Suggested Models of Ecotaxopathy in Lymphoreticular Malignancy

A Role for Iron-Binding Proteins in the Control of Lymphoid Cell Migration

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In the present paper we apply the "ecotaxis hypothesis " to the analysis of lymphocyte distribution in Hodgkin's disease and other forms of lymphoid malignancy. The results lead us to consider the possibility that metal-binding proteins, namely ferritin, transferrin and lactoferrin, play a role in lymphocyte ecotaxopathy. It is suggested that in Hodgkin's disease, a failure of lymph node and spleen monocytes to handle iron normally could explain most of the hematologic, immunologic, pathologic, and epidemiologic features of the disease. (Am J Pathol 90:497–520, 1978)

T AND B LYMPHOCYTES circulate continuously between blood and lymph at well-defined tempos and through well-defined sites of the peripheral lymphoid organs.[1-5] Basically, two theories have been suggested to explain the mechanism of control of lymphocyte circulation: in the first theory, originally proposed by Gesner and Ginsburg [6] and subsequently supported by the work of Woodruff,[7-10] the central point of control resides in the interaction of lymphocytes with the endothelium of the postcapillary venules (PCV) in the lymph nodes; the second theory was formulated as the "ecotaxis hypothesis"[2] and was subsequently expanded by the work of Freitas [11-16]: as lymphocytes differentiate and express a number of surface phenotypes, they acquire, at the same time, the surface make-up that will determine the nature of their interactions with other cells in the periphery. In the light of the second theory, control of lymphoid cell positioning and circulation will thus result not only from the single interaction with lymph node PCV but also from other interactions with circulating and resident cells in the organs traversed by lymphocytes in their continuous circulation from blood to lymph to blood.[4,11]

Neither theory has been formally applied to the analysis of diseases of

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the lymphoid system in humans. The present paper is a preliminary account of results of one study of lymphoproliferative malignancies in the light of the ecotaxis hypothesis.

Our starting questions were thus formulated: if normal lymphoid cell positioning and circulation are controlled by the interaction of the circulating cells with other cells, a) will it be possible to clarify the nature of these interactions by the study of lymphomas and b) will it be possible to find in lymphoid malignancy evidence of breakdown of the mechanism that normally "keeps lymphoid cells going"?

Acknowledgment of Collaborators

The work to be presented was possible as the result of our close collaboration with the Hemopoietic Tumor Research Group headed by Clarkson and the support of the Tumor Procurement Service headed by Hirshaut. The cases studied and the names of the colleagues whose direct or indirect collaboration makes then rightful coauthors of the present work are listed in Table 1.

At the time of the 1977 FASEB meeting (Chicago, April), some of the results which were presented at the Symposium on Leukemias and Lymphomas and which constitute the basis of the present paper were fresh and poorly understood. Work for the past 3 months has considerably clarified their meaning. The present paper includes data presented at the symposium and results of relevant work done since that time.

Materials and Methods

Clinical Material

Details of childhood cases of Hodgkin's disease (HD) have been published elsewhere.[17] In addition, we have examined six spleens removed at staging laparotomy from 6 adults: 5 with the diagnosis of HD and 1 with the diagnosis of "splenic lymphoma." Details of

Table 1—List of Cases Studied and Co-workers in Study

Cases studied	Clinician	Others
Childhood Hodgkin's disease	C. Tan	D.A. Filippa (pathology, surface markers)
Acute lymphocytic leukemia	M. Haghbin	J. Beck
		D. Yao, J. Hansen (mitogen stimulation: peripheral blood lymphocytes)
Adult Hodgkin's disease	B. Koziner	G. Munn (mitogen stimulation: spleen cell suspensions)
	S. Kempin	
		M. Vitale (electron microscopy)
Splenic lymphoma	B. Koziner	E. de Harven (electron microscopy)
Lymphomatoid papillosis	S. Kempin	
	B. Safai	

