First Hodgkin Cell Line L428 and the CD30 Antigen
Their Role for Diagnostic and Treatment of CD30-positive Neoplasms

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WHY WAS IT ESSENTIAL TO ESTABLISH A HODGKIN CELL LINE?
When the histology of Hodgkin disease (HD) was investigated (1898 first by Sternberg1 and 4 years later by Reed2), an unusual cellular composition for a fatal disorder was observed (Fig. 1). Most of the cells in the affected lymph nodes proved to be nonatypical bystander cells (ie, lymphocytes, plasma cells, histiocytes, neutrophilic and/or eosinophilic granulocytes, and others). The atypical large mononuclear and multinuclear blastoid cells, called Hodgkin and Reed-Sternberg (HRS) cells, were found in the minority, ranging from 0.1% to 1% of all cells present in the specimen.3 Because of this, it was thought that HD is more likely an infectious disease or an inflammatory

KEYWORDS

- Hodgkin lymphoma
- Establishment of the cell line L428
- Detection of the CD30 Antigen

KEY POINTS

- Hodgkin’s disease (HD) is a fatal disorder with the unique histologic features of few dysplastic Hodgkin- and Reed-Sternberg (HRS) cells surrounded by an abundance of nonatypical bystander cells in primary biopsies.
- By using the first Hodgkin cell line L428 the cytokine receptor CD30 was discovered.
- CD30 proved to be an excellent target for the diagnoses of CD30+ malignancies and for monoclonal antibody therapy in patients with these malignancies because of its highly restricted expression in healthy individuals.
- Recently, a new anti-CD30-toxin-drug-conjugate consisting of an anti-CD30 monoclonal antibody bound to the nonimmunogenic toxin auristatin E with a newly designed linker was generated.

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process than a true neoplasm. The rarity of the HRS cells and the abundance of surrounding bystander cells made the investigation of HRS cells with special techniques impossible.

THE ESTABLISHMENT OF THE L428 CELL LINE

To obtain pure HRS cells in large quantities, many laboratories tried to establish long-term in vitro cultures of primary HRS cells when in the 1960s and 1970s suitable culture conditions like RPMI media together with fetal calf serum became available. All attempts by Zech and colleagues,\(^4\) Kaplan and Gartner,\(^5\) and many others were unsuccessful. The same was true for the 427 culture attempts by Volker Diehl and his group between 1969 and 1978 at the Radiumhemmet/Karolinska Sjukhuset in Stockholm/Sweden and later in Hannover/Germany. Most of the culture attempts resulted in Epstein-Barr virus-transformed lymphoblastoid cell cultures. After his relocation to Hannover, Diehl continued with the attempts to establish in vitro cultures of Hodgkin-Reed-Sternberg cells and succeeded with his team in 1979 in growing for the first time a permanently growing Epstein-Barr virus-negative cell line from a pleural effusion of a young female patient with nodular sclerosing HD (Table 1).\(^{6,7}\)

Features of the L428 Hodgkin Cell Line

This cell line was designated L428, because it was the 428th culture attempt. The in vitro proliferating cells proved to be aneuploid, carrying several marker chromosomes (1p+, 2p+, 6q+, 7q+, 9p+, 11q−, 13p+, 21q−). The total number of chromosomes per cell amounted to 48–50.\(^7\) These findings demonstrated that the L428 cell-line cells were monoclonal and derived from a very atypical cell population. Immunophenotypical studies revealed an absence of B-cell-, T-cell-, and macrophage markers (Fig. 2A).\(^7\) Considering these findings, it was tempting to assume that the
L428 cells were direct derivatives from HRS cells. In addition, the disease report of the donor patient (see Table 1) of the L428 cell line supported this conclusion. Despite the mentioned findings and especially because of the disappointing publications by Kaplan and Gartner and Long and colleagues, there was general hesitation in the scientific community to accept the L428 cell line as a true Hodgkin cell line.

In this situation Diehl approached Stein with the request to establish arguments that could help to show that the L428 cells are real derivatives from in vivo HRS cells.

What Stein did first was to incubate the L428 cells with his own T cells. This experiment showed that the L428 cells bind T cells in rosette formation like primary HRS cells (Stein H, unpublished observation, 1980) and was an important further criterion for the assumption that the L428 cells represent true HRS cells.

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Table 1
Clinical data of patient E.M. from whom L-428 cells were established

<table>
<thead>
<tr>
<th>Year</th>
<th>Histology Stage</th>
<th>Therapy</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/1972</td>
<td>Nod. sclerosis II, B suprACL, LN, mediast. LN</td>
<td>Ext. RT</td>
<td>CR</td>
</tr>
<tr>
<td>2/1974</td>
<td>Relapse, NS IVE, B, chest wall infiltration, pleural effusion</td>
<td>Chemo-radiotherapy: COPP-ABVD+ RT</td>
<td>PR → Progressive disease</td>
</tr>
<tr>
<td>11/1974</td>
<td>Progression</td>
<td>Palliation Pleural tappings 2–4 wk before death</td>
<td>Death</td>
</tr>
</tbody>
</table>

*a Time points of pleural tappings for in vitro cell cultures.

