**Haematology and Blood Transfusion** Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler


# Evidence for the Origin of Hodgkin and Sternberg-Reed Cells from a Newly Detected Small Cell Population

H. Stein, J. Gerdes, U. Schwab, H. Lemke, and V. Diehl

## A. Summary

To clarify the origin of Hodgkin (H) and Sternberg-Reed (SR) cells, frozen sections of lymph nodes from 25 patients with Hodgkin's disease were immunostained with a large panel of monoclonal antibodies reactive with cells of lymphoid tissue and granulopoiesis. The results showed that (a) H and SR cells are devoid of markers specific to, or characteristic of B cells, macrophages, dendritic reticulum cells, or interdigitating reticulum cells, and (b) the vast majority of H and SR cells contain granulocyte-related antigens detectable with the monoclonal antibodies TÜ9 and 3C4, but constantly lack other granulocytic cell markers (such as peroxidase and chloroacetate esterase). Monoclonal antibodies raised against a Hodgkin's disease-derived cell line included one, Ki-1, that was found to be selectively reactive with H and SR cells and a minute, but distinct, cell population in normal lymphoid tissue and bone marrow. The latter hitherto unknown cell population appears to be the normal equivalent of H and SR cells.

## B. Introduction

Hodgkin's disease is one of the most common types of malignant lymphoma, with approximately 1.5 cases per 100,000 population per year [19]. The Hodgkin (H) and Sternberg-Reed (SR) cells are the most characteristic morphologic elements of the disease. With the recognition and acceptance of H and SR cells as a distinctive neoplastic cell form, there followed a fierce dispute over their derivation. Many diverse theories have been propounded. The more recent ones have related H and SR cells to B cells [1, 4, 7, 10, 11, 20], macrophages [6, 8, 9], dendritic reticulum cells [2], or interdigitating reticulum cells ([5, 12]; Kadin 1981, personal communication).

To test these possibilities, we applied an immunohistologic technique using monoclonal antibodies that are selectively, or nearly selectively, reactive with B cells, macrophages, dendritic reticulum cells, and interdigitating reticulum cells. These studies provided a bulk of negative information, i.e., findings showing the cell types from which H and SR cells probably do not originate.

In an attempt to obtain positive results, we raised monoclonal antibodies against cells of the L428 cell line, which had been shown to share all the investigated conventional immunologic and enzyme markers with H and SR cells [3, 13]. The results of immunostainings with one of the anti-L428 cell hybridoma antibodies suggest that H and SR cells are derived from a unique, as yet unidentified cell population of the lymphoid system.

## C. Material and Methods

Fresh unfixed biopsy specimens were obtained from the University Hospitals in Kiel and various other hospitals in northwestern Germany. The tissue was frozen in liquid nitrogen within 12 h of surgical removal.

All the monoclonal antibodies and methods applied in the present study are described in detail elsewhere [14–18].

## D. Results and Discussion

The reactivity of H and SR cells of 25 cases of Hodgkin's disease with a large set of cell type-specific or characteristic monoclonal antibodies is shown in Table 1. With the exception of granulocytic cell-reactive antibodies, none of the antibodies reacted with H and SR cells of any case. This is a strong argument against a close relationship between H and SR cells and B cells, macrophages, dendritic reticulum cells, or interdigitating reticulum cells. The reactivity of H and SR cells with antibodies that recognize only cells of granulopoiesis [15] suggests that H and SR cells may be related to cells of the granulocytic series. It appears unlikely that H and SR cells are directly derived from granulopoietic cells, however, because H and SR cells are constantly devoid of peroxidase, chloroacetate esterase, and lysozyme, whereas cells of granulopoiesis constantly express these three markers.

On the whole, the results of our multiple marker analyses clearly show that the antigen and enzyme profile of H and SR cells does not correspond to that of any of the cell types mentioned above. We conclude from these findings that H and SR cells represent a unique cell type that differs from all other cell types identified in the lymphoid system.

This conclusion was substantiated by studies using the Hodgkin's disease-derived cell line L428. First, L428 cells and H and SR cells were immunostained with the same set of monoclonal antibodies. The staining patterns were identical (Table 1).

