Sternberg-Reed Cells in the Peripheral Blood of Patients with Hodgkin's Disease

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It is well known that Hodgkin's disease can originate in lymph nodes, spleen, liver and other organs of the reticuloendothelial system. From there it spreads throughout the body by local invasion, by lymphatic dissemination, and by hematogenous dissemination.

The possibility of the hematogenous dissemination of Hodgkin's disease was first suggested by Dorothy Reed in her classic description of the morbid pathology of Hodgkin's disease in 1902: "These giant cells occur in great numbers in the large lymph sinuses of the gland and occasionally occur in blood vessels." In 1926, Jeanselme and Marchal reported Sternberg-Reed cell emboli observed within blood vessels in biopsied and postmortem organs of a patient who had died from Hodgkin's disease. In addition, these giant cells were described as being frequent in perivascular locations and in some sections appeared to be passing through a vessel into the parenchyma.

These early observations support the possibility of hematogenous dissemination of Hodgkin's disease. However, the presence of Sternberg-Reed cells in routine peripheral blood smears is a rare occurrence and only 8 isolated cases have been reported in the world's literature. Among 890 patients studied in our institution, Sternberg-Reed cells were found in the routine peripheral blood examinations in only 2 patients with advanced generalized disease.

In this report, we present the results of our search for Sternberg-Reed cells in the smears of leukocyte concentrates in 135 patients with confirmed diagnoses of Hodgkin's disease. We also report our comparative observations of leukocyte concentrates in the splenic vein and splenic artery of 3 splenectomized patients who had Hodgkin's disease.

Materials and Methods

Method of Study

Among the numerous methods described for the demonstration of cancer cells in the circulating blood, we chose a simple, but not quantitative, method which is a modification of the silicone flotation technique of Seal. This method takes advantage of the differences in the specific gravity of the various cells.

Dow-Corning Silicone 555 and 710 are blended to achieve 5 ml. of solution of a specific gravity 1.075. Five ml. of fresh heparinized blood is carefully layered above the silicone
solution and the tube centrifuged at 1500 rpm for 15 minutes. The Sternberg-Reed cells, being lighter than 1.075, come to lie on the surface of the silicone and form a distinct buffy coat which is easily aspirated with a fine Pasteur pipette. Smears are prepared, giving special care to achieving readily visible edges and are stained with Wright’s stain. Scanning of 10 slides for each blood sample is carried out by an expert technician. Suspicious cells are marked on the slide with a microlocator and are then personally examined and photographed by the author.

Several tests were first employed to determine the effectiveness of the above technic: (1) Ascites tumor cells were obtained from the abdominal cavity of an infected mouse and “seeded” in normal human blood. This sample was processed following our technic. The ascites tumor cells were present in stained smears in proportionate numbers to the “seeded” cells. (2) Areas grossly involved with Hodgkin’s disease from fresh lymph nodes, obtained at surgery, were scraped and mixed with compatible blood from normal individuals and then samples were processed following the described silicone technic. Sternberg-Reed cells were found in the stained smears proportionally to the amount of the scraped material of the Hodgkin’s lymph node mixed with the normal blood. From the results of the above 2 experiments, we concluded that our technic is effective in isolating Sternberg-Reed or similar cells.

Material

Employing the described silicone technic we have examined, for the past 4 years, the peripheral blood of 135 patients who had confirmed pathologic diagnoses of Hodgkin’s disease. During this 4-year period of observation, from 1 to 49 determinations were done on individual patients during the course of their illness. The total number of determinations performed was 1060. Seventy-eight patients were males and 57 were females. The age range was from 8 to 91 years. A group of 50 healthy individuals were used as normal controls. Another control group consisted of 22 patients with proved diagnoses of lymphosarcoma.

In each of 3 patients with Hodgkin’s disease who underwent splenectomy for secondary hypersplenism, 3 samples were obtained at the time of surgery. The first sample was obtained from the splenic artery, the second sample from the splenic vein, and the third sample from the splenic vein after manual squeezing of the spleen by the surgeon. A total of 15 ml. of blood was obtained per sample and was divided into 3 tubes. Ten slides were made from each tube and reviewed for the presence of Sternberg-Reed cells. A similar experiment was carried out in three splenectomized control patients; in 1 the diagnosis was hereditary spherocytosis, and in the other 2 it was idiopathic thrombocytopenia purpura.

