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Recent Efforts to Establish an *in vivo* Model as a New Experimental Tool in the Study of Hodgkin's Disease

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INTRODUCTION

HODGKIN'S DISEASE is defined by the presence of characteristic Hodgkin (HD)-cells and Reed–Sternberg (RS)-cells surrounded by normal reactive cells including lymphocytes, benign histiocytes, plasma cells and eosinophils. The HD- and RS-cells are thought to be the actual malignant cells. Despite intensive investigation the cell of origin of RS-cells has not been definitively identified. These cells only constitute 0.1–1% of the cells in involved tissues. Thus, a number of groups have attempted to establish Hodgkin- and RS-cell lines. Ten tumour cell lines are currently recognised as being Hodgkin derived [1–10] (see Table 1). Although heterogeneous, they all fulfill the criteria of monoclonality and aneuploidy. However, it is difficult to prove that these lines are true counterparts of HD- and RS-cells *in vivo*. An animal model, in which the biological conditions are more equivalent to the microenvironment of HD- and RS-cells, might be advantageous for further studies of Hodgkin's disease.

Heterotransplantation of haematological malignancies has proved to be difficult [11, 12]. Extensive immunosuppression of the athymic nude mice with treatment such as irradiation [13] is necessary for successful transplantation of non-Hodgkin-lymphomas (NHL) and leukaemia derived cell lines. Otherwise the tumour must be injected intracranially or into the anterior chamber of the nude mouse eyes [11, 14]. Except intracranially *in vivo* growth of any of the 10 available Hodgkin's lymphoma derived cell lines or of primary biopsy specimen has not been achieved until now in nude mice [2, 15]. Only one mutant subline (L540 cy, see below) transplanted subcutaneously into X linked immunodeficient/beige/nude (BNX) mice led to reproducible tumour growth and could be employed for testing the antitumour effects of ricin A-chain immunotoxins [16]. BNX mice and severe combined immunodeficient (SCID) mice appear to be better recipients for human tumours [17, 18].

The BNX mouse is a cross between three murine immunodeficient strains. It is homozygous for the thymus (T cell) deficiency nude (nu)gene on chromosome 11 and the lysosomal (NK cell) defect beige gene on chromosome 13. Furthermore it carries the X chromosomal B cell regulatory gene defect. In BNX mice three specific immune defence effector cells are affected. However, there may be some remaining activity of B cells since BNX mice still have detectable levels of serum immunoglobulin. Widespread use of BNX mice is impeded by difficulties with breeding.

Severe combined immunodeficient (SCID) mice bear a VDJ recombinase gene defect on chromosome 16 [19] resulting in loss of peripheral B and T cell function but retention of natural killer cell (NK) function. In comparison with other immunodeficient mouse strains, the SCID-mouse appears to provide favourable growth conditions for lymphatic cells [20–23]. The reconstitution of SCID-mice with a human immune system has been described [24]. Therefore, these mouse strains were selected for heterotransplantation of Hodgkin's disease derived cell lines and primary material.

MATERIALS AND METHODS

Subcutaneous and intraperitoneal injection of Hodgkin's disease derived cell lines

Six Hodgkin's disease derived cell lines (L428, L540, L591, DEV, HD-LM2, KM-H2) and the mutant subline 540 cy, established from a nude mouse after intramuscular injection of L540 cells and subsequent cyclophosphamide treatment [25], were tested by subcutaneous and intraperitoneal injection into BNX and SCID-mice [26].

Of each cell line 2.5×10^7 cells were injected subcutaneously or intraperitoneally into 5 male or female mice at the age of 6 weeks. When there was a palpable tumour of more than 20 mm or detectable abdominal swelling, worsening overall condition of the animal or after an uneventful observation period of 120 days, the animals were killed and the tissues were subjected to histological and cytogenetic examination. Tumour cells were identified immunohistochemically by the detection of CD30 antigen on cryostat sections which were immunostained employing the APAAP technique [27].

