Immunological Membrane Markers of Hodgkin's Cells

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Summary

Reed-Sternberg and other Hodgkin's giant cells derived from involved spleens and lymph nodes of patients with Hodgkin's disease were examined for surface markers of T and B cells and macrophages. Attachment and phagocytosis of untreated (E) or sensitized (EA and EAC) sheep red blood cells and yeast by Reed-Sternberg cells did not occur. IgG frequently detected at the membrane of Reed-Sternberg cells was partially removed by incubation and washing at 37 °C. Fluorescence with a specific anti-T cell serum was not seen on Reed-Sternberg and other Hodgkin's giant cells. These studies indicate that Reed-Sternberg and other Hodgkin's giant cells lack most detectable normal human lymphoid cell markers, but do exhibit membrane bound immunoglobulin possibly of exogenous origin.

Introduction

Morphological criteria alone are inadequate for the recognition of the Hodgkin's cell. These criteria lack specificity since Reed-Sternberg cells may be observed in a number of other disorders including infectious mononucleosis (1), and they do not permit identification of the perhaps more proliferative precursors of the Reed-Sternberg cell (2). It would be attractive to find a membrane marker or combination of markers to characterize these cells. Therefore, we have examined isolated viable Hodgkin's cells for the presence of membrane markers typical for normal histiocytes and lymphocytes.

Initial studies in our laboratory (3) and by Braylan et al (4) indicated that the cytologically malignant giant cells in Hodgkin's tissues lack normal T cell markers. We have extended these observations and further examined these cells for receptors for sheep erythrocytes sensitized with IgG, or IgM and complement which are surface markers for histiocytes and B lymphocytes. We have also studied their capacity for the phagocytosis of opsonized yeast and latex particles. Finally, we have looked for surface immunoglobulins and are currently investigating the possible exogenous origin of the surface IgG which we have detected.

Methods

All of the experiments were performed with cells sterily isolated from Hodgkin's lesions of nine involved lymph nodes and six spleens by careful mincing in RPMI 1640 culture medium. Erythrocytes were lysed in 0.83 % NH_4Cl and the resulting cell suspension washed 3 times in media. Cells were then resuspended in media containing 10 % fetal calf serum at 5×10^6 cells/ml for the rosetting and phagocytosis studies. The cell viability as determined by trypan blue dye exclusion was at least 90 %.

Rosetting and phagocytosis studies

T lymphocytes were detected by the spontaneous formation of rosettes with washed sheep red blood cells (SRBC) using the method of Jondal et al (5). Briefly, 0.25 ml cells from tissue suspensions were incubated with 0.25 ml washed SRBC (1 % in Hank's balanced salt solution – 10 % FCS) at 37° for 5 minutes. The cells were then centrifuged at 1000 rpm for 10 minutes at room temperature and stored overnight at 4°. Tubes were inverted gently, cells were added to trypan blue, and 200 viable cells were counted with the hemocytometer. A rosette was defined as a cell having 3 or more SRBC bound to its surface. Cytocentrifuge preparations were also made and stained with Wright-Giemsa.

Fc and complement receptors were detected by the method of Shevach et al (6). Fresh SRBC (E) were washed and sensitized either with 7S (IgG) rabbit anti-SRBC (7S EA) or with 19S (IgM) rabbit anti-SRBC (19S EA). The anti-SRBC reagents are from Cordis Laboratories, Miami, Florida. For complement receptor detection, the 19S EA was further incubated with fresh mouse complement diluted 1:20 with gelatin-veronal buffer. 0.25 ml of the appropriate reagent – 7S EA (for Fc receptor), 19S EA (for non-specific binding), 19S EAC (for C_3 receptor) was mixed with 0.25 ml tissue cell suspension and put on a rotator at 37° for 30 minutes. Hemocytometer counts were made as for the T-rosettes, with the additional observation of any phagocytic cells. Cytocentrifuge preparations were also made and were the best means of observing phagocytosis.

Normal peripheral blood lymphocytes did not demonstrate Fc receptors with the 7S EA reagent; normal and malignant histiocytes did rosette and phagocytose 7S EA. Normal peripheral blood lymphocytes (10–25 %) and most monocytes rosetted with 19S EAC as did the majority of CLL cells. Monocytes and histiocytes also phagocytosed the 19S EAC reagent. T-rosettes were formed by 50–70 % of peripheral blood lymphocytes and by 99 % of normal thymocytes.