Table 2—Summary of Patients Studied

Diagnosis	Collaborator	Patient	Sex	Stage	Histopathology	Specimen
Hodgkin's disease	S. Kempin	N.A.	M	IIIA	NS	Spleen
	B. Koziner	J.S.	F	IIA	MC	Spleen
	B. Koziner	N.M.	M	IIIB	MC	Spleen
	T. Gee	R.S.	M	IA	LP	Spleen
	C. Tan	D.K.	F	IIIB	LP	Spleen
Splenic lymphoma	B. Koziner	F	M	—	—	Spleen
Acute lymphocytic leukemia	M. Haghbin	J.H.	M			PBL
Lymphomatoid papillosis	M. Kempin	V.R.	F	—	—	Skin

LP = lymphocyte predominant; NS = nodular sclerosis; MC = mixed cellularity; PBL = peripheral blood lymphocytes.

clinical stage and histopathology and collaborators in the study of the case are summarized in Table 2. The peripheral blood from 1 child with the diagnosis of acute lymphocytic leukemia (ALL) typed by Beck as a T-cell leukemia and the skin from 6 patients with mycosis fungoides and 1 with lymphomatoid papillosis were also studied.

Cell Suspensions and Mitogen Stimulation

Details of methods used in preparation of cell suspensions and stimulation by mitogens have been published elsewhere.[17,18]

Microscopy

Thin (5-μ) frozen tissue sections were fixed in methanol for 10 minutes. Stained or unstained sections were examined on a Leitz Ortholux II microscope fitted with an Osram HBO 200-watt mercury vapor lamp and Ploemopak 2 incidence illuminator for fluorescence examination and a 50-watt tungsten halogen lamp and a Type 402A condensor for transmitted light, dark-field, and phase contrast examination. Two filter block combinations were normally used with the Ploemopak illuminator, ie, the wide-band blue light (H) and the green light (M) blocks. The incorporated filters of these blocks are shown in Table 3.

Autofluorescence Examination

Unstained fixed tissue sections were examined with each filter block. Absorption of blue light usually resulted in an orange or yellow autofluorescent emission; green light illumination gave a deep red fluorescence. Counts of autofluorescent cells were done under oil immersion (× 100 objective).

Table 3—Incorporated Filters of Blocks Used in Immunofluorescence Studies

Filter system	Exciting filter	Dichroic beam splitting mirror	Suppression filter
Wide-band blue light (H)	2 × KP 490	TK 510	K 515
Green light (M)	2 mm Bg 36 + S 546	TK 580	K 580

Immunofluorescence Examination

Antihuman ferritin, antihuman transferrin, and antihuman lactoferrin antiserums produced in rabbits and normal rabbit serum were supplied by Dako Immunoglobulins (Accurate Chemical and Scientific Corporation, Hicksville, NY). Fluorescein-conjugated (FITC) goat antirabbit immunoglobulin was obtained from Meloy Laboratories (Springfield, Va). Ferritin, transferrin, and lactoferrin were stained by the indirect or double-layer technique. Thin (5-μ) tissue sections were rehydrated in phosphate-buffered saline (PBS), pH 7.2, for 1 minute and the slide was wiped dry except for an area overlying the section. Twenty-five microliters of the appropriate antiserum was layered over the section, and the slide was left in a horizontal moisture chamber for 30 minutes. After five washes in PBS, the slide was again wiped dry except on the section and 25 μl of FITC-goal antirabbit immunoglobulin was layered onto the tissue. After an additional 30 minutes, the slides were washed in PBS five times and mounted in a mixture of glycerol–glycine buffer, pH 8.6.[19] As a control, normal rabbit serum was used in place of antiserum in the first layer of the double-layer technique, followed by the second layer of goat antirabbit immunoglobulin.

