PAPERS AND ORIGINALS

Observations on Abnormal Cells in the Peripheral Blood and Spleen in Hodgkin's Disease

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Summary

The occurrence of abnormal cells in the peripheral blood of patients with Hodgkin's disease has been described in the literature. In the present investigation several varieties of cells were found, two of which are believed to be typical of the disease. The significance of these cells in the peripheral blood is not yet clear, but there seems to be a correlation between the presence of these characteristic cells in the blood and involvement of the spleen by the disease as determined by microscopical examination. In 11 patients both abnormalities proved to be absent; 13 out of 14 other patients showed both the abnormal cells in the blood and Hodgkin lesions in the spleen.

If circulating abnormal cells are indeed an indication of the presence of Hodgkin's disease in the spleen, involvement of this organ is likely to be due to or to give rise to haematogenous dissemination. The other possibility remains that both the occurrence of abnormal cells in the peripheral blood and splenic involvement are due to a multicentric origin of the disease. It seems most unlikely that the splenic lesions are consistent with localized disease still restricted to the lymphoid system. These findings challenge the validity of the present widely used so-called Rye classification of clinical stages in Hodgkin's disease.

Introduction

Reports of cures in some cases of Hodgkin's disease have made the choice of therapy of the utmost importance. The therapeutic approach is usually based on the clinical stage of the disease and an extensive search for diseased tissues, supported by new techniques, is now routine (Peters, 1966; Ripault and

Dumont, 1971). In the past two years laparotomy and splenectomy have been widely accepted as helpful tools. In the literature and in our experience the results of microscopical examination of the spleen have not always been what were expected preoperatively (Glatstein *et al.*, 1969; Lowenbraun *et al.*, 1970). Furthermore, in some of these cases the abnormal histological appearances of the spleen could hardly be explained on the basis of lymphatic spread from a unicentric focus (Rosenberg, 1966; Rosenberg and Kaplan, 1966). A multicentric origin or a metastatic spread by blood or both have to be borne in mind. In this respect reports of abnormal cytological findings in the peripheral blood of patients with Hodgkin's disease seemed of interest (Stofberg, 1962, 1963; Bouroncle, 1966). Although the exact nature of these cells has not been clarified it seems possible that they have some significance in the spread of the disease. During our investigation on peripheral blood leucocyte concentrates abnormal cells were found in the blood of a patient who was later shown microscopically to have definite lesions in the spleen not hitherto suspected. This led to the examination of a correlation between the presence of abnormal blood cells and the histological appearances of the spleen.

Materials and Methods

Hodgkin's disease was diagnosed by lymph node biopsy and histologically classified according to the criteria of the Rye conference 1965 (Lukes and Butler, 1966; Lukes *et al.*, 1966). Investigations to assess the extent of the disease in each patient included physical examination, routine blood and urine profiles, bone marrow biopsy, liver function tests, scanning of the liver and spleen with ^{99m}Tc and of the abnormal lymph nodes with ^{75}Se -selenomethionine, chest x-ray examination, lymphography, x-ray examination of the bones, and, if indicated, scanning (^{85}Sr or ^{87m}Sr) of the bones, and intravenous pyelography.

Patients under the age of 60 selected for possible curative radiotherapy were subjected to laparotomy with splenectomy, inspection, and, if possible, biopsy of abdominal lymph nodes and wedge biopsy of the liver. The tissues were examined histologically for the presence of abnormalities and classified by the pathology laboratory. Cytological examinations were done in imprint preparations in the haematology laboratory for comparison.

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Concentrates of peripheral blood leucocytes were made before laparotomy and the start of therapy. The concentrates were prepared according to the method described by Stofberg (1963) but with edetic acid 3% 1 : 10 substituted for heparin. Smears were stained with May-Grünwald-Giemsa, Sudan black, non-specific esterase, and methyl green-pyronine and examined for the presence of abnormal cells as described by Stofberg (1963), Bouroncle (1966), and others.

