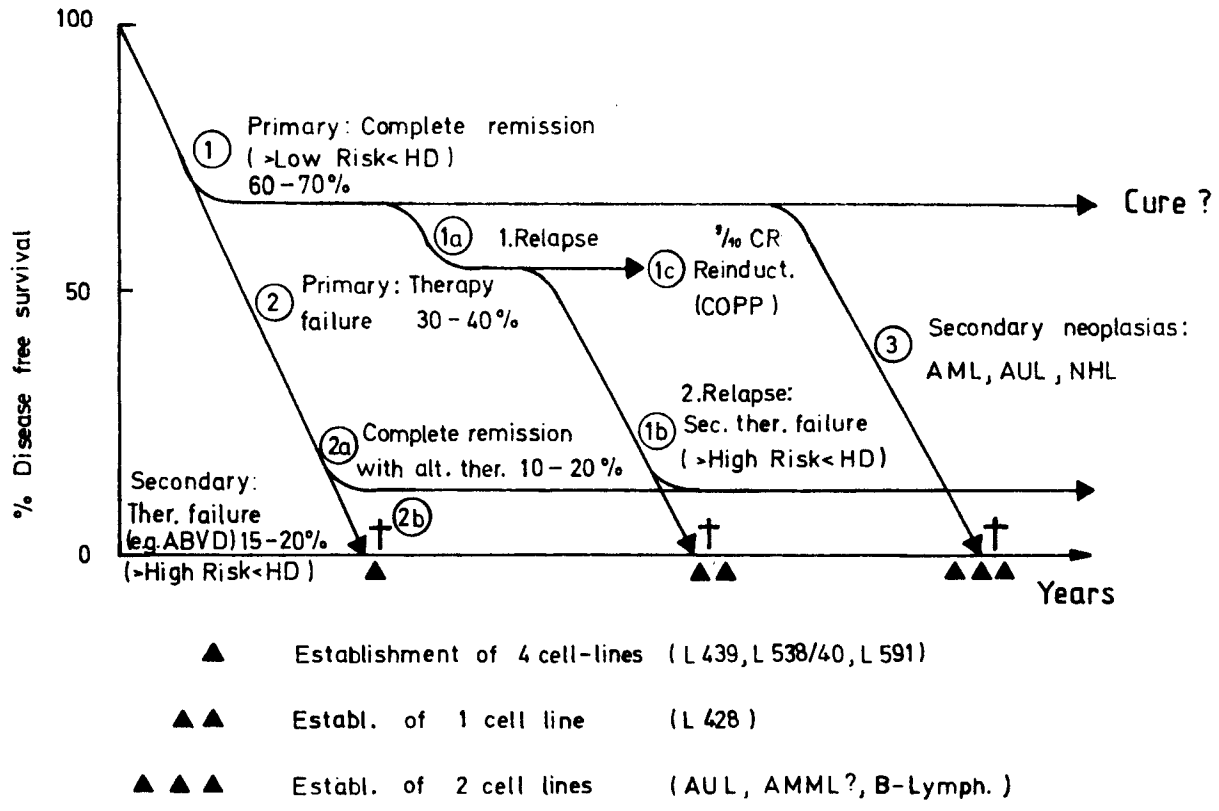

Fig. 1. Hodgkin's disease: clinical course and secondary neoplasias

L 591) with in vivo H- and SR-cells and represent a cell type previously unknown according to the methods used for cellular discrimination [15].

The neoplastic nature of the five HD tumor cell lines is indicated by aneuploidy, except in one line (L 591), and multiple structural and numerical chromosome abnormalities associated with a monoclonal pattern of multiple marker chromosomes.

A comparison of the characteristic features of the in vitro cultured HD cells with H- and SR-cells from freshly obtained biopsies is shown in Table 1. All cultured cells lacked surface- or cytoplasmic-Igs. IgG present in fresh biopsy H- and SR-cells was not found in vitro. Ia-like antigens, receptors for human T cells, acid phosphatase, and acid naphthol acetate esterase were present in all cultured lines. EBV specific receptors were found in two out of two tested lines, EBV genomes and EBV-induced antigens, however, only in one line (L 591). All HD cell lines as well as fresh biopsy H- and SR-cells are devoid of HTLA receptors for C3b, C3d, IgG-Fc, mouse-E, or sheep-E and of lysozyme, peroxidase, and chloracetate esterase (Table 1).