Iron Staining

Frozen sections of spleen, lymph nodes, and skin and spleen cell suspensions were prepared and fixed in methanol for 10 minutes. Ferric (Fe[III]) and ferrous (Fe[II]) iron deposits in tissues were stained by the Prussian blue and Turnbull blue methods, respectively, and counterstained with 0.5% nuclear fast red solution.

Results

Hodgkin's Disease

Hodgkin's disease is perhaps the human lymphoma most studied by interdisciplinary means. Hodgkin first described 2 cases in 1832.[20] Since then, pathologists have laid down strict rules of microscopic diagnosis;[21] immunologists have reported anomalies of immunologic function;[22] hematologists have described abnormalities of iron metabolism;[23,24] and epidemiologists have found evidence consistent with viral transmission [25] and have discussed possible links of the disease with tonsillectomy,[26] environmental and genetic factors,[27] and close contact with rabbits.[28] Diagnosis of extent of the disease has become more precise with the use of ^{67}Ga scanning,[29] and progress in the quality of chemotherapy has placed it in the group of curable malignancies. Thus, we started by examining the pertinence of our question in a disease whose multiple clinical and laboratorial facets are well known.

One of the aspects that became clear early in the immunologic study of patients with HD is that they have a failure of cell-mediated immunity.[30] Early findings of low numbers of peripheral blood T lymphocytes in patients with HD, which were used to explain that deficiency,[31] were not confirmed; in adults, however, a progressive blood lymphopenia parallels progression of the disease.[32,33]

But does a blood lymphopenia reflect an absolute decrease in numbers of circulating lymphocytes or simply their sequestration in some other tissue compartment?

With the advent of splenectomy as part of the staging procedure of the disease, it became possible to answer this question with some precision. Both from our studies of peripheral blood and spleen in children [34] and from the studies of adults by other investigators,[35-37] it became apparent that unusually high numbers of T lymphocytes are present in spleens of patients with HD, with simultaneous peripheral blood depletions. The finding of high numbers of E-rosette–forming cells in the spleen has been confirmed by Filippa's analysis of cell surface markers in the spleen lymphocytes of the adults included in the present report (Table 4).

If the spleen is acting as a sequestration site, two questions follow: a) Will removal of the spleen improve peripheral blood T-cell function? b) Does the cause of lymphocyte sequestration relate to the cause of the disease itself?

Effect of Splenectomy on Peripheral Blood T-Cell Function

We chose to look at children with HD diagnosed clinically at Stage IA, who were treated with involved field irradiation therapy, ie, confined to the area covering the involved lymph node, to minimize errors from the effect of the therapy. The peripheral blood lymphocyte (PBL) response to PHA stimulation was examined as the indicator of T-cell function, at diagnosis, prior to splenectomy, and at several intervals following splenectomy.

The results in two children, splenectomized (J.E.) and nonsplenectomized (B.W.), of comparable sex and age, are summarized in

Table 4—Percentages of E-rosette-Forming Cells and Cells with Surface Immunoglobulin in Spleens of 4 Adults With Hodgkin's Disease

Patient	Spleen involved	ERFC	SIg
N.A.	+	90	2
R.S.	−	54	4
L.M.	+	84	0
I.K.	+	51	18
Mean (range)		69.7 (51-90)	6 (0-18)
Literature controls*	−	36.5	45

Determined by D. A. Filippa.
EFRC = sheep E-rosette-forming cells; SIg = cells bearing surface immunoglobulins.
* Habeshaw JA, Stuart AE: T and B cells in human spleens. Lancet 1:1164, 1974

Text-figure 1. The PHA response fell within the control range at 18 months after splenectomy; in the nonsplenectomized child it was still significantly lower than the control at 22 months after therapy in spite of slight improvement of the response to the higher mitogen concentration.