Results

Peripheral Blood Studies

The results of our study have demonstrated that in leukocyte concentrate slides from patients with Hodgkin’s disease a marked increase in monocytes is present. Many of them are large and young forms, and some have vacuoles. Such increase in monocytes is more pronounced during the periods of activity or in the advanced stages of their disease. This finding confirms our observations in routine peripheral white blood cell differential counts from patients with Hodgkin’s disease.

In addition to the usual normal cells seen on the routine differential slides, other cells were found, which are rarely or never found in routine blood smears. We have arbitrarily classified these cells in 3 groups:

1. Atypical cells which are rarely found in routine slides but are found in low number in leukocyte concentrates of normal subjects.
2. Abnormal cells which are not found in either routine slides or leukocyte concentrates of normal subjects.

3. Neoplastic cells of the Sternberg-Reed type which are only found in patients with Hodgkin's disease.

Among the atypical cells we found:

a. Atypical mononuclear cells which stand out because of their dark blue, strongly basophilic cytoplasm. They frequently have a clear perinuclear area near the nucleus. The chromatin structure of the nucleus can be either coarse or delicate. Nucleoli are usually distinguished. They most likely represent altered monocytes. We have found these cells in the slides prepared from normal subjects but in greater numbers in patients with Hodgkin's disease (Fig. 1).

b. Cells in mitosis were very rarely observed in the leukocyte concentrate slides of our normal control subjects, but they were more frequently found in patients with Hodgkin's disease. These mitotic cells were at different stages of mitosis, and they seem to belong to the atypical mononuclear cells (Fig. 2).

c. Small binucleated cells were also found occasionally in the normal buffy coat; they were numerous and very frequently found in the buffy coat from patients with Hodgkin's disease (Fig. 3).

d. Nuclei of megakaryocytes of different sizes and shapes were found frequently in the leukocytes concentrates of normal subjects, sometimes surrounded by normal platelets. However, intact megakaryocytes were rare. On the contrary, in patients with Hodgkin's disease, numerous intact megakaryocytes were found, in some patients as many as 2 or 3 per oil immersion field (Fig. 4). The high incidence of megakaryocytes in leukocyte concentrates has been reported recently in patients with carcinoma.

e. Histiocytes, also described as macrophages or reticulum cells, were found in very small numbers in the leukocyte concentrates of some of the normal subjects, being found more frequently in patients with Hodgkin's disease. They appeared as large cells with abundant cytoplasm, which is pale gray-blue with a delicate edge, so that the cell membrane is difficult to discern, giving the appearance of a veil. The cytoplasm contained vacuoles of various sizes, granules, cellular debris, phagocytosed red cells, white cells or platelets. The nucleus is oval, kidney shaped or round. The nuclear structure has a honey-comb appearance and stains to a light purple color (Fig. 5).

f. Endothelial cells, often arranged in small clusters surrounded by a large field of cytoplasm of grayish-blue color, were seldom found in leukocyte concentrates of normal subjects; however, they were seen in patients with Hodgkin's disease.

We found that patients with Hodgkin's disease present other abnormal cells which are not present in either routine slides or leukocyte concentrates of normal subjects. They include:

a. Large atypical mononuclear cells are 2 to 4 times larger than a normal monocyte having large nuclei, with irregular structure and one or several nucleoli and basophilic cytoplasm (Fig. 6).
Plate 1.—Leukocyte concentrates of peripheral blood of patients with Hodgkin’s disease. Fig. 1: Atypical mononuclear cell. Fig. 2: Cell in mitosis (metaphase) probably an atypical mononuclear cell. Fig. 3: Small binucleated cell. Fig. 4: Intact megakaryocyte. Fig. 5: Histiocyte. Fig. 6: Large atypical mononuclear cell. Fig. 7: Large atypical mononuclear cell with numerous cytoplasmic vacuoles. Fig. 8: Large atypical binucleated cell. Reduced 20 per cent from magnification 2400 ×.
b. Large atypical mononuclear cells with numerous vacuoles (Fig. 7).

c. Large atypical binucleated cells, some of which are elongated with the same cytoplasmic and nuclear characteristics of the cells described above (Fig. 8).

These three groups of abnormal cells resemble pathologically altered reticulum cells, and morphologically we can recognize the characteristics of the transitional stages of reticulum cells.