The identity of the in vivo and in vitro H- and SR-cells was shown by congruent morphological, functional, and immunological markers. The strongest proof for the derivation of the cultured cells from H- and SR-cells in vivo was the demonstration of cross-reacting surface and cytoplasmatic constituents on the in vivo and in vitro cells by means of absorbed polyclonal (rabbit anti L 428 cells) (Table 1) and mouse monoclonal (anti L 428 cells) antibodies (Ki 1, Ki 24, Ki 27) (Table 2). Furthermore, monoclonal antibodies directed against granulopoietic cell determinants (3C4, TÜ 9) were also present on biopsy HD cells and the cell lines L 428 KS and L 540, but were absent on the L 591 cells.

In an attempt to determine the origin and nature of the cultured H- and SR-cells a multitude of monoclonal antibodies directed against human lymphoid and hematopoietic differentiation markers were tested against these cells. Table 3 summarizes the results by showing the most commonly accepted markers as specific attributes of the different cell types.

As demonstrated in this table, the reactive pattern of the cell line L 428 and partially of the cell line L 540 was identical

Table 1. Properties of in vivo and in vitro H- and SR-cells

	HD and SR cells in biopsies	HD cell lines			
		428	439	538/540	591
Surface staining for					
IgG, IgM, IgA	–	–	–	–	–
Lysozyme	–	–	–	–	
Ia-like antigen	+	+	+	+	+
Cytoplasmic staining for					
IgG	+	–	–	–	–
Lysozyme	–	–	–	–	–
Rosette assays with					
EAC 3b, EAC 3d	–	–	–	–	60% – 70%
IgG – EA	–	–	–	–	–
Human T cells	+	+	+	–	+
Immunphagocytosis					
C-3B-coated E, IgG-coated E	–	–	–	–	–
EBV-specific antigens					
EBV-receptors	N.T.	+	N.T.	N.T.	+
EBNA, EA, VCA	N.T.	–	–	–	+
Cytochemical staining					
Naphthol chloracetate esterase, Peroxidase Alkaline phosphatase	–	–	–	–	–
Acid-α-naphthyl acetate esterase Acid phosphatase	+	+	+	+	+
Reactivity with heterologous L 428 antiserum	+	+	+	+	+

with that of the H- and SR-biopsy cells, lacking markers characterizing B cells, T cells, monocytes, dendritic reticulum cells, interdigitating reticulum cells, and Null cells. They carried, however, granulopoietic cell determinants, as shown by the reactivity of antibodies 3C4, TÜ 9, and Vim D5, but they were unreactive for the peroxidase and chloracetate esterase cytochemical staining.

The L 540 cells reacted like the L 428 cells, but were positive with the monocyte 1 antibody. The L 591 cells showed a very peculiar pattern insofar as they lacked

Table 2. Reactivity of HD and SR cells in biopsies and in vitro (lines) with monoclonal L 428- and granulopoietic cell antibodies

HD material	Antibodies				
	L 428 Antibodies			Granulopoietic cell antibodies	
	KI 1	KI 27	KI 24	3C4	TÜ 9
HD biopsies	+	+	+	+	+
HD cell lines					
L 428	+	+	+	+	+
L 428 KS	+	+	+	+	+
L 428 KSA	+	–	+	N.T.	N.T.
L 540	+	–	–	+	+
L 591	+	+	+	–	–

Table 3. Differential characteristics of HD and SR cells and HD cell lines

Cell Type	Markers/monoclonal Antibodies	H- and SR-cells in Biopsies	HD-derived cell lines		
			L 428	L 540	L 591
B cells	Anti-IgM-D	–	–	–	–
	Ig surface	–	–	–	–
	Ig cytoplasm	–	–	–	–
	EBV antigens	–	–	–	+
T cells	OKT_{11}	–	–	–	+
	LYT_3	–	–	–	+
Monocytes	OKM_1	–	–	–	–
	$Monocyte_1$	–	–	+	–
	$Monocyte_2$	–	–	–	–
	Lysozyme production	–	–	–	–
	Phagocytosis	–	–	–	–
Granulopoietic cells	3C4	+	+	+	–
	TÜ 9	+	+	+	–
	VIM-D5	+	+	N.T.	N.T.
	Peroxidase	–	–	–	–
	Chloracetate esterase	–	–	–	–
Dendritic reticulum cells	R 4/23	–	–	–	–
Interdigitating reticulum cells	$NA_{1/34}$	–	–	–	–
	$T\text{-}ALL_2$	–	–	–	–
Null cells (NK cells?)	OKM_1	–	–	–	–
HD-derived cells (H- and SR-cells)	Ki 1 } anti 428 cells	+	+	+	+
	Ki 24 } anti 428 cells	+	+	–	–
	Ki 27 } anti 428 cells	+	+	–	–
	Heterologous anti 428 serum	+	+	+	+