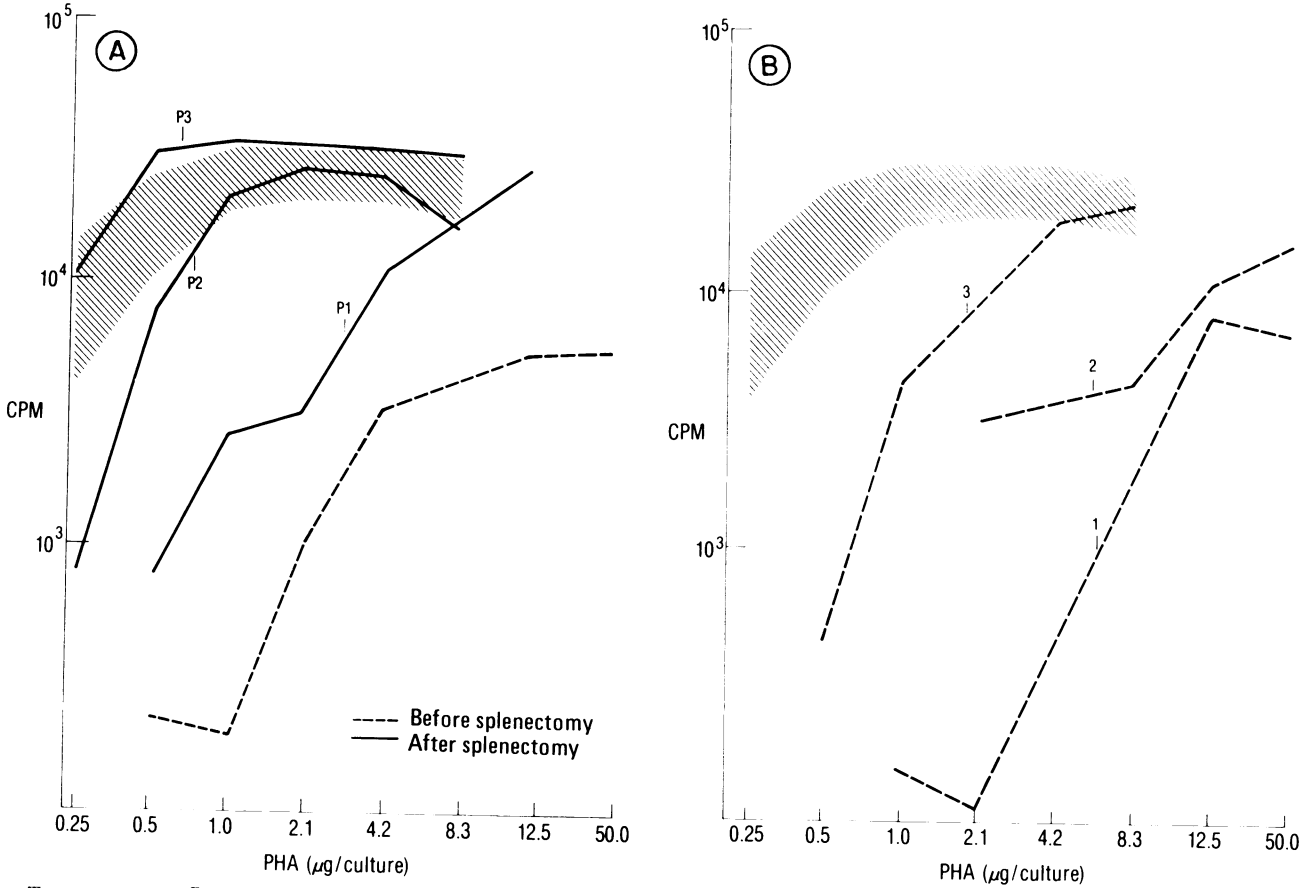
It has been our experience in following up the PBL response to PHA in patients with HD that, on the whole, patients at all stages of the disease, who are doing well clinically, show a return of the PHA response toward normal. By contrast, those who have done poorly or relapsed have persistently low responses to PHA.