The next group consisted of definitely neoplastic cells and was found only in the leukocyte concentrates of patients with Hodgkin's disease. They are different morphologic variations of typical Sternberg-Reed cells. Some of them are as large as 50 microns in diameter. They are mono, binucleate or multinucleated. The nucleus is large, sometimes showing lobulations; the nuclear chromatin is of variable thickness and staining. The nucleoli are very large, irregular in shape, and usually deeply stained (Figs. 9-16).

Among the 135 patients with Hodgkin's disease studied, 85 (63 per cent) had negative leukocyte concentrates for abnormal cells or Sternberg-Reed cells. Fifty (37 per cent) were positive. Of this latter group, 25 patients (18.5 per cent) of the total number of patients studied showed the presence of typical Sternberg-Reed cells sometime during the course of their illness.

The abnormal cells described were found mainly during the time of activity of Hodgkin's disease, but occasionally large atypical binucleate and large atypical mononuclear cells were found during the time of clinical remission.

Sternberg-Reed cells were found only in patients showing generalized and advanced stages of their Hodgkin's disease. In 18 patients, these cells were found from 4 months to 1 day prior to death. However, 4 patients in whom Sternberg-Reed cells were found in the peripheral blood still responded to therapy and obtained further remissions of 1½ to 3 years duration. Three patients who had Sternberg-Reed cells in their leukocyte concentrates are still alive and responding to therapy.

We have also observed that the presence of Sternberg-Reed cells has been more frequently associated with the involvement of abdominal organs by Hodgkin's disease.

Splenic Studies

Of interest are the results of our observations in leukocyte concentrates from samples obtained at the time of splenectomy on 3 patients with Hodgkin's disease. Patient 1 was in an advanced stage of Hodgkin's disease at the time of surgery, and patients 2 and 3 developed secondary hemolytic anemia while they were in earlier stages of their disease.

Patient 1 illustrates the results of our splenic studies. We found that after careful scanning of 10 slides prepared from each of 3 samples, no Sternberg-Reed cells were found in the first blood sample obtained from the splenic artery, and only 1 large atypical binucleate cell was seen. In the second sample, obtained from the splenic vein, 12 abnormal cells and 5 Sternberg-Reed cells were found. In the third sample, obtained from the splenic vein
Plate 2.—Leukocyte concentrates of peripheral blood of patients with Hodgkin's disease. Sternberg-Reed cells: Fig. 9: Binucleated and elongated. Fig. 10: Binucleated with prominent nucleoli. Fig. 11: Binucleated with nucleoli. Fig. 12: Binucleated, lobulated with cytoplasmic vacuoles. Fig. 13: Two cells in the same field. Fig. 14: Trinucleated. Fig. 15: Binucleated with lobulated nuclei and vacuoles. Fig. 16: Multinucleated with prominent nucleoli and with vacuoles. Reduced 20 per cent from magnification 2400 x.
after manual squeezing of the spleen by the surgeon, 21 abnormal cells and 12 Sternberg-Reed cells were detected (Figs. 17-24). Leukocyte concentrates from peripheral blood, obtained before surgery, revealed the presence of only 1 large binucleated cell. The day after surgery 2 binucleated cells and 1 large atypical mononuclear cell with vacuoles were found; no Sternberg-Reed cells were seen.

Pathologic examination of the spleen revealed gross infiltration by Hodgkin's disease. The spleen weight was 919 Gm. Microscopic examination of the spleen revealed generalized infiltration by Hodgkin's disease (Fig. 25).

Special search for the presence of Sternberg-Reed cells in the capillary vessels of the spleen revealed that many of the sinusoidal vessels of the spleen contained large atypical reticulum cells which may possibly represent Sternberg-Reed cells.

Similar studies done on patient 2 revealed neither abnormal cells nor Sternberg-Reed cells in either the splenic artery or the splenic vein samples. In the samples obtained after mechanical squeezing of the spleen, 22 abnormal cells and 4 Sternberg-Reed cells were present. Patient 3 showed no abnormal cells present in the sample obtained from the splenic artery, and 10 abnormal cells were found equally divided between the splenic vein samples obtained directly and after squeezing of the spleen.

As control studies, similar splenic samples were obtained in 3 splenectomized patients, 2 of idiopathic thrombocytopenic purpura and 1 of hereditary spherocytosis. No abnormal cells or Sternberg-Reed cells were found in either the splenic artery or splenic vein samples obtained before and after manual squeezing of the spleen. The only occasional large cells found had the morphologic characteristics of histiocytes. Similar cells were also found in the splenic studies of the splenectomized patients with Hodgkin's disease (Fig. 5).