Table 4. Biological activities of HD line supernatants

	HD cell lines					Control lines	
	L 428	L 428 KS	L 428 KSA	L 540/538	591	LCL	BL lines RAMOS, HRIK
Granulocyte colony stimulating activity (CSA)	+	++	+++	++	(+)	0	0
Interleukin 1 activity	+	+	+	+	+	0	0
Accessory function for mitogen-induced T-cell proliferation	+	+	+	N.T.	N.T.	0	0
Stimulation of mixed lymphocyte reaction	+	+	N.T.	N.T.	N.T.	0	0
Enhancement of EBV-induced spontaneous B-cell transformation	0	0	+	0	0	–	–

B-cell markers like Ig production or Ig surface reactivity (anti IgM, anti IgD), but carried C3b and C3d receptors, as well as EBV receptors and EBV antigens. Furthermore, they reacted positively with T-cell antisera like OKT-11 and Lyt 13, but lacked monocyte, granulopoietic cell, dendritic reticulum cell, and interdigitating reticulum cell, as well as Null cell markers.

The cell lines produced a variety of substances which are known to be mediators of immune response and granulopoiesis (Table 4):

Conditioned media of all cell lines contained high amounts of granulocyte colony stimulating activity [6]. Furthermore, supernatants of all HD-derived cell lines showed pronounced Interleukin 1 activity. The L 428 line and its sublines (for origin see [6]) exhibited an accessory function for mitogen (CON-A)-induced T-cell proliferation, as well as a stimulation of mixed lymphocyte reaction [7, 8]. Conditioned medium of the TPA-treated L 428 KSA adherent subline [6] enhanced the EBV-induced B-cell transformation to immortalized, continously growing lymphoblastoid cell cultures, when using EBV-positive healthy donors.

E. Conclusions

1. L 428 respresents in vitro the in vivo H- and SR-cell population. The other reported lines exhibit most but not all markers of in vivo H- and SR-cells. The non-LCL character of L 591 is still the subject of discussion.

2. The HD cells (in vivo and in vitro) represent no known cell type or any cell class so far identified by common immunological, functional, and morphological tests.

3. The in vitro HD cells exhibit biological activities regulating and/or promoting immune response and granulopoiesis.

F. Hypothetic Pathogenesis of Hodgkin's Disease

Figure 2 summarizes all the data obtained on the described HD-derived cell lines in an attempt to propose a hypothesis for some of the pathogenetic mechanisms involved in Hodgkin's disease:

The origin of H- and SR-cells in unknown. It is possible that the Ki 1 antibody not only recognizes H-SR-specific determinants, but also depicts a previously