Relationship Between the Cause of the T-Cell Sequestration and the Disease

The finding that T cells are sequestered in the spleens of patients with HD at a time of no detectable involvement of that organ by the disease led us to look for some early abnormality of the spleen environment that could explain the delay of the T cells in this organ.

Initially, we looked at unstained, methanol-fixed frozen spleen sections under the fluorescence microscope. This resulted in the observation of large numbers of autofluorescent cells in the spleens of patients with HD. Control tissues included spleens from patients with Gaucher's disease, CLL, hairy cell leukemia, ALL, and splenic lymphoma. Autofluorescent cells were also seen in these controls but in small numbers as compared with the number seen in patients with HD. We were examining the sections under ultraviolet light with exciting filter systems in the regions of 490 to 510 nm (filter H) and 546 to 580 nm (filter M). These are the established conditions for detection of porphyrins in tissues,[38] and a preliminary quantitative analysis kindly carried out by Rifkind from Cornell Medical School confirmed the presence of protoporphyrin in one involved bone marrow and one uninvolved spleen in amounts considerably higher than those found in a spleen from a patient with hairy cell leukemia and bone marrow of a patient with ALL. Coproporphyrin was also present in an involved lymph node from a patient with HD.

The location of the autofluorescent cells in the spleen sections was not random; they were seen in the red pulp and in the white pulp, but in the latter they were confined precisely to the area surrounding the central arteriole (Figure 1), which is known to contain mostly thymus-derived (T) cells. Autofluorescent cells have been described in the rat and human thymus,[39,40] although no details of the conditions of excitation under which they were first detected are available. We have observed sections of human thymus and confirmed the presence of cells with autofluorescence (yellow and red) characteristics similar to those seen in the tissues from patients with HD. However, in the Hodgkin's disease cases examined,



TEXT-FIGURE 1—Effect of splenectomy on the PHA response. PHA dose response curves of peripheral blood lymphocytes from two HD children of same sex (M), age (5 years), clinical stage (IA), and histopathology (lymphocyte predominant). Both patients received radiotherapy (4000 rad) confined to a small area (irradiated field of 10 sq cm over the area involved by the disease). Note that recovery of the response to values within the control range (shaded area: mean ± SD) occurred more rapidly in the splenectomized patient (JE). **A**—JE. Dose response curve prior to splenectomy and therapy and at 2 (*P1*), 14 (*P2*), and 18 (*P3*) months after diagnosis. **B**—BW. Dose response curves at 9 (*1*), 13 (*2*), and 22 (*3*) months after diagnosis.

the presence of these cells was not confined to the spleen. They were also seen in the thymus-dependent area of lymph nodes and in the liver in 1 patient with HD in that organ, suggesting a close association between thymus-derived (T) cells and autofluorescent cells.

In April 1977, we concluded that we did not know how the autofluorescent cells relate to the Reed–Sternberg cell or to the finding of a suppressor factor in the blood of patients with Hodgkin's disease.[22] We would like to think that they related to the pathogenesis of the disease, although we were prepared for the possibility that they would turn out to be another red herring in the HD maze,[41] only different from others for their rightful claim to be red.

Since then, we have reached the conclusion that their link to the pathogenesis of the disease may not be direct. We are interpreting their presence and the presence of increased numbers of T cells in the spleen and other sites involved by the disease as symptomatic of another feature which we suspect can be directly linked to pathogenesis of Hodgkin's disease: abnormal handling of iron by macrophages in lymph nodes and spleen. (See *Discussion*.) The reasoning and results that led us to that conclusion follow.