Table 1. Hodgkin's disease derived cell lines

Cell line	Stage, Source	Phenotype (Markers)	Genotype (rearrangements)	Ref.
L428	IV, PE		B ($Ig_{H,L}$, TCR β)	1
L540	IV, BM	T (CD2, CD4)	T (TCR)	2
L591	IV, PE	B (CD19, CD20)	B (Ig)	3
CO	IV, LN	T (CD3, CD5, CD7)	T (TCR β,δ)	4
DEV	II, PE	B (CD19, CD20)	B ($Ig_{H,L}$)	5
HD-LM2	IV, PE	T (CD2)	T (TCR)	6
KM-H2	IV, PE	B (CD19, CD20)	B (Ig_H)	7
HO	II, LN	T (CD3, CD4, CD7)	T (TCR β,δ)	8
ZO	II, PE		B ($Ig_{H,L}$)	9
SUP-HD1	III,PE		B ($Ig_{H,L}$, TCR β)	10

PE: pleural effusion, BM = bone marrow, LN = lymph nodes.

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Table 2. Tumorigenicity of Hodgkin's disease derived cell lines in nu/nu, BNX and SCID mice

Mouse strain	Growth enhancer treatment	HD cell lines: L428 s.c.	L428 i.p.	L540 s.c.	L540 i.p.	L591 s.c.	L591 i.p.	DEV s.c.	DEV i.p.	HD-LM2 s.c.	HD-LM2 i.p.	KM-H2 s.c.	KM-H2 i.p.
nu/nu	Untreated	0/5		0/5		0/5							
BNX	Untreated	0/5	0/5	0/5	0/5	0/5	0/5	4/5	0/5	0/5	0/5	0/0	0/0
	HT1080	0/5	0/5	5/5	4/5	3/5	0/5			0/5	0/5		
	Pristane	0/5	4/5	4/5	2/5	0/5	4/5			0/5	0/5		
	α-Asialo-antibody	0/5	0/5	4/5	2/5	0/5	0/5			0/5	0/5		
SCID	Untreated	3/5	3/5	3/5	1/5	1/5	0/5	3/5	1/5	2/5	1/5	3/5	2/5
	HT1080	4/5	2/5	4/5	0/5	0/5	0/5			1/5	1/5		
	Pristane	4/5	4/5	5/5	3/5	5/5	2/5			0/5	3/5		
	α-Asialo-antibody	1/5	1/5	5/5	2/5	4/5	3/5			1/5	0/5		

s.c. = subcutaneous injection; i.p. = intraperitoneal injection.

Tumour growth enhancers

Tumour growth enhancers were employed in order to improve the take rates and because their use might help to identify relevant host factors interfering with the tumorigenicity of HD cell lines. Cotransplantation of the fibrosarcoma derived line HT1080 is believed to induce local angiogenesis factor production [28]. HT 1080 human fibrosarcoma cells were sublethally irradiated with 60 gy for 30 min. and coinjected with untreated HD-cells.

The intraperitoneal injection of pristane causes peritoneal inflammatory granulomas leading to the production of IL-6 and probably other growth factors [29]. The animals were injected 6 days before tumour cell inoculation with 500 μl of pristane (Sigma).

Host NK-cell activity can be specifically suppressed by α-asialo-antibody treatment [30]. α-Asialo-antibody solution (100 μl) (Wako, Osaka, Japan) was injected intraperitoneally 3 days before and every 5 days until the day 17 post tumour cell injection.

Intravenous inoculation of HD-cells

In 400 μl RPMI 1640 (Gibco) 1×10^7 cells of the Hodgkin's disease derived cell line 540 and the same number of cells of the subline 540 cy were injected into the tail vein.

Transplantation of primary Hodgkin material into SCID mice

Material from diagnostic biopsies in 13 Hodgkin patients has been transplanted into the mice. In 10 instances small cubes of lymph nodes and spleen tissue (2 mm^3) were transplanted under the kidney capsule and in 5 cases a cell suspension prepared from a lymph node was injected into the liver.

Tumours grown after xenotransplantation were retransplanted into different mice employing the same technique as in the first passage and also cultured *in vitro* after mincing in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum.