Phagocytosis was studied further in two cases by prolonging incubation with 7S EA or 19S EAC to 150 minutes. At the end of the incubation period aliquots were taken and on one portion the percentage of rosetted and phagocytic cells were counted. Cytocentrifuge preparations were also made. The other portion of the incubation mixture was treated with NH_4Cl to lyse the SRBC in order to differentiate between ingested and external SRBC's since ingested SRBC's are not lysed by NH_4Cl. Rosetting and phagocytosis was assessed by hemocytometer counts and cytocentrifuge preparations. Opsonized Baker's yeast was also used as a phagocytic stimulant at a ratio of 100 yeast particles to one white cell. This

mixture was incubated on a rotator at 37° for one hour and cytocentrifuge preparations made to assess the degree of phagocytosis.

Immunofluorescence

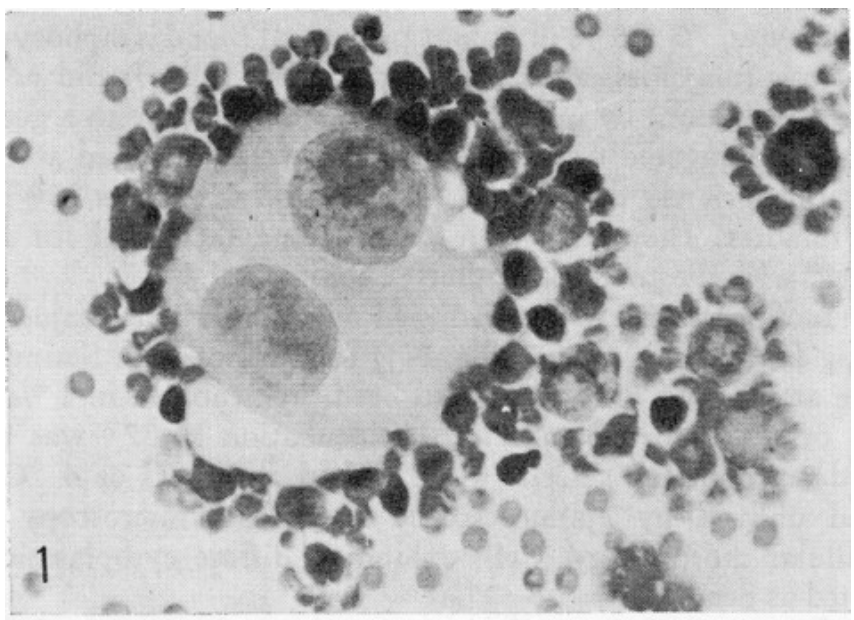
In four cases cells from tumor tissues were examined by indirect immunofluorescence with an antiserum specific for human T-cells. This antiserum was prepared by immunizing rabbits with pooled fetal thymocytes and rendered specific for T-cells by repeated absorptions with chronic lymphocytic leukemia cells (B-cells) and pooled human RBC. The antiserum was heat inactivated for 30 minutes at 56° and stored at –40 °C until used. The antiserum detected 95–100 % of thymocytes, 70 ± 5 % of human peripheral blood lymphocytes, malignant cells from mycosis fungoides, and Sternberg sarcoma cells. It did not detect CLL B-cells. All tests were done by indirect immunofluorescence with a goat-anti-rabbit Fc (IgG) reagent conjugated to fluorescein. Cells were incubated at 4 °C with the anti-T serum appropriately diluted in 1 % BSA-PBS with 2.5×10^{-5} molar sodium azide for 30 minutes. They were then washed, and incubated for an additional 30 minutes at 4° with the goat-anti-rabbit Fc conjugate.

Direct immunofluorescence was performed with fluorescein-conjugated anti-IgG serum (Behring Diagnostics, Somerville, NJ) for surface IgG. Staining with anti-IgG was done at 4 °C on cells which had been preincubated in 1 % BSA-PBS at either 37 °C or 4 °C for 30 minutes. Preincubation at 37° was performed to elute absorbed antibodies (7). Cells were washed at 37 °C or 4 °C respectively and examined unfixed by epifluorescence and phase microscopy in order to distinguish cellular morphology. Cells exhibiting diffuse cytoplasmic fluorescence were interpreted as dead.

Latex particles were added to cell suspensions at the beginning of the incubation period in an attempt to facilitate the distinction of Hodgkin's cells from histiocytes, monocytes, and reticulum cells. This proved to be of limited value because in many instances the latex particles seemed to adhere to the irregular surface of the Hodgkin's giant cells. Furthermore, these cells had prominent cytoplasmic granulations not readily distinguished from the latex particles. The Hodgkin's giant cells were best differentiated from histiocytes and reticulum cells by their much larger, more irregular nuclei and prominent, often huge, nucleoli. Hodgkin's giant cells with multiple separate nuclei or distinct nuclear lobulations, each with individual nucleoli, were judged to be Reed-Sternberg cells. Both Reed-Sternberg and mononuclear Hodgkin's cells exhibited long spike-like and foot-shaped projections. Multiple small lymphocytes frequently were attached to the surface of these Hodgkin's giant cells. Fluorescence was therefore judged only on those cells with adequate free cell membrane and those without attached lymphocytes. In the anti-T serum preparations an additional difficulty encountered was a tendency towards leukagglutination. We did not attempt to judge fluorescence of clumped cells.