Anomalies of iron handling by the phagocytic system in Hodgkin's disease were first described by Jacobs' group in Cardiff, tracing the release of radiolabeled iron dextran in untreated patients.[23] In 15 cases studied, only 2 fell within the control range. Failure to release the labeled iron dextran was already detectable in Stage I patients, indicating that it is a basic feature of the disease. Other abnormalities of phagocytic function include more rapid clearance of ^{125}I-labeled aggregated human serum albumin in more advanced stages of the disease,[42] and in a more recent study of bactericidal function, macrophages from patients with Hodgkin's disease were found to be more avidly phagocytic than normal.[43]

In addition, increased amounts of stainable ferric iron have been found in lymph nodes of patients with HD,[44] and unusually high amounts of ferritin, once thought to represent a tumor-associated antigen,[45] have been detected in tissues from patients with HD.[46]

We have started to examine the question of iron deposition and distribution of iron-binding proteins in spleens involved by the disease. In addition to ferritin, the iron-storage protein, we are also examining the distribution of the two major iron-carrying proteins, ie, transferrin and lactoferrin. The results are of considerable interest.

Spleen Sections

Deposits of ferric iron have been found in association with areas of sclerosis. The immunofluorescence analysis revealed the presence of large

numbers of ferritin-containing cells in areas of the spleen involved by the disease, but similar cells were seen in the red pulp of spleens with no evidence of involvement by the disease (Patients RS and JS). Faintly positive transferrin-containing cells were observed, with a distribution pattern similar to that of the more brightly positive ferritin-containing cells. The most striking result of the immunofluorescence study was the finding of large numbers of brightly positive lactoferrin-containing cells in uninvolved and involved spleens. The cells were mostly in the marginal zone and splenic red pulp; their distribution is illustrated in Figure 2. The only clear-cut difference between involved and uninvolved spleens was the presence of more cells with positive nuclear staining for lactoferrin in the involved than in uninvolved organs. In sections, the lactoferrin-positive cells were so bright that it was difficult to determine their morphology.

Spleen Smears

In the spleen smears, it was much easier to analyze the morphology of the iron-containing cells and of the cells containing the different iron-binding proteins. Generally, the cells containing iron were binucleated, with a large cytoplasm (Figure 3); these cells were much more frequent in the smears of involved spleens. In addition, in the smears of involved spleens, many more heavily iron-loaded cells were seen, some with bizarrely shaped nuclei. The immunofluorescence analysis of the spleen cell suspensions confirmed the analysis of the sections. In the smears, however, it was possible to discern that the majority of the lactoferrin-positive cells were myeloid cells.

Lymph Node Section

The finding of iron-containing cells in the spleen is generally met with reservation because of the major role played by the spleen in iron metabolism. Recently, however, we had the opportunity of examining paraffin sections of an involved lymph node from a patient studied by Horta at the Lisbon Medical School; similar large, binucleated cells with bizarre shapes, containing intracytoplasmic and intranuclear iron, were seen (Figure 4). These cells were distributed in the area of sclerosis, where a diffuse layer of stainable iron was also observed.

Ecotaxopathy in a Case of Splenic Lymphoma?

We were encouraged by the results of the study of tissues from patients with HD to look for iron deposits and distribution of the three major iron-binding proteins in tissues from patients with other lymphomas.

In a case with the pathologic finding of monoclonal (κ chain) B-cell

lymphoma consisting of lymphoid cells expressing all different Ig classes (IgG: 70%, IgM: 97%, IgD: 63%, IgA: 60%) and only 10% E-rosette forming cells (case studied by Filippa), the thymus-dependent areas (TDA) of the spleen were completely replaced by macrophages heavily loaded with ferric and ferrous iron (Figure 5). This distribution is markedly different from that seen in the spleens of patients with HD, in whom the Fe(III) appears in diffuse, lakelike areas or as intracellular dark blue clumps but confined to the marginal zone and red pulp.

In splenic lymphoma, all the iron was in dark blue clumps localized within cells distributed in the TDA, ie, around the central arterioles (Figure 5).

The immunofluorescence analysis revealed large numbers of brightly positive ferritin-containing cells and few transferrin-positive cells in the periarteriolar locations. In this case, few lactoferrin-positive cells were found among the lymphoma cells, away from the TDA.

Ecotaxopathy in Cutaneous Lymphomas

Infiltration of skin by malