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Immunohistological Analysis of Hodgkin's and Sternberg-Reed Cells:

Detection of a New Antigen and Evidence for Selective IgG Uptake in the Absence of B Cell, T Cell and Histiocytic Markers*

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Summary. To help clarify the origin and nature of Hodgkin's (H) and Sternberg-Reed (SR) cells, three different sets of experiments were performed. First, it was shown that cytoplasmic γ, $\varkappa$, and λ occur not only in H and SR cells, but also in polymorphic tumor cells of epithelial, neurogenic, and lymphoid origin. Furthermore, human IgG that was injected i.v. into rats penetrated many rat liver cells, whereas injected human α_1-antitrypsin did not. Second, staining of frozen sections revealed that H and SR cells lack surface immunoglobulin and peripheral T-cell antigen. Third, an antiserum raised against the L 428 cell line (derived from Hodgkin's disease) and absorbed with human serum and normal cells did not react with any cells of tonsil tissue (lymphoid cells, histiocytes, and interdigitating reticulum cells), whereas it reacted strongly with the L 428 cell line cells and with H and SR cells of 10 different cases. In all ten cases, the antiserum stained the surface of H and SR cells; in two cases, it also stained the nucleoli and some chromatin spots in H and SR cells.
The results obtained in these experiments indicate that H and SR cells are not closely related to lymphoid cells, histiocytes, or interdigitating reticulum cells. The findings also suggest that H and SR cells express one or more antigens that have not yet been detected on or in normal cells.

Key words: Immunoperoxidase – Hodgkin's disease – Significance of polytypic cytoplasmic IgG – Histiocytic, B and T markers – Antiserum to Hodgkin's and Sternberg-Reed cells

Introduction

The many purely morphological studies of Hodgkin's (H) and Sternberg-Reed (SR) cells performed in the past provided arguments for both a histiocytic and a

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Abbreviations: H Cell = Hodgkin's cell; SR cell = Sternberg-Reed cell; C = cytoplasmic; Ig = immunoglobulin

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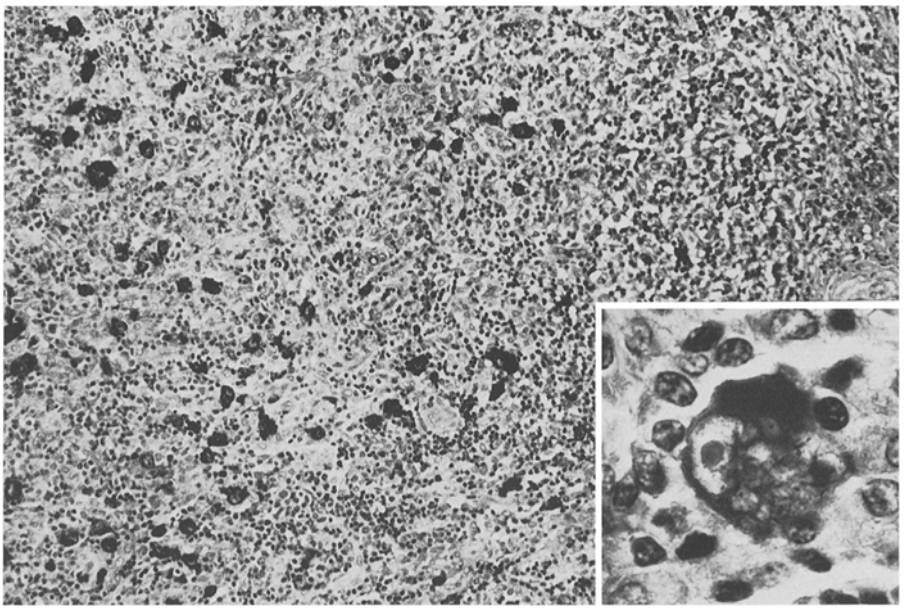


Fig. 1. Paraffin section of a sample from a patient with Hodgkin's disease stained for IgG. The Hodgkin's and Sternberg-Reed cells are positive. *Inset:* IgG-positive Sternberg-Reed cell

lymphocytic origin of these cells (Rappaport 1966; Mori and Lennert 1969; Carr 1975; Dorfman et al. 1973; Glick et al. 1976). The electron-microscopic study of H and SR cells done by Hansmann and Kaiserling (1981) may explain these contradictory results, since these authors were able to show that H and SR cells may have features of either immunoblasts or macrophages, or even interdigitating reticulum cells. Thus, purely morphologic studies proved to be of no help in the derivation of H and SR cells.