Fig. 2. Features of the L428 Hodgkin cell line. (From Schaadt M, Diehl V, Stein H, et al. Two neoplastic cell lines with unique features derived from Hodgkin’s disease. Int J Cancer 1980;26(6):723–31; with permission.)
DISCOVERY OF THE CD30 ANTIGEN AND ITS APPLICATION IN RESEARCH, DIAGNOSTIC, AND THERAPY

Detection of HRS-cell-related Antigens by Rabbit Polyclonal Antisera

To detect HRS-cell-specific antigens, Stein’s team immunized rabbits with the L428 cell-line cells. Two rabbit antisera selectively immunostained HRS cells following absorption with neutrophils and Daudi-cell-line cells. One antiserum stained the nuclei and the other antiserum stained the cytoplasm of HRS cells (Fig. 3).^{10,11}

Detection of the Proliferation-Associated Antigen Ki-67 and the HRS-cell-Related Antigen Ki-1

Ki-1 by the monoclonal antibody approach

To generate a permanent source of antibodies that react selectively with HRS cells, the L428 cells were subjected to the monoclonal antibody (moab) approach. More than 3000 hybridoma supernatants were screened by immunostaining frozen sections of a Hodgkin case, a tonsil, and L428 cells. Two monoclonal antibodies reactive with HRS cells could be identified. One moab called Ki-67 did not only react with the nuclei of HRS cells but also reacted with the nuclei of germinal center cells.^{12} Further studies confirmed that Ki-67 is a proliferation-associated antigen that is expressed throughout the whole cell cycle but not in resting cells.^{13} The other moab, called Ki-1, confirmed the existence of an antigen that is highly restricted to HRS cells and is present on the surface membrane and the cytoplasm of HRS cells (Fig. 4A).^{14,15} In normal lymphoid tissues the Ki-1 antigen was encountered only on a few mononuclear blastoid cells usually located in the perifollicular area of secondary follicles (Fig. 4B).

CD30 as a Diagnostic Target: Identification of a New Lymphoma Entity and Its Detection in Formol-fixed and Paraffin-embedded Tissue Sections

With the aid of the Ki-1 antibody, Stein and colleagues^{16,17} detected a new lymphoma disease, which they designated anaplastic large cell lymphoma (ALCL). Because the Ki-1 antibody did not work on formol-fixed and paraffin-embedded sections, a broad diagnostic application of this antibody was not possible. Therefore, the Stein team generated new monoclonal antibodies to the Ki-1 antigen. Among the 10 new

![Fig. 3. 1980/01: First detection of an HRS-cell restricted cytoplasmic/membrane antigen by immunostaining of a frozen section from a classical Hodgkin lymphoma with a rabbit polyclonal antiserum raised against the L428 cell line and absorbed with neutrophils and Daudi cell line cells.](image-url)
monoclonal antibodies directed to the Ki-1 antigen, there was one, designated BerH2, which selectively and reliably immunostained HRS cells in routinely formol-fixed and in paraffin-embedded tissue sections (see Fig. 4A). Thanks to the availability of Ber-H2 and further anti-Ki-1 antibodies, the monoclonal anti-Ki-1 antibodies were clustered at the Leukocyte Typing workshop at Oxford in 1986 and received the cluster designation CD30. With the availability of the monoclonal anti-CD30 Ber-H2, CD30 became the most important marker for CD30-associated diseases with special reference to HD and ALCL. The broad screening of normal tissues confirmed the highly restricted expression of CD30 in healthy individuals. Labeling of biopsies from patients with classical Hodgkin lymphoma (CHL) for CD30 revealed that HRS cells exhibit a greater morphologic variability as previously known and are often much more frequent, ranging up to 30% of the cells in the affected tissue (Fig. 5). All nuclei in the multinucleated RS cells are Ki-67 positive, indicating that all the nuclei present in these multinucleated cells take part in the proliferation process (insert in Fig. 5).

**Biosynthesis and Structure of CD30 and Shedding as a Soluble Form**

The CD30 molecule is synthesized as a 90-kDa precursor protein, which is expressed as a 120-kDa at the surface membrane. It can be shed in a soluble form. Molecular cloning of the CD30 gene revealed in 1992 that the CD30 protein is a cytokine receptor of the tumor necrosis factor receptor family. Structurally, CD30 proved to be a type 1 transmembrane protein that contains 6 cysteine-rich repeat motifs in its extracellular domain. The cytoplasmic tail contains several tumor necrosis factor receptor-associated factor–binding sequences that can mediate activation of the nuclear factor kB (Fig. 6). The CD30 gene has been assigned to chromosome 1p36.
The cellular origin and nature of HRS cells were an enigma since their first description. Moab to CD30 enabled the isolation of single HRS cells from frozen sections (Fig. 7) and their investigation by a single-cell PCR assay (see Fig. 7). In 1994 and the following years, this approach led to the finding that HRS cells represent disabled monoclonal and thus neoplastic B cells since they consistently harbour monoclonal immunoglobulin (IG) rearrangements. The HRS cells are disabled because of a defect.