RESULTS

Subcutaneous and intraperitoneal growth of Hodgkin's disease derived cell lines in BNX- and SCID-mice

Transplantation of tumour material into SCID and BNX mice showed a difference in frequency and latency of detectable tumour growth (Table 2). The HD cell line DEV was the only line growing in BNX mice. After subcutaneous inoculation, 4/5 tested animals showed progressive but locally restricted tumour growth. In contrast, all cell lines formed tumours in SCID mice. The tumorigenicity of the individual cell lines varied between 1/5–3/5 injections. The subcutaneous route, with an overall take rate of 14/30 was more efficient than intraperitoneal injection (overall take rate 6/30). The latency period of tumour growth ranged from 7 to 20 days. The mutant subline L540 cy led to subcutaneous tumour growth in 100% of the examined BNX and SCID mice. Intraperitoneal growth of this subline was only detectable in one SCID mouse.

Tumorigenicity of HD cell lines in combination with tumour growth enhancers

Injections of L540 cells which did not grow in untreated BNX mice led to subcutaneous tumours in 4/5 cases after pristane or α-asialo-antibody treatment and in 5/5 cases after cotransplantation of the cell line HT1080. Intraperitoneal tumours developed after pristane or α-asialo-antibody treatment in 2/5 cases and in 4 out of 5 animals when HT1080 was cotransplanted. In BNX mice pristane injection supported intraperitoneal growth of L428 and L591 cells (Table 2). L591 became subcutaneously transplantable in the presence of irradiated HT1080 fibrosarcoma cells. Simultaneous injection of fibrosarcoma cells did not lead to a detectable change in frequency or latency of successful tumour growth in SCID mice. Pristane preconditioning of SCID mice and NK-cell inactivation by α-asialo-antibodies only improved subcutaneous and intraperitoneal growth of the cell line L591.

No HD derived cell line showed any alteration in morphology, immunocytology or karyotype when recultivated after *in vivo* passaging.

Disseminated growth of the Hodgkin's disease derived cell line 540 *and its subline* 540 *cy in SCID mice*

The distribution of the cell lines after subcutaneous or intraperitoneal injection into immunodeficient mice did not correspond with the pattern of tumour dissemination in patients suffering from Hodgkin's disease. In order to establish an animal model that better represents Hodgkin's disease in man, SCID mice were engrafted intravenously. The animals developed tumours with a latency period of 5–7 weeks. The subline 540 cy

Table 3. Macroscopic growth of the intravenously transplanted HD-cell lines L540 and L540 cy in SCID mice

Cell lines	L540	L540 cy
Number of mice injected	10	21
Number of mice with positive tumour growth	4	13
Take (%)	40	62
Sites of visible tumour growth		
Lymph nodes (total)	4/4	13/13
Cervical lymph nodes	3/4	12/13
Liver	0/4	9/13

showed a higher degree of tumorigenicity with 13/21 tumour takes (62%) after intravenous injection compared with the original cell line 540 (4/10 cases, 40%). Tumours reproducibly developed in the lymph nodes with a significant preference for the cervical nodes (Table 3). Tumours were found in the liver of 9/13 animals with visible tumour growth after injection of the subline 540 cy. No macroscopic tumour growth was seen in the spleen. Tumour cells were seen microscopically in the bone marrow of 4 mice xenotransplanted with L540 cy. Thus, intravenous injection of Hodgkin's disease derived cells led to tumour growth resembling the distribution of Hodgkin tumours in patients.