In contrast to the variations in morphology, the vast majority of H and SR cells resemble each other in their stainability for cytoplasmic (C) IgG (Fig. 1; Taylor 1974; Garvin et al. 1974).

Until recently it was assumed that when CIg was demonstrated in a cell it was also produced by that cell (Coons et al. 1955; Fitch and Wissler 1965; Seligmann et al. 1973). Accordingly, several authors regarded the CIgG staining of H and SR cells as a hint of the B-cell nature of these cells. Doubt was cast on this hypothesis when it was shown that H and SR cells contain both κ and λ simultaneously (Taylor 1974, 1976; Garvin et al. 1976; Landaas et al. 1977; Papadimitriou et al. 1978; Mason et al. 1980). Simultaneous staining of cells for κ and λ contradicts the concept of allelic exclusion in the production of Ig light chains. For this reason, many research workers have assumed that the CIgG demonstrable in H and SR cells is not produced, but instead phagocytosed by them (e.g., Kaplan and Gartner 1977). Since we were able 3 years ago to show that most H and SR cells do not contain other serum proteins than Ig in significant amounts (Papadimitriou et al. 1978), a finding that has now been reproduced by others (e.g., Mason et al. 1980), it was

concluded that IgG incorporation by H and SR cells is mediated by specific mechanisms, such as immunophagocytosis via IgG Fc receptors. Because immunophagocytosis is a property of phagocytes, the hypothesis of a histiocytic origin of H and SR cells became more and more attractive. Since a histiocytic origin of H and SR cells, however, does not harmonize with the immunohistological findings showing that lysozyme is absent in morphologically typical H and SR cells (Taylor 1976), and since previous studies on the surface markers of suspended H and SR cells led to contradictory results (Leech 1973; Kadin et al. 1974; Boecker et al. 1975; Hayhoe et al. 1978; Kadin et al. 1978; Biniaminov and Ramot 1974; Stein et al. 1978), we performed three sets of experiments. The first set was designed to clarify the question whether the presence of IgG (and κ and λ) in the cytoplasm of a cell is a marker for the histiocytic nature of that cell. In the second set of experiments the question was investigated whether H and SR cells in situ express B- and T-cell-specific markers. In the third set we raised an antiserum against the L 428 cell line cells that were established from pleural fluid cells of a patient affected with Hodgkin's disease and studied the reactivity of this antiserum against H and SR cells in situ.

Materials and Methods

Reagents

α_1-Antitrypsin was purchased from Sigma, Munich (FRG). Human IgG was prepared from pooled human serum by fractionation with ammonium sulfate precipitation followed by chromatography on DEAE cellulose equilibrated with 0.0175 *M* phosphate buffer, pH 7.0. Rabbit IgG against human IgG, IgM, IgD, κ and λ, lysozyme, α_1-antitrypsin, swine IgG against rabbit IgG and rabbit anti-mouse IgG peroxidase conjugate were obtained from Dako (Boehringer Ingelheim, Munich, FRG). Monoclonal antibodies specific for peripheral T cells were secreted by a mouse myeloma hybrid (Warnke and Levy 1980; Englemann et al. 1981). An antiserum against L 428 cells (Schaadt et al. 1979, 1980) was prepared by immunizing a rabbit with repeated i.v. injections of 1×10^8 L 428 cells and simultaneously with s.c. injections of 1×10^8 L 428 cells emulsified with complete or incomplete Freund adjuvant. The antiserum was exhaustively absorbed with glutaraldehyde-insolubilized human serum protein, thrombocytes, and tonsil cells or homogenates of tonsil tissue, and partially with homogenates of human thymi.

Immunostaining for Intracellular (Cytoplasmic) and Surface Antigens

Paraffin or frozen sections were immunostained either by the PAP technique, as described recently (Stein et al. 1980), or by the indirect immunoperoxidase method using a rabbit anti-mouse IgG peroxidase conjugate. For demonstrating human IgG and α_1-antitrypsin in rats, antisera were used that were exhaustively preabsorbed with glutaraldehyde-insolubilized rat serum proteins.