Fig. 5. CD30 immunostaining of a classical Hodkin lymphoma reveals that more than 30% of the cells present in the affected tissue can be HRS cells and demonstrates that HRS cells can be much more frequent than assumed before the availability of anti-CD30 antibodies. The double labeling for CD30 in red and Ki-67 in black (insert) shows that HRS cells are Ki-67-positive and thus in proliferation and not silent.

Derivation and Molecular Characteristics of HRS Cells

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Fig. 6. Molecular structure and function of CD30.
in IG transcription. This is not due to crippled rearranged IG genes since 75% of the rearranged IG genes are functional but because of a lack of the transcription factors Oct2a and BOB.1.26 These findings induced the editorial team of the World Health Organization lymphoma classification to change the term Hodgkin’s disease into classical Hodgkin lymphoma.3

Hodgkin Lymphoma Includes 2 Different Entities

Investigations using CD30 and CD20 as cell markers confirmed that HD is composed of 2 different lymphoma disease entities: one is the CD20+ CD30– nodular lymphocyte-predominant HL and the other the CD30+ CD20– CHL.

CD30 as a Therapeutic Target

Studies with naked anti-CD30 antibodies and the first anti-CD30-drug conjugate

CD30 is an excellent target for moab therapy in patients with CD30+ malignancies because of its highly restricted expression in healthy individuals. Therefore, many times it was attempted to target CD30+ lymphoma cells with naked anti-CD30 antibodies.27–29 Significant responses were not observed. Therefore for the first time in 1992 the anti-CD30 antibody was conjugated to a toxin, which was saporin, a potent single-chain ribosome-inactivating protein from Saponaria officinalis (Fig. 8).30 With this conjugate a rapid but only transient tumor reduction was achieved in 3 of 4 patients with advanced CHL (Fig. 9). The administration of the conjugate could not be repeated because the patients developed high antibody titers to both parts of the conjugate. Notwithstanding this problem, this result of an antibody-mediated tumor
The toxin saporin is linked to the Ber-H2 anti-CD30 antibody

Fig. 8. Structure of the first monoclonal anti-CD30 antibody-toxin conjugate.

reduction of an advanced Hodgkin tumor represented, to the best of the authors’ knowledge, the first proof of principle of targeted therapy in CHL. Another important finding of this study was that the anti-CD30–saporin-conjugate injected into the patient intravenously found its way to the HRS cells despite the presence of a very strong fibrotic tissue of nodular sclerosing HL after the preceding radiotherapy and chemotherapy, which was demonstrated by the fact that the HRS cells could be immunostained with an antimurine IgG antibody on a biopsy taken 24 hours following intravenous injection of the Ber-H2-saporin conjugate.

**Breakthrough in CD30-targeted antibody therapy**

It took more than 20 years until the potent nonimmunogenic toxin auristatin E was developed from the Indian Ocean sera hare, *Dolabela auricularia*.

Auristatin E exerts its toxin activity through inhibiting tubulin polymerization. A further important step was the development of a special linker (valin-citrulline dipeptide) by which the auristatin E is conjugated to the anti-CD30 antibody (Fig. 10). The binding by this linker provides good stability of the conjugate in the plasma. As long as the auristatin E is linked

Fig. 9. Treatment result achieved with the anti-CD30-saporin conjugate in a patient with classical Hodgkin lymphoma.

Relapse Problems:
-the injection of the saporin-Ber-H2 anti-CD30 conjugate could not be repeated because of the production of antibodies to saporin und Ber-H2
to the anti-CD30 antibody, it is not toxic. When the anti-CD30 antibody-auristatin con-
jugate has reached the target cell and is internalized, auristatin E is released from the
antibody and becomes toxic and kills the cell. There is evidence that not only is the
targeted CD30-positive cell killed, but also CD30-negative cells in the neighborhood.33
The presumed mechanism is that the internally released auristatin E can diffuse out of
the killed targeted cell and then kill CD30-negative cells in close proximity to those
that have internalized and processed the conjugate.

Taken together, CD30 proved to be the most specific marker for HRS cells of CHL
and, furthermore, of the pathognomonic tumor cells of the ALCL. The identification of
the CD30 antigen and its sequence analysis contributed significantly to the clarifica-
tion of the origin of the HRS cells. More than 25 years later, CD30 could be exploited
as a therapeutic target by development of an anti-CD30-antibody-drug-conjugate
(Senter and colleagues elsewhere in this issue) that has a high potential and specific
antilymphoma cell activity without having the limitations of the first-generation
conjugates.

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