**Discussion**

The presence of Sternberg-Reed cells in routine smears of patients with Hodgkin's disease is extremely rare. In numerous periodic observations of a total of 890 patients in our institution, over the past 15 years, such cells were found only on 2 occasions, or 0.2 per cent. In the world's literature, only 8 isolated cases have been reported.3-10

The presence of Sternberg-Reed cells in routine needle bone marrow aspirations is also rare. In our institution Sternberg-Reed cells have been found in routine examinations of the bone marrow in 43 of 890 patients studied, or 4.8 per cent. These findings agree with other reports.20,21 This is probably due to the scattered focal lesions which may not be aspirated by the sternal needle and also because the specific lesions in the bone marrow tend to have much fibrous tissue, making their aspiration more difficult. This explains the practically constant finding of Hodgkin's invasion of bone marrow in fatal autopsy cases.22

The periodic examination of the leukocyte concentrates in 135 patients with Hodgkin's disease, conducted over the past 4 years in our laboratory, has
Plate 3.—Leukocyte concentrate of splenic vein samples from patients with Hodgkin’s disease. Sternberg-Reed cells: Fig. 17: Two cells in same field. Fig. 18: Cell with indented nucleous and cytoplasmic vacuoles. Fig. 19: Cell with cytoplasmic vacuoles. Fig. 20: Mononuclear cell with prominent large nucleoli and cytoplasmic vacuoles. Fig. 21: Mononuclear cell with large nucleous and prominent nucleoli. Fig. 22: Multinucleated. Fig. 23: Multilobulated. Fig. 24: Bimucleated with irregular cytoplasmic membrane and vacuoles. Magnification for Fig. 17 reduced 20 per cent from 2000 ×; magnification for Figs 18–24 reduced 20 per cent from 2400 ×.
revealed the presence of abnormal cells in 50 patients, or 37 per cent. Of this group, 25 (18.5 per cent) also revealed the presence of typical Sternberg-Reed cells sometime during the course of their illness. A few reports of similar results in small numbers of patients have been published. The cells we have classified and described previously as abnormal cells resemble morphologically transitional forms from reticuloendothelial cells. In previous studies we have supported the view that Sternberg-Reed cells derive from the reticuloendothelial system, and we have described transitional forms in electron microscopy observations of lymph nodes of patients with Hodgkin’s disease. This view is generally accepted. The abnormal or transitional cells were found mainly during the time of activity of Hodgkin’s disease, but on some occasions they were also present during periods of clinical remission. The Sternberg-Reed cells were found only in patients with generalized advanced stages of Hodgkin’s disease. In 17 of the 25 patients, these cells were found from 4 months to 1 day prior to death. However, 7 patients still responded to therapy and obtained further remissions of from 1½ to 3 years duration. Three of the 7 obtained even longer remissions since they are still alive and continuing to respond to therapy. Consequently, the presence of Sternberg-Reed cells in the peripheral blood must be considered as indicating an advanced stage of the disease but not necessarily predicting an immediately fatal outcome.
In spite of the fact that the technic employed in our study was qualitative, the incidence of abnormal or transitional cells and Sternberg-Reed cells has been as high as 40 cells per total number on periodic observations per each individual patient and from 1 to as many as 30 cells per 5 ml. sample per individual patient. It is then apparent that the total Sternberg-Reed cells and transitional cells in the peripheral blood must, at times, be quite high.

Both the abnormal or transitional cells and the typical Sternberg-Reed cells were found individually isolated in the leukocyte concentrate smears, in contrast to cancer cells which are usually found in clusters. The viability of the exfoliated cells and the ability to overcome the patient's defenses to form metastases were not studied.

In some patients who responded to therapy, the Sternberg-Reed cells disappeared from the peripheral blood. However, this has occurred in some instances despite any objective evidence of a remission. It is also important to emphasize that in our group of 135 patients, 85 (63 per cent) gave negative findings. Nineteen of the negative patients had far-advanced disease, and they did not clearly differ in terms of clinical or laboratory findings from the similar positive group. In some of them, even determinations obtained immediately prior to death were negative.