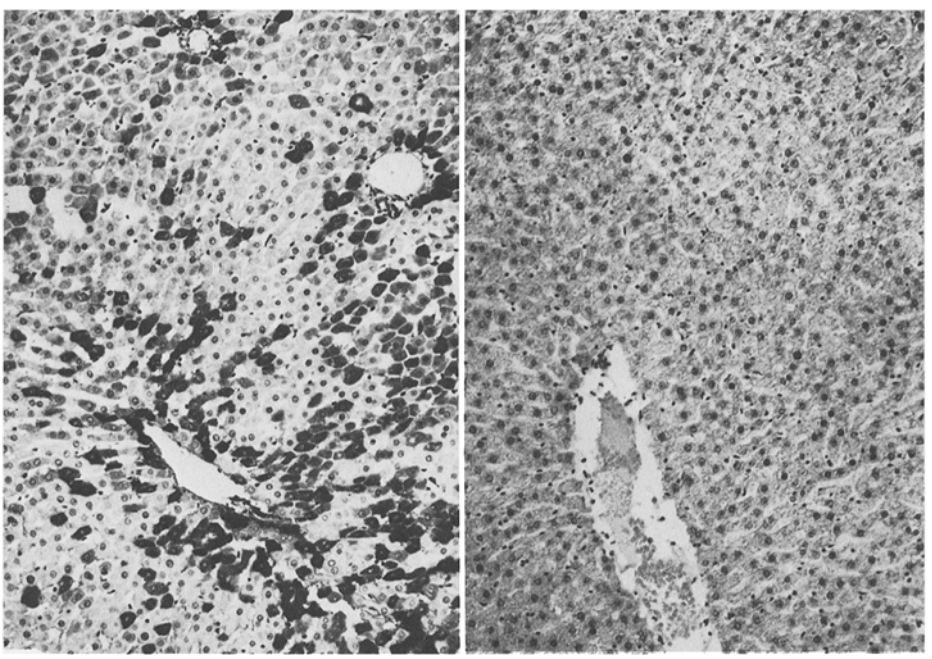
Results and Discussion

The Significance of Cytoplasmic IgG with both Light Chain Types (κ and λ)

To determine whether the simultaneous presence of γ, κ and λ is a specific feature of cells of the histiocytic cell series, we performed several immunostainings.

First we looked for CIgG in nonneoplastic histiocytes and in various neoplasms. With the exception of sinus histiocytes, nearly all histiocytes remained unstained by anti-IgG sera. Surprisingly, we found positive staining for IgG in some polymorphic astrocytomas, liver cell carcinomas (Fig. 2), and other polymorphic neoplasms.

Fig. 2. Paraffin section of a poorly differentiated liver cell, carcinoma stained for IgG. Note that many polymorphic cells and the giant cell in the upper left corner are strongly stained



a b

Fig. 3. a Paraffin section of a rat liver stained for human IgG. The rat received 200 mg human IgG i.v. 18 h before killing. Many liver cells display strongly positive staining, whereas Kupffer's cells remained unstained. **b** Paraffin section of a rat liver stained for human α_1-antitrypsin. The rat received 200 mg human α_1-antitrypsin 18 h before killing. Not a single cell in the liver was stained