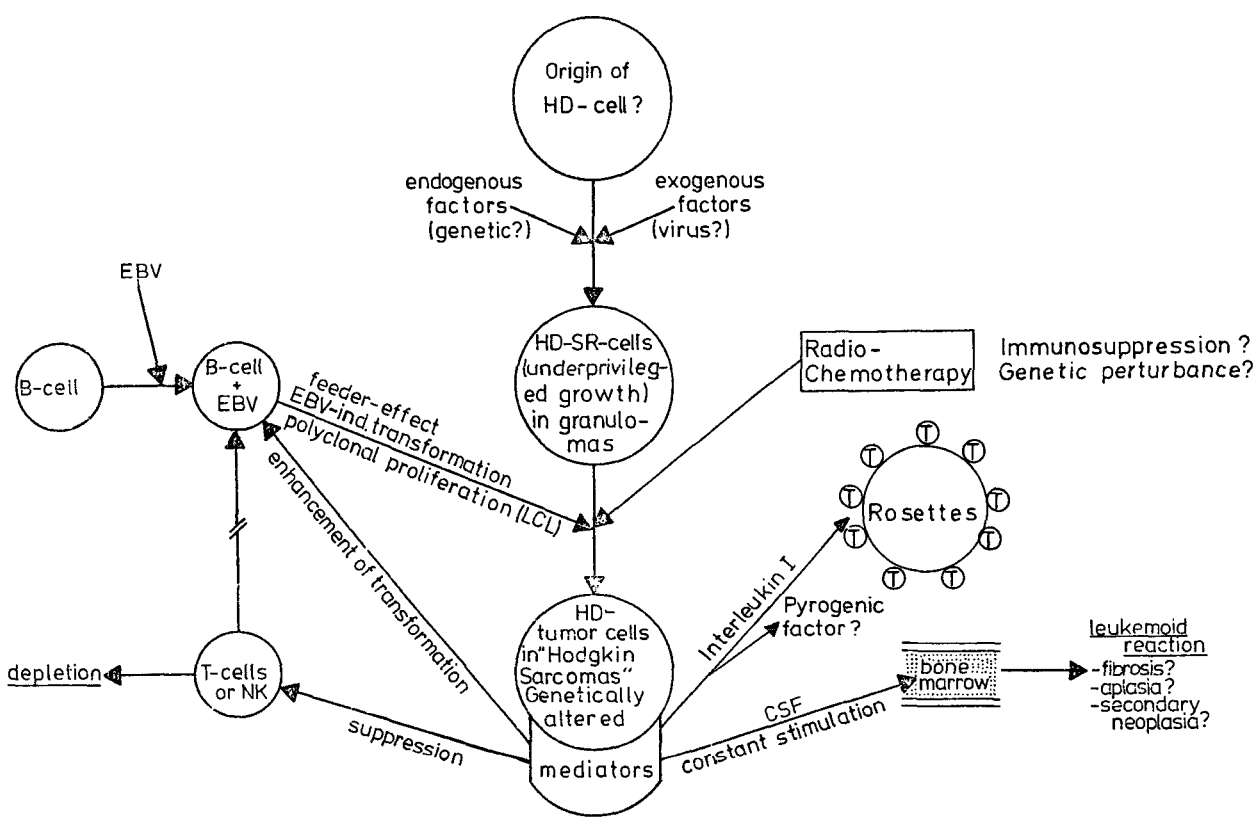

Fig. 2. Hypothetic pathogenesis of Hodgkin's disease

undefined cell in normal tissue [13, 15], which could be the normal counterpart of the "malignant" H- and SR-cells (see Stein, this volume).

The pathogenetic mechanisms involved in the transformation of a normal cell, possibly playing some role in immune and hematopoietic regulation, is unknown. Endogenous (genetic?) and exogenous (viruses, chemical agents, both?) might induce a gradual "evolution" from a primarily nonproliferating, biologically active cell, which by its products (CSF, Il 1) might create the clinically not very aggressive "Hodgkin's lymphoma", to a genetically altered (Fonatsch et al. unpublished results) more malignant cell, embedded in the histological entity of a "Hodgkin sarcoma." Radiochemotherapy might act as a cofactor in this process of gradual malignization. Of the HD patients, however, 60%–90% are cured by radio- and/or chemotherapy in the early stages of this process before genetically altered cells have chance to commence rapid proliferation and possibly exert resistance to cytoreductive therapy.

The variance in the histological presentation of Hodgkin's disease could reflect this gradual malignization process: Paragranuloma and/or lymphocytic predominance and lymphocyte-enriched nodular sclerosis would identify a stage of "low risk", with a high functional activity of the H- and SR-cells, producing mediators like CSF, Interleukin 1, but still restricted in cellular proliferation. If cytoreductive therapy is carried out at this stage, cure is possible in up to 90% of cases ([10], Schellong, personal communication).

If the HD cells withstand therapy by either genetically inherent or resistance mechanisms acquired during treatment, the patient will present a picture of a more malignant Hodgkin's sarcoma with a higher number of rapidly proliferating H- and SR-cells. These cells could still have retained their biological mediator production, but the balance might be toward more production of immune suppressive and EBV transformation enhancing factor.

The fact that many Hodgkin's disease patients develop high antibody serum titers against EBV antigens and give rise to EBV-induced lymphoblastoid cell cultures significantly more than normal individuals [4] could be explained not only by T-cell immunosuppression but also by a direct influence of an EBV transformation enhancing factor. The resulting polyclonal lymphoblastoid transformation could "feed" or protect the tumor cell, possibly under a concomitant protection of the rosetting OKT-4-positive T-helper cells, attaching to the H- and SR-cells. These protection mechanisms might enable an a priori "low-grade malignant" HD cell to "sneak through" to a higher malignant proliferating tumor cell, which in 15%–20% of the clinical outcome could eventually kill the patient. Most Hodkgin's disease patients, however, do not die of tumor cell proliferation, but of biological side effects of immune deficiency and hematological complications, possibly due to some of the described factors.

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