Transplantation of primary Hodgkin's material into SCID mice

In the first tumour passage 52 mice were inoculated. Tumour growth could be observed in 9 animals with macroscopic dissemination into lymph nodes, liver, spleen and thymus. The material transplanted into these tumour bearing animals was derived from 4 different patients. In 2 cases the tumours grown in SCID mice were of human origin, whereas in the other two cases lymphomas of mouse origin were induced. The tumours were retransplantable in further mice. *In vitro* culture of the tumours led to permanent growing cell lines. These cell lines showed a B-cell phenotype and expressed CD30-antigen, Epstein–Barr virus (EBV)-antigens, like LMP and EBNA2. Therefore in many respects they resemble lymphoblastoid cell lines (LCL's). However, in contrast to recently published reports by Cannon *et al.* [31], Rowe *et al.* [32] and Purtilo *et al.* [23], who transplanted EBV transformed LCL's derived from healthy individuals into SCID mice, the cell lines showed numerical and structural chromosomal abnormalities in a very high frequency. The cytogenetic aberrations were very heterogeneous as has been described in primary material from Hodgkin patients [33, 34].

Preliminary histological examination demonstrates three kinds of lesions: (a) Lymphoproliferative disorders (LPD) as described previously [23, 31, 32] after inoculation of normal peripheral blood lymphocytes into SCID mice; (b) anaplastic large cell lymphomas with very bizarre cells; (c) Hodgkin like lesions with large CD30 positive Reed–Sternberg cells with a B cell phenotype surrounded by reactive cells which were mouse cells in the lymphocyte-depleted SCID mice. The Reed–Sternberg cells were also EBV positive, whereas the Hodgkin- and Reed–Sternberg-cells in the primary biopsy tissue of one patient were EBV negative. A few EBV positive lymphocytes could be found surrounding the Reed–Sternberg cells in the primary tumour specimen. This observation raises the question, whether precursor cells of Hodgkin and Reed–Sternberg cells exist among the bystander cells, which under certain conditions develop the appearance of Reed–Sternberg cells.

DISCUSSION

Our experiments show that HD cell lines which could not be propagated in nude mice and produce only a few tumours in BNX mice, grow in SCID mice. Intravenous inoculation of the cell line 540 and its subline 540 cy resulted in tumour growth which mimics the natural pattern of spread of Hodgkin's disease in man. Similar results could be achieved by intravenous injection of human acute lymphoblastic leukaemia cells [20]. Also, cells of the Daudi–Burkitt lymphoma line heterotransplanted intravenously into SCID mice showed a pattern of tumour growth reminiscent of that observed in patients [35] with Burkitt's lymphomas. Tumour growth has also been observed following injection of primary Hodgkin tumour material into SCID mice.

Thus the SCID mouse model provides a useful experimental *in vivo* system for studying host–tumour cell interactions in Hodgkin's disease and for testing new therapeutic approaches such as specific immunotherapy.