It is possible to hypothesize that in patients with active Hodgkin's disease Sternberg-Reed cells, transitional cells, other abnormal cells and monocytes are stimulated in their production from cells of the reticuloendothelial system, in the lymph nodes, spleen, liver and other organs of the reticuloendothelial system. The stimulating agent which initiates this process is presently unknown. However, the production of these various cells from the reticuloendothelial system seems to increase simultaneously. These cells gain entry into the general circulation. The reason the number of Sternberg-Reed cells is very small and frequently absent in the peripheral blood is that their large size militates against their remaining in circulation. They are presumably filtered by the capillaries of the lungs. Only those cells which escape the first filtering effect of the capillaries would be found transiently in the peripheral blood. The concept of a filtering action by the lungs of large cellular elements which may gain entrance into the general circulation is one which was suggested by Aschoff and applied by Kiyono to the explanation of the low histiocyte count in arterial blood. Simpson has demonstrated this same phenomenon to be true for macrophages in experimental studies done in rabbits. The monocytes, due to their smaller size, are able to circulate in the peripheral blood and are found to be markedly increased during the active or advanced stages of Hodgkin's disease.

This hypothesis is further supported by our studies on the incidence of Sternberg-Reed cells and abnormal or transitional cells in the three splenectomized patients with Hodgkin's disease. Samples of circulating blood obtained at the time of surgery showed no Sternberg-Reed cells in the samples obtained from the splenic artery. However, they were present in the samples obtained from the splenic vein, and in greater number in the samples obtained...
from the splenic vein after manual squeezing of the spleen. Because of the
distribution of the circulation of the spleen, it is possible that the Sternberg-
Reed cells and transitional cells, after they have reached the spleen or after
they are produced in the spleen, find easier access to the circulation. This
might also explain the higher incidence of Sternberg-Reed cells in the periph-
eral blood of patients who had abdominal involvement with Hodgkin’s
disease. In view of their higher circulating number more cells are able to
escape the effect of the capillary filtration. The microscopic study of the path-
ologic sections of the spleen from these patients confirmed the presence of
large atypical reticulum cells inside the splenic veins.

It should be emphasized that the search for Sternberg-Reed cells in the
leukocyte concentrates of patients with Hodgkin’s disease is essentially a re-
search technic and should not be considered as a screening test for Hodgkin’s
disease or a definite prognostic test.

Summary

Our observations of 135 patients indicate that 37 per cent of those suffering
from Hodgkin’s disease exhibit abnormal cells in the leukocyte concentrates of
the peripheral blood during the course of their illness. Typical Sternberg-Reed
cells were found in 18.5 per cent of patients and were present only in the
advanced stages of generalized Hodgkin’s disease.

The presence of Sternberg-Reed cells in the peripheral blood indicates an
advanced stage of the disease but does not necessarily predict an immediately
fatal outcome.

Comparative studies, searching for Sternberg-Reed cells in the splenic circu-
lation, showed no Sternberg-Reed cells to be present in the splenic arteries of
patients with Hodgkin’s disease; but numerous Sternberg-Reed cells were pres-
ent in the splenic vein, particularly after mechanical squeezing of the spleen.

A possible hypothesis is given to support the evidence for the circulation of
Sternberg-Reed cells and an explanation for their lower incidence in the pe-
ripheral blood.

Our observations support the hematogenous metastasis of Hodgkin’s disease.

Summario in Interlingua

Nostre observationes in 135 patientes indica que 37 pro centa del casos de
morbo de Hodgkin es characterisate durante le curso clinic per cellulas
anormal in le concentratos leucocytic del sanguine peripheric. Typic cellulas
de Sternberg-Reed esseva trovate in 18,5 pro cento del patientes e esseva
presente solmente in le stadios avanitate de generalisate morbo de Hodgkin.

Le presentia de cellulas de Sternberg-Reed in le sanguine peripheric indica
un stadio avanitate del morbo sed non predice necessarimente un imminente
esito mortal.

Studios comparative, cercante cellulas de Sternberg-Reed in le circulation
splenic, revelava nulle tales in le arterias splenic de patientes con morbo de
Hodgkin, sed numerose cellulas de Sternberg-Reed esseva presente in le vena
splenic, particularmente post compression mechanic del splen.
STERNBERG-REED CELLS

Es formulate un hypotese in supporto del evidentia pro le circulation de cellulas de Sternberg-Reed e un explication pro lor plus basse incidentia in le sanguine peripheric.

Nostre observationes supporta le conception del metastase hematogene de morbo de Hodgkin.

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REFERENCES


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