**Table 1.** Antigen and enzyme profile of the most important known cell types of the hematopoietic system, cells of the Hodgkin's disease-derived cell line L428, and in situ Hodgkin (H) and Sternberg-Reed (SR) cells of Hodgkin's disease

| | B cells | Monocytes macrophages | DRC[a] | IDC[b] | Granulopoietic cells | L428 cells | H and SR cells |
|---|---|---|---|---|---|---|---|
| SIgM[c] | + | – | + | – | – | – | – |
| SIgD[c] | + | – | – | – | – | – | – |
| To15[c] (pan-B cell) | + | – | – | – | – | – | – |
| C3RTo5[c] (C3b receptor) | + | + | + | – | +/– | – | –/(+) |
| OKM1[c] | – | + | –/+ | – | + | – | – |
| Monocyte 1[c] | – | + | + | – | – | – | – |
| Monocyte 2[c] | – | + | –/+ | – | – | – | – |
| TÜ2[c] | – | + | ? | – | –/+ | – | – |
| Lysozyme[d] | – | + | – | – | + | – | – |
| R4/23[c] (dendritic reticulum cells) | – | – | + | – | – | – | – |
| TÜ9[c,d] (granulocytic cells) | – | – | –/+ | – | + | + | +/– |
| 3C4[c,d] (granulocytic cells) | – | – | +/– | – | + | + | +/– |
| Peroxidase[e] | – | –/(+) | – | – | + | – | – |
| Chloroacetate esterase[f] | – | –/(+) | – | – | + | – | – |
| Ki-1[c] | – | – | – | – | – | + | + |
| Ki-24[c] | – | – | – | – | – | + | +/– |
| Ki-27[c] | – | – | – | – | – | + | +/– |

[a] DRC, dendritic reticulum cells
[b] IDC, interdigitating reticulum cells
[c] Demonstrated by immunoperoxidase staining of frozen sections or acetone-chloroform fixed cytocentrifuge slides
[d] Demonstrated by immunoperoxidase staining of paraffin sections or acetone-chloroform fixed cytocentrifuge slides
[e] Demonstrated by diaminobenzidine staining of frozen sections or unifixed cytocentrifuge slides
[f] Demonstrated by naphthol-AS-D-chloroacetate esterase staining of paraffin sections or methanol-formol fixed cytocentrifuge slides

+ = all cells positive; +/– = some cells positive, other negative; –/(+) = most cells negative and only few cells positive; – = all cells negative

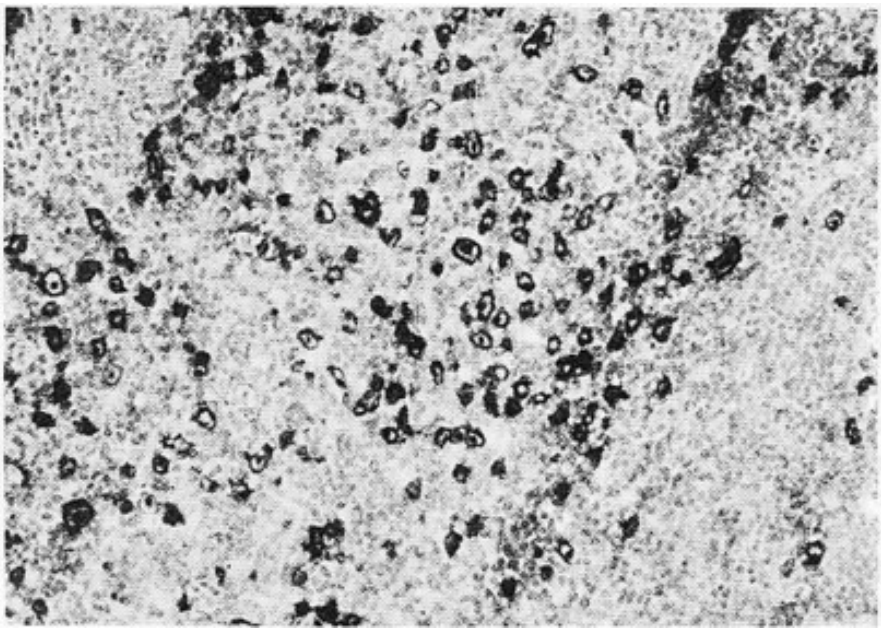

**Fig. 1.** Frozen section of Hodgkin's disease of mixed cellularity type immunostained with the monoclonal antibody Ki-1. All the Hodgkin and Sternberg-Reed cells are strongly stained, whereas all other cell types failed to react