Results

Several kinds of abnormal cells were found in the leucocyte concentrates. They were classified preliminarily according to their morphology and staining properties as shown in Table I

TABLE I—Cells in Peripheral Blood of Patients with Hodgkin's Disease. Present Classification of Cells compared with Bouroncle's (1966) Classification

Present Study	Bouroncle (1966)
(1) Monocytes: (a) Normal diameter, differentiation to more mature and activated forms (b) Large diameter, elongated large vacuoles, cytoplasm, irregular nucleus	"Abnormal cells," described as large atypical mononuclear cells with or without vacuoles, sometimes binucleated
(2) Dark basophilic cells: Diameter 12–25 µm; large nucleus, almost round, sometimes indented, chromatin corpuscular; nucleoli not always apparent; cytoplasm dark blue, irregular	Atypical cells? (Designated as immunoblasts by Crowther <i>et al.</i> , 1968)
(3) Moderately basophilic cells: Diameter >15 µm; nucleus variable shape; nucleoli large, not always apparent; chromatin somewhat reticulated; cytoplasm smooth blue-grey, with clear zone mostly on one side of nucleus	Not described as separate category, perhaps characterized as atypical cells or neoplastic cells
(4) Sternberg-Reed cells: Multilobulated or multinucleated giant cells	Neoplastic cells

Moderately basophilic cells in leucocyte concentrates of Cases 1 (Top) and 3 (Bottom).

and compared with the cells described by Bouroncle. Most of the cells found in the leucocyte preparations could be fitted into one of the categories described by Bouroncle. A number of the larger cells, however, could be divided into groups that did not quite correspond to this classification; these are summarized in Table I. Some of the large abnormal cells were designated as atypical monocytes (Table I, category 1b) and others as immunoblasts (category 2) (Crowther *et al.*, 1968). The third category consisted of large blast-like cells up to 40 µm in diameter with a finely structured moderately basophilic cytoplasm, a nucleus varying in appearance from oval to lobulated with a somewhat reticulated chromatin network, and one or more large irregularly-shaped nucleoli (see Fig.).

Sternberg-Reed cells according to the classical description were considered to be a separate category; they were seldom found in our preparations. With the exception of the above-mentioned moderately basophilic blast-like cells and the Sternberg-Reed cells all groups of cells could be found with a varying frequency in leucocyte concentrates from normal persons or patients with viral infections. We therefore considered the presence of these moderately basophilic cells in the peripheral blood to be important in determining the extent of the disease in so far that they could play a part in the way it spreads. The correlation between the presence of moderately basophilic cells and Sternberg-Reed cells on the one hand and the existence of disease of the spleen on the other is summarized in Table II. As these cells are rare,

TABLE II—Correlation of Categories of Abnormal Cells in Peripheral Blood and Diseased Spleen

Case No.	Peripheral Blood Category				Spleen Abnormality
	Abnormal Monocytes	Dark Basophilic Cells (Immunoblasts)	Moderately Basophilic Cells	Sternberg-Reed Cells	
1	+	+	+	±	+
2	+	+	±	±	+
3	+	+	+	±	+
4	±	±	—	—	—
5	+	+	—	—	—
6	+	+	+	—	+
7	+	+	+	±	+
8	+	+	±	—	+
9	+	+	±	—	+
10	+	+	±	—	+
11	+	+	±	—	+
12	+	+	±	—	+
13	±	±	—	—	+
14	+	+	+	—	+
15	+	+	±	—	+
16	+	±	±	—	—
17	±	±	—	—	—
18	±	+	±	—	+
19	±	+	—	—	±
20	±	+	—	—	+
21	±	±	—	—	—
22	±	±	±	—	—
23	—	±	—	—	—
24	—	±	—	—	—
25	+	+	+	—	±

*Only in cells from imprint preparations.
+ = Present. ± = Sporadic or scarce. — = Absent.

even in leucocyte concentrates, their presence had to be expressed in terms of absent, sporadic, or present.