Results

Cell suspensions derived from involved lymph nodes and spleens of patients with Hodgkin's disease contained Reed-Sternberg and mononuclear Hodgkin's giant cells, frequently with adherent small lymphocytes. The majority of the lymphocytes were identified as T-cells by their linear membrane fluorescence with specific anti-T cell serum and their capacity to form spontaneous rosettes with non-sensitized SRBC (E rosettes) (Fig. 1). By contrast, the Hodgkin's giant cells never formed E rosettes (Fig. 2) and showed no linear membrane fluorescence with the anti-T cell serum. Fluorescence at the interface of a Hodgkin's cell and an



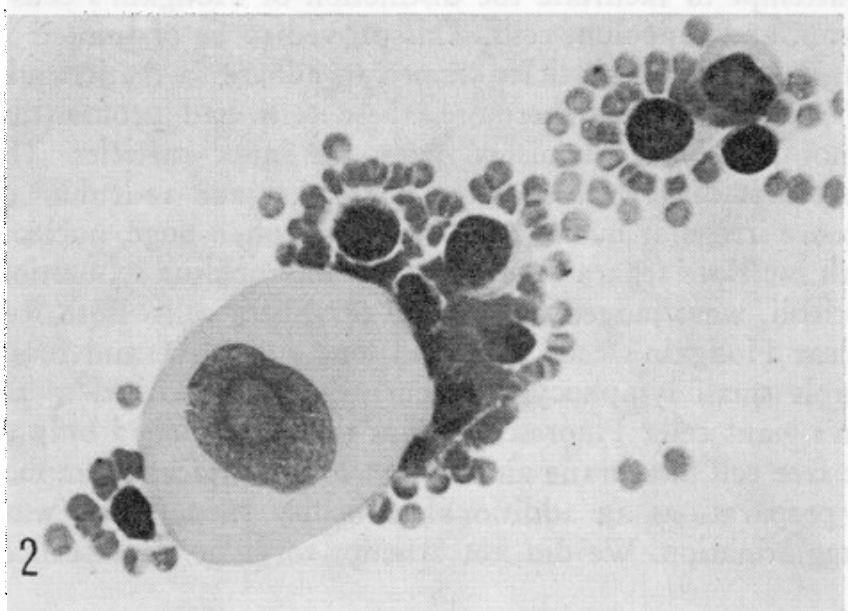


Fig. 1: A large binucleate Reed-Sternberg cell surrounded by small lymphocytes identified as T-cells by their rosetting with untreated sheep erythrocytes (E).
Fig. 2: A mononucleate Hodgkin's giant cell is partially surrounded by rosetted T-lymphocytes but has no T-cell marker (E) attached to its membrane.

adherent lymphocyte was disregarded since it was assumed to be a property of the lymphocyte.

When the large Hodgkin's cells were examined for Fc and complement receptors no rosettes were formed. Normal B-lymphocytes and histiocytes did rosette, and histiocytes frequently showed phagocytosis. In some cytocentrifuge preparations, a few large Hodgkin's cells appeared to contain one or more erythrocytes and large vacuoles, possibly with digested erythrocyte material. Since we considered that this might be an artifact caused by the impingement of erythrocytes on the much larger Hodgkin's cells in the cytocentrifuge, phagocytosis was further examined by lysis of non-ingested erythrocytes prior to cytocentrifugation. Figure 3 shows that no erythrocytes were found in Hodgkin's cells after such treatment whereas erythrocytes phagocytosed by macrophages were not lysed. Hodgkin's cells were also unable to phagocytose heat-killed Baker's yeast.