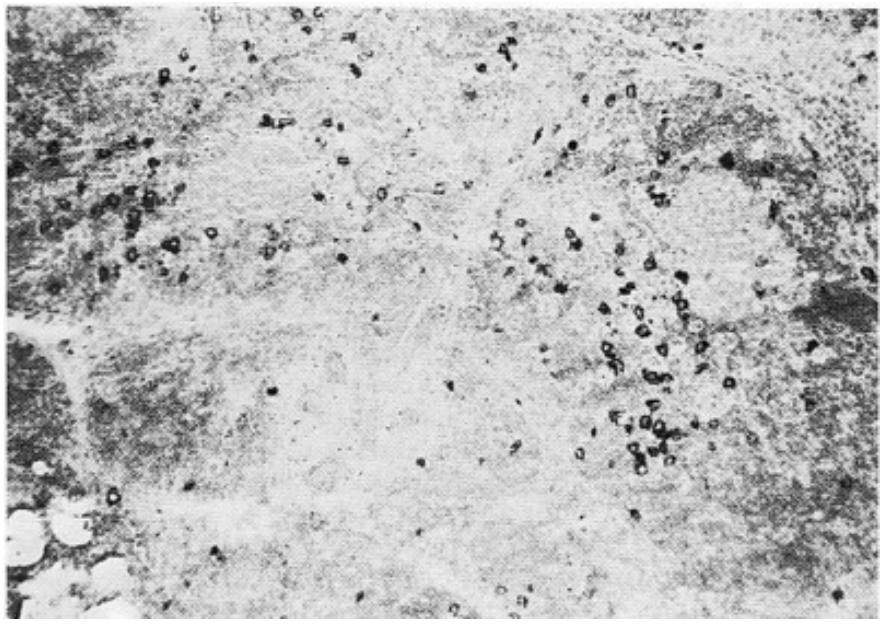

**Fig. 2.** Frozen section of Piringer's lymphadenitis immunostained with the monoclonal antibody Ki-1. There is a relatively large number of Ki-1 reactive large cells around the germinal centers and between the lymphoid follicles

In a second set of experiments, we raised monoclonal antibodies against the L428 cells and selected the antibodies that reacted with L428 cells, but not with B cells, T cells, macrophages, or B or T region specific reticulum cells. So far, we have obtained three such monoclonal antibodies: Ki-1, Ki-24, and Ki-27. All three antibodies recognize H and SR cells. Ki-24 also reacted with cells of most cases of centroblastic lymphoma, but not with cells in normal lymphoid tissue. Ki-27 recognized not only H and SR cells, but also endothelial cells and smooth muscle cells.

The Ki-1 antibody [14] proved to be the most important and interesting one, because it constantly reacted with H and SR cells (Fig. 1), but not with any of the other known cell types. The nonreactivity of the Ki-1 antibody with cells other than H and SR cells was substantiated by the negative reaction of tumor cells of more than 50 cases of non-Hodgkin's lymphoma of various types and two cases of lysozymet malignant histiocytosis. The Ki-1 antibody also did not react with cells of normal peripheral blood, skin, liver, kidney, lung, or brain, or with macrophages of different types or stages of differentiation.

Unexpected results were obtained, however, when normal and inflamed tonsils and lymph nodes were stained with the Ki-1 antibody using a highly sensitive three-layer immunoperoxidase technique.

A small population of cells located at the outer rim of and between cortical lymphoid follicles was labeled. The distribution of the Ki-1 reactive cells differed from that of all other known cell types. Ki-1+ cells were also detectable, although in small numbers, in the B-cell areas of spleen, the medulla of thymus, and bone marrow.

In cases of certain types of lymphadenitis, e.g., Piringer's lymphadenitis, the number of Ki-1+ cells was found to be remarkably increased (Fig. 2). This finding is of particular interest, because Piringer's lymphadenitis shows some similarities to Hodgkin's disease (e.g., the presence of epithelioid cell clusters and myeloid cells). By staining serial sections of lymphadenitis with increased numbers of Ki-1+ cells, we found that the nonneoplastic cell population expressing Ki-1 antigen did not react with antibodies to B cells, T cells, macrophages, dendritic reticulum cells of the B-cell region, or interdigitating reticulum cells of the T-cell region. These findings suggest that the Ki-1 antibody recognizes a new, as yet unidentified cell population in normal lymphoid tissue. It is tempting to assume that the Ki-1+ cell population is the normal equivalent of H and SR cells.

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