With one exception (Case 22) cells believed to be typical for Hodgkin's disease—that is, the moderately basophilic blast cells and Sternberg-Reed cells—were not found in the peripheral blood of patients whose spleen proved to be without macroscopic or microscopical signs of abnormal infiltration. All other patients had abnormal cells in the spleen as well as in the peripheral blood.

Discussion

The significance of abnormal cells in the peripheral blood of patients with Hodgkin's disease has not yet been clarified. Some of these cells can be classified according to Bouroncle (1966) but no correlation between circulating cells and features of the

disease was found by her. Our first investigations permitted the distinction of certain groups of cells, one consisting of Sternberg-Reed cells and the other of large moderately basophilic blast-like cells, which we considered to be typical for patients with Hodgkin's disease. Morphological examination and staining procedures by light microscopy showed some characteristics that resembled those of so-called Hodgkin cells in lymph nodes.

In contrast to Bouroncle (1966) we found a correlation between the presence of these characteristic cells and the Hodgkin lesions of the spleen. This could indicate that splenic involvement is not due to lymphogenic dissemination of the disease. Ripault and Dumont (1971) have already pointed out that retrograde lymphatic spread is not the most probable pathway for involvement of the spleen. The correlation mentioned above makes haematogeneous spread more likely in these cases. Vascular invasion in lymph nodes has been described (Rappaport and Strum, 1970; Strum *et al.*, 1971), and Rappaport and Strum indicated the increased risk of the occurrence of extranodal or non-adjacent lymphoid involvement in the disease. Even if the cells are wandering from the spleen to other organs it still means that the disease is not restricted to lymphoid organs any more.

As the exact nature of these cells has not been elucidated it is also possible that both features indicate a role in a multicentric origin of the disease or the influence of a lymphotropic agent as mentioned by Teillet (1971) and others.

All possibilities throw doubt on the validity of the Rye classification of the clinical stages in Hodgkin's disease and on its principles of unicentric origin and lymphogenic contiguous spread, on which the extensive irradiation regimen was founded by Kaplan (1966) and others. In cases as described above this method leaves a high risk of dissemination of the disease to non-adjacent and at that moment non-irradiated areas. Indeed this could be an explanation for some of the unexpected disappointing results in the treatment of patients with splenic

Hodgkin's disease by irradiation (Halie *et al.*, 1972). Generalized therapy—for instance, with chemotherapeutic agents or perhaps low-dose irradiation of large fields—is then preferable.

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Curative Radiotherapy in Hodgkin's Disease: Significance of Haematogenous Dissemination Established by Examination of Peripheral Blood and Spleen

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Summary

Studies of peripheral blood leucocyte concentrates in patients with Hodgkin's disease showed two types of cells believed to be typical for the disease in a number of patients. Involvement of the spleen as diagnosed after splenectomy and histological examination showed a close correlation with the presence of these characteristic cells in the peripheral blood. This is believed to be an argument for haematogenous spread or a multicentric origin of the disease in these cases. The results of attempted curative high-voltage radiotherapy with total node irradiation in 24 patients seem to support this concept. On the basis of

the Rye classification of clinical stages the results of radiotherapy are not predictable. Six patients in stage II and seven in stage III were in remission, one in each of stages II and IV, and six in stage III had recurrences of the disease within one year. A division into localized or disseminated forms of the disease based on the investigations of blood and spleen showed all localized cases in remission; of the disseminated cases one reached a remission and all others had recurrences. In three patients the therapy could not be completed. These preliminary treatment results are believed to support the idea of a special role of the spleen in the dissemination of the disease. A new classification of clinical stages in Hodgkin's disease is proposed.

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Introduction

The principles of unicentric origin and lymphatic spread in Hodgkin's disease, as formulated by Kaplan (1968) and others, have led to the application of radiotherapy on both involved