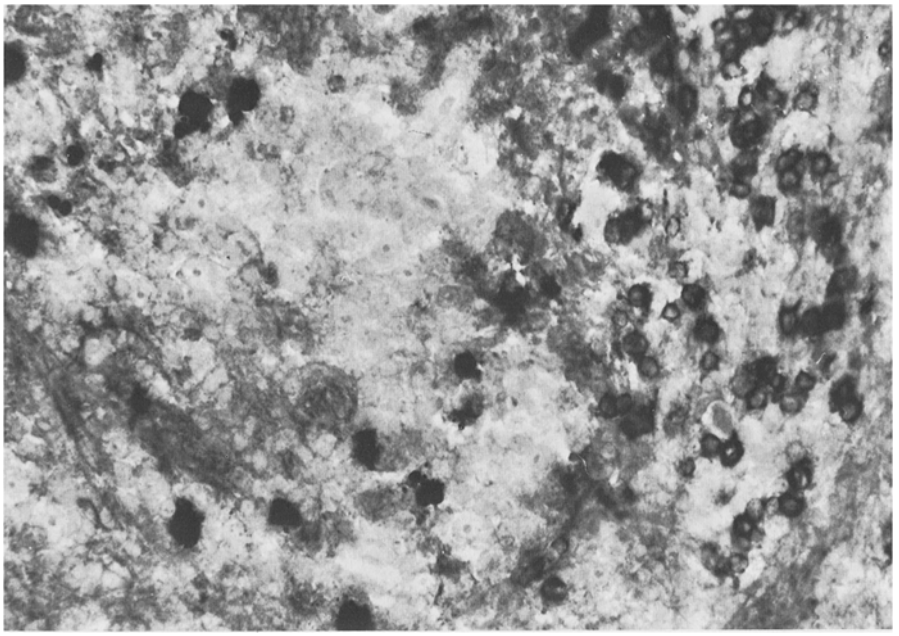


Fig. 4. Frozen section of a sample from a patient with Hodgkin's disease stained for IgM. The strongly stained B cells show a ring-shaped reaction product. H and SR cells are unstained. The dark brown diffusely stained cells are neutrophils rich in endogenous peroxidase

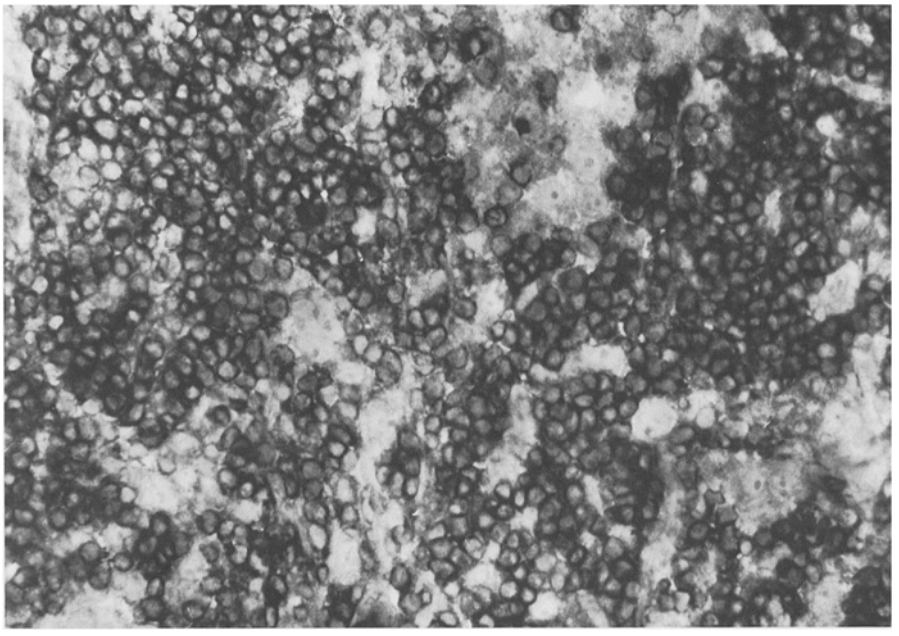
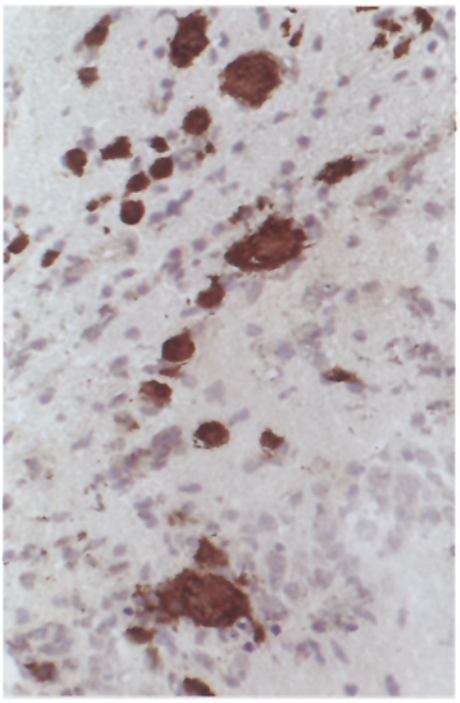


Fig. 5. Frozen section of a sample from a patient with Hodgkin's disease stained with a monoclonal antibody that is directed against peripheral T cells. Most of the lymphoid cells surrounding the H and SR cells were identified as T cells. H and SR cells did not react with the monoclonal anti-T cell antibody