1. Schaadt M, Fonatsch C, Kirchner HH, Diehl V. Establishment of a malignant, Epstein–Barr-virus (EBV)-negative cell-line from the pleural effusion of a patient with Hodgkin's disease. *Blut* 1979, **38**, 185–190.
2. Diehl V, Kirchner HH, Schaadt M, Fonatsch C, Stein H, Gerdes J, Boie C. Hodgkin's disease. Establishment and Characterization of four *in vitro* cell lines. *J Cancer Res Clin Oncol* 1981, **101**, 111–124.
3. Diehl V, Kirchner HH, Burrichter H, *et al.* Characteristics of Hodgkin's disease derived cell lines. *Cancer Treat Rep* 1982, **66**, 615–632.
4. Jones DB, Scott CS, Wright DH, *et al.* Phenotypic analysis of an established cell line derived from a patient with Hodgkin's disease (HD). *Hematol Oncol* 1985, **3**, 133–145.
5. Poppema S, De Jong B, Atmosoerodjo J, Idenburg V, Visser L, De Ley L. Morphologic, immunologic, enzyme histochemical and chromosomal analysis of a cell line derived from Hodgkin's disease. Evidence for a B-cell origin of Sternberg–Reed cells. *Cancer* 1985, **55**, 683–690.
6. Drexler HG, Gaedicke G, Lok MS, Diehl V, Minowada J. Hodgkin's disease derived cell lines HDLM-2 and L428: Comparison of morphology, immunological and isoenzyme profiles. *Leuk Res* 1986, **10**, 487–500.
7. Kamesaki H, Fukuhara S, Tatsumi E, *et al.* Cytochemical, immunologic, chromosomal, and molecular genetic analysis of a novel cell line derived from Hodgkin's disease. *Blood* 1986, **68**, 285–292.
8. Jones DB, Furley AJW, Gerdes J, Greaves MF, Stein H, Wright DH. Phenotypic and genotypic analysis of two cell lines derived from Hodgkin's disease tissue biopsies. *Recent Res Cancer Res* 1989, **117**, 62–66.
9. Poppema S, Visser L, de Jong B, Brinker M, Atmoserodjo J, Timens W. The typical Reed–Sternberg phenotype and Ig gene rearrangement of Hodgkin's disease derived cell line ZO indicating a B-cell origin. *Recent Res Cancer Res* 1989, **117**, 67–74.
10. Naumovski L, Utz PJ, Bergstrom SK, Morgan R, *et al.* SUP-HD1: A new Hodgkin's disease-derived cell line with lymphoid features produces interferon-α. *Blood* 1989, **74**, 2733–2742.
11. Fidler IJ. Rationale and methods for the use of nude mice to study the biology and therapy of human cancer metastasis. *Cancer and Metastasis Reviews* **3**, 29–39.
12. Epstein AL, Herman MM, Kim H, Dortman RF, Kaplan HS. Biology of the human malignant lymphomas. Intracranial heterotransplantation in the nude athymic mouse. *Cancer* 1976, **37**, 2158–2176.
13. Igarashi T, Oka K, Miyamoto T. Human non-Hodgkin's malignant lymphomas serially transplanted in nude mice conditioned with whole-body irradiation. *Br J Cancer* 1989, **59**, 356–360.
14. Les White AT, Norris MD, Tobias V, Sosula L, Marshall GM, Stewart BW. Heterotransplantation of human lymphoid neoplasms using a nude mouse intraocular xenograft model. *Cancer Res* 1990, **50**, 3078–3086.
15. Schaadt M, Kirchner H, Fonatsch CH, Diehl V. Intracranial