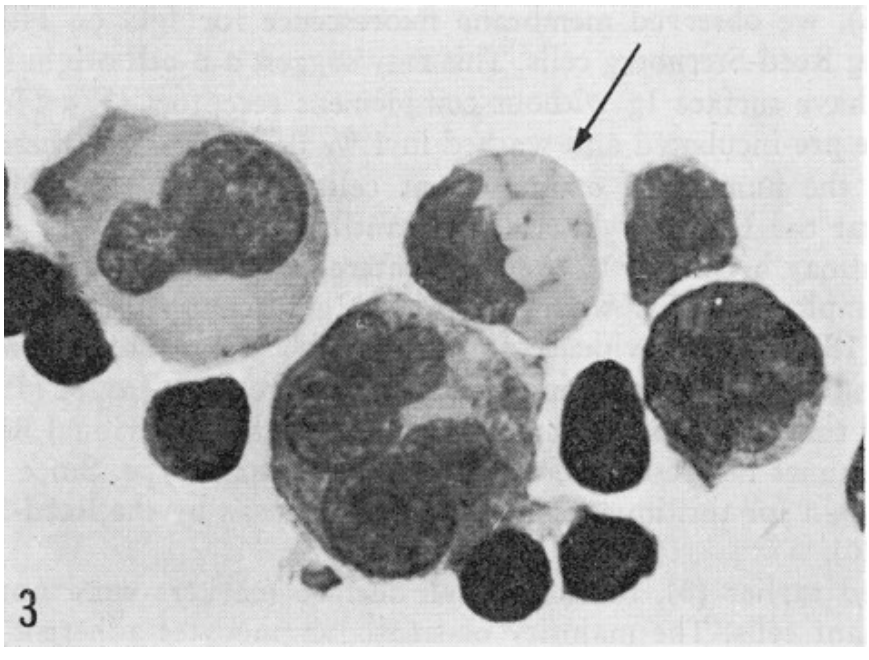


Fig. 3: The splenic macrophage (arrow) has phagocytosed several complement-coated sheep erythrocytes (EAC) while the adjacent Hodgkin's cells from a case of nodular sclerosis have not phagocytosed this reagent.

Examination of surface immunoglobulins by direct immunofluorescence revealed speckled membrane fluorescence with anti-IgG on 54 % of large Hodgkin's cells but not on the surrounding T-lymphocytes. To determine whether this surface IgG might be absorbed antibody, which could be eluted at 37 °C, immunofluorescence studies were also carried out following incubation and washing of the cells at 37 °C (7). Following this treatment the proportion of Hodgkin's cells staining was reduced to 26 % and the degree of residual fluorescence on stained cells was moderately diminished.

Discussion

The derivation of the Reed-Sternberg cell cannot be determined solely on morphological grounds. Various morphological observations have suggested its relationship to either a macrophage (8, 9, 10) or transformed lymphocyte (1, 11, 12). We have therefore sought to characterize these giant Hodgkin's cells in terms of immunological markers. Except for IgG which could be partially eluted at 37 °C, we found no markers of normal lymphocytes or macrophages on these cells. Reed-Sternberg cells showed no avidity for sensitized erythrocytes or opsonized yeast and therefore could not qualify as "professional" phagocytes (13). Their ability to ingest smaller latex particles could not be accurately determined with the light microscope and awaits ultrastructural studies.

In these studies, the complement receptor, frequently detected on benign and malignant B cells (14, 15) was not observed on Hodgkin's cells. As shown earlier by Leech (16), we observed membrane fluorescence for IgG on Hodgkin's giant cells including Reed-Sternberg cells. This may suggest a B-cell origin for these cells; B cells may have surface Ig without complement receptors (15). However, when the cells were pre-incubated and washed in 1 % BSA at 37 °C, there was a 50 % reduction in the number of stained giant cells and the degree of fluorescence, indicating that the IgG may be absorbed antibody which may be eluted (7, 17). The situation may be similar to that encountered by Preud'homme and Seligmann in some examples of CLL where absorbed IgG removed by trypsin was not regenerated (18). Further evidence for the possible exogenous origin of the SIgG on Reed-Sternberg cells may be inferred from the work of Taylor (19). He clearly demonstrated that cytoplasmic immunoglobulins within individual Reed-Sternberg cells are sometimes not restricted to a single light chain type. Since this implies a polyclonal origin for this immunoglobulin, its synthesis by the Reed-Sternberg cell is unlikely (20).

As reported earlier (3), normal T cell surface markers were not detected on Hodgkin's giant cells. The majority of small lymphocytes adherent to the Reed-Sternberg cells rosetted with nonsensitized sheep red blood cells and showed membrane fluorescence with antithymocyte serum. *In vivo* ultrastructural observations indicate that this close apposition of the small T-lymphocytes is correlated with significant cytotoxic changes in the large neoplastic cells (21).

If the Reed-Sternberg cell were derived from a T-cell, the detection of T cell surface markers may be prevented by the presence of a blocking antibody. Cytotoxic IgG antibodies have been demonstrated in the serum and on the peripheral blood lymphocytes of patients with Hodgkin's disease (22), where they may be produced in large part by the spleen (23). Such antibodies can inhibit formation of E rosettes, PHA responsiveness, and allograft rejection by normal T cells (24–26). If directed against an antigen shared by the peripheral blood T cells and Reed-Sternberg cells these antibodies could account for the impaired cellular immunity found in early stages of Hodgkin's disease and our present inability to detect normal T cell surface markers on the Reed-Sternberg cell.

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Abbreviations used

FCS	=	Fetal calf serum
BSA-PBS	=	bovine serum albumin-phosphate buffered saline
CLL	=	chronic lymphocytic leukemia
SIgG	=	surface-bound immunoglobulin G