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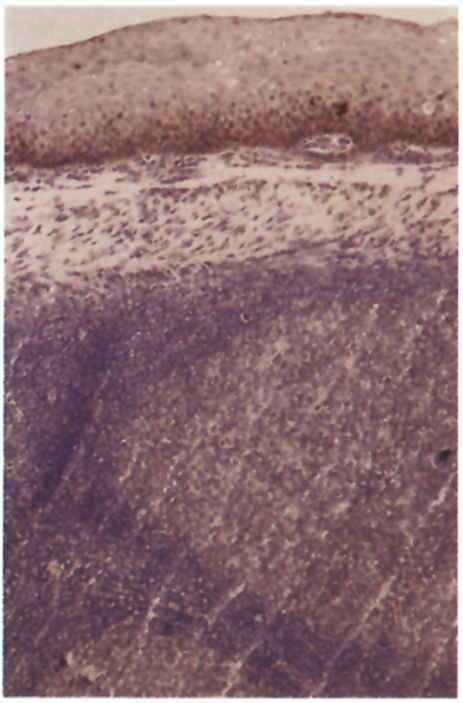

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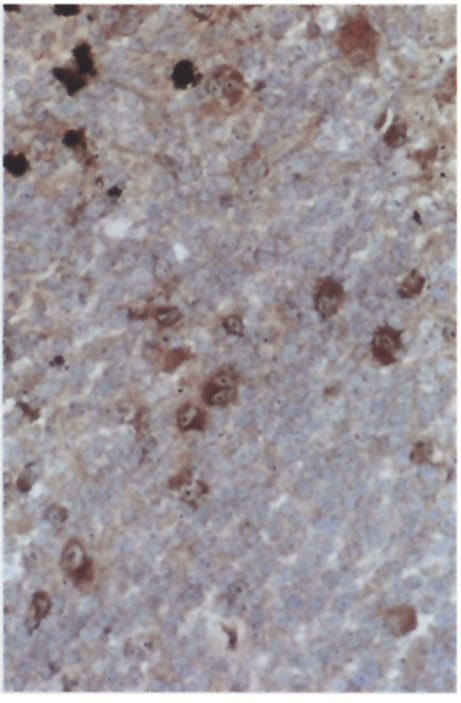

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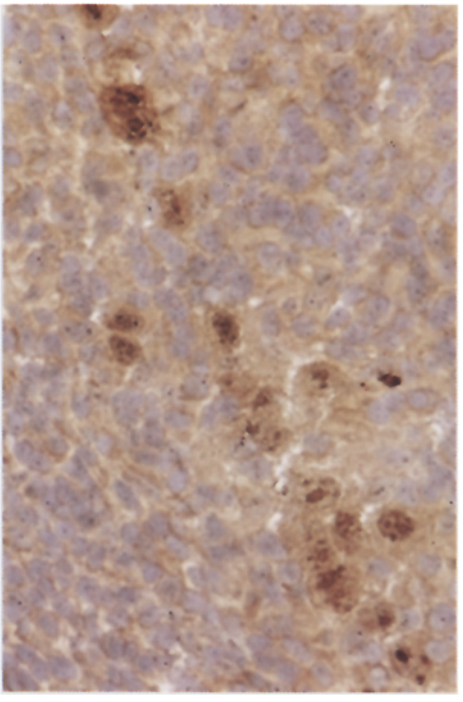

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These findings stimulated us to inject human IgG and α_1-antitrypsin into rats i.v., and to analyze the fate of these human substances 18 h later by immunostaining the rat organs. As Fig. 3 shows, the human IgG was picked up by many liver cells, whereas α_1-antitrypsin was not. It is interesting to note that Kupffer's cells did not incorporate IgG or α_1-antitrypsin either, at least within this period. We conclude from these results that the mere presence of IgG in cells cannot be used as a marker for histiocytes. In other words, the presence of IgG in H and SR cells does not confirm a histiocytic origin of H and SR cells. This is in line with the results of our immunostainings for lysozyme in Hodgkin's disease. Since in a previous study of 103 cases of Hodgkin's disease, we found one case in which H and SR cells immunostained for lysozyme (Papadimitriou et al. 1978), we reinvestigated 50 cases of Hodgkin's disease, including the one that was previously lysozyme-positive, for the presence of lysozyme using an improved PAP technique applied to paraffin sections pretreated with trypsin. Clear-cut staining for lysozyme was not obtained in any H and SR cells of the cases investigated.