heterotransplantation of human hematopoetic cells in nude mice. *Int J Cancer* 1979, **23**, 751–761.
16. Engert A, Burrows F, Jung W, *et al.* Evaluation of Ricin A chain containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. *Cancer Res* 1990, **50**, 84–88.
17. Phillips RA, Jewett MAS, Gallie BL. Growth of human tumors in immune-deficient SCID mice and nude mice. *Curr Topics Microbiol Immunol* 1989, **152**, 259–263.
18. Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. *Science* 1988, **242**, 1706–1709.
19. Schuler W, Bosma MJ. Nature of the SCID defect: a defective VDJ recombinase system. In: Bosma MJ, Phillips RA, Schuler W, eds. *The SCID Mouse Characterisation and Potential Uses*. Heidelberg, Springer, 1989, 55–62.
20. Kamel-Reid S, Letarte M, Sirard C, *et al.* A model of acute lymphoblastic leukemia in immune-deficient SCID mice. *Science* 1989, **246**, 1597–1600.
21. Charly MR, Tharp M, Locker J, *et al.* Establishment of a human cutaneous T-cell lymphoma in C.B-17 SCID mice. *J Invest Dermatol* 1990, **94**, 381–384.
22. Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* 1988, **335**, 256–259.
23. Purtilo DT, Falk K, Pirruccello SJ, *et al.* SCID mouse model of Epstein–Barr virus induced lymphomagenesis of immunodeficient humans. *Int J Cancer* 1991, **47**, 510–517.
24. McCune JM, Namikawa R, Kaneshima H, Schultz LD, Lieberman M, Weissman IL. The SCID-hu-mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 1988, **241**, 1632–1639.
25. Engert A. Experimentelle zytostatische behandlung menschlicher tumorzellen in der nude maus: bietet das intrakranielle Testsystem eine Alternative? Dissertation 1985, Medizinische Hochschule Hannover.
26. von Kalle C, Wolf J, Becker A, *et al.* Growth of Hodgkin cell lines in severely combined immunodeficient mice. (Submitted)
27. Schwonzen M, Kuehn N, Vetten B, Diehl V, Pfreundschuh M. Phenotyping of acute myelomonocytic (AMMOL) and monocytic (AMOL) leukemia: Association of T cell related antigens and skin infiltration in AMOL. *Leukemia Res* 1989, **10**, 893–898.
28. Ziegler HW, Frizzera G, Bach F. Successful transplantation of a human leukemia cell line into nude mice: conditions optimizing graft acceptance. *J Natl Cancer Inst* 1982, **68**, 15–17.
29. Nordan RP, Potter M. A macrophage-derived factor required by plasmocytomas for survival and proliferation *in vitro*. *Science* 1986, **233**, 566–569.
30. Yoshioka T, Sato S, Fujiwara H, Hamaoka T. The role of anti-asialo GM1 antibody-sensitive cells in the implementation of tumor-specific T cell-mediated immunity *in vivo*. *Japan J Cancer Res* 1986, **77**, 825–832.
31. Cannon MJ, Pisa P, Fox RI, Cooper NR. Epstein–Barr virus induces aggressive lyphoproliferative disorders of human B cell origin in SCID/hu chimeric mice. *J Clin Invest* 1990, **85**, 1333–1337.
32. Rowe M, Young LS, Crocker J, Stokes H, Henderson S, Rickinson AB. Epstein–Barr virus (EBV)-associated lymphoproliferative disease in the SCID mouse model: Implications for the pathogenesis of EBV-positive lymphomas in man. *J Exp Med* 1991, **173**, 147–158.
33. Tilly H, Bastard C, Delastre T, *et al.* Cytogenetic studies in untreated Hodgkin's disease. *Blood* 1991, **77**, 1298–1304.
34. Schouten HC, Sanger WG, Duggan M, Weisenburger DD, MacLennan KA, Armitage JO. Chromosomal abnormalities in Hodgkin's disease. *Blood* 1989, **73**, 2149–2154.
35. Ghetie MA, Richardson J, Tucker T, Jones D, Uhr JW, Vitetta ES. Disseminated or localized growth of a human B-cell tumor (Daudi) in SCID mice. *Int J Cancer* 1990, **45**, 481–485.

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ChlVPP: Reducing Toxicity in the Treatment of Hodgkin's Disease

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COMBINATION CHEMOTHERAPY for Hodgkin's disease was first introduced at the National Cancer Institute in 1964. This consisted of mustine, vincristine, procarbazine and prednisolone (MOPP). Approximately 80% of patients achieved complete remission with a 5-year relapse-free survival of 68% [1, 2, 3]. Because of these impressive results, MOPP has always been the gold standard on which other treatment regimens for Hodgkin's disease have been based. However, the combination is not without toxicity. The predominant acute toxicity includes nausea, vomiting, superficial thrombophlebitis, peripheral neuropathy, bone marrow suppression and alopecia.

Over the last 20 years various changes have been made to the MOPP regimen in order to reduce the toxicity but without detracting from the clinical efficacy. ChlVPP (Table 1) was first introduced at the Royal Marsden Hospital in 1976 with the aim of avoiding the gastrointestinal toxicity, thrombophlebitis, alopecia and myelosuppression by substituting chlorambucil for mustine, and the peripheral neuropathy by substituting vinblastine for vincristine. Alternative variations along the same line include MVPP (substituting vinblastine for vincristine) [4, 5], LOPP (substituting mustine for chlorambucil) [6], and BCVPP (omitting mustine, substituting vinblastine for vincristine and incorporating carmustine and cyclophosphamide) [7], to name but a few. Early results using ChlVPP suggested

Table 1. ChlVPP chemotherapy

Chlorambucil	6 mg/m^2 (max. 10 mg)	Orally days 1–14
Procarbazine	100 mg/m^2 (max. 150 mg)	Orally days 1–14
Prednisolone	40 mg/m^2	Orally days 1–14
Vinblastine	6 mg/m^2	IV days 1 and 8

Cycle repeated every 4 weeks

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