Immunostaining of Hodgkin's and Sternberg-Reed Cells for Surface Ig and Peripheral T Cell Antigen

Attempts to phenotype H and SR cells in cell suspensions from biopsies have led to varying results. Some authors found SIg on H and SR cells (Leech 1973; Kadin et al. 1974, 1978; Boecker et al. 1975; Hayhoe et al. 1978). Others did not (Biniaminov and Ramot 1974; Stein et al. 1978). The same holds true for immunophagocytosis (Kadin et al. 1978; Stein et al. 1978).

We are of the opinion that these varying results are due to the difficulty in isolating H and SR cells as enriched and viable populations from biopsy material and to the difficulty in identifying these cells in vital cell preparations, which are necessary for immunofluorescent staining. To overcome these difficulties, we investigated surface antigens of H and SR cells in situ, using the immunoperoxidase method applied to frozen sections.

Figure 4 shows a frozen section of Hodgkin's disease stained for IgM. B cells are strongly positive, whereas H and SR cells are completely negative. The surface

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Fig. 6. Frozen section of a nude mouse brain tumor stained with an anti-L 428 cell line serum. The brain tumor was produced in nude mice by intracerebral inoculation of L 428 cell line cells. The intracerebrally growing L 428 cell line cells stained strongly

Fig. 7. Frozen section of a human tonsil stained with the anti-L 428 cell line serum. The entire section did not show any specific staining

Fig. 8. Frozen section of a sample from a patient with Hodgkin's disease stained with the anti-L 428 cell serum. The H and SR cells were strongly stained, whereas the other cells were not. The dark brown cells in the upper left corner are neutrophils

Fig. 9. Frozen section of a sample from a patient with Hodgkin's disease stained with a more highly diluted anti-L 428 cell serum that was absorbed only with intact cells. This antiserum selectively stained the nucleoli and some nuclear chromatin spots in H and SR cells

of H and SR cells could not be stained with antisera directed against IgD, IgA, IgG, κ, and λ either.

Figure 5 shows a frozen section immunostained with a monoclonal antibody that is directed against peripheral T cells. The staining pattern reveals that most of the lymphoid cells are T cells. H and SR cells did not react with the anti-T-cell antibodies.

Preparation of an Antiserum with Selective Reactivity Against Hodgkin's and Sternberg-Reed Cells

Since the immunostainings for conventional cytoplasmic and surface markers did not help to establish the nature of H and SR cells, we raised an antiserum against the L 428 cell line cells, whose properties were described recently (Schaadt et al. 1980). Some preliminary results obtained with this antiserum follow.

After exhaustive absorption with normal human cells, the anti-L 428 cell serum no longer stained tonsil sections (Fig. 6), but it strongly stained brain tumors produced in nude mice by intracerebral inoculation of the L 428 cell line cells (Fig. 7). It is notable that the absorbed anti-L 428 serum did not stain lymphoid cells or histiocytic cells either.

Figure 8 shows a frozen section from a lymph node affected with Hodgkin's disease stained with the anti-L 428 cell serum. Only large cells morphologically identifiable as H and SR cells were stained, whereas small cells were not stained. Thus, the staining pattern obtained with the anti-L 428 cell serum differs from that obtained with the anti-Ig sera and the anti-T cell antibodies.

In two cases of Hodgkin's disease we obtained the staining pattern seen in Fig. 9 by using a more highly diluted anti-L 428 cell serum that was absorbed only with intact cells. The nucleoli and some nuclear chromatin spots in the H and SR cells were stained, whereas the surface of the lymphoid cells and also of the H and SR cells remained unstained.

Conclusions

Our findings may be summarized as follows:

1. H and SR cells do not express markers known to be specific to B cells, T cells, histiocytes, or interdigitating reticulum cells. Thus, the nature of H and SR cells remains open.
2. The L 428 cells and H and SR cells share antigenic site(s) that have not yet been detected on normal cells of lymphoid tissue. This finding substantiates the derivation of the L 428 cell line cells from H and SR cells.
3. The anti-L 428 cell serum contains two kinds of antibody, one directed against surface membrane antigens, and one against nuclear antigens of H and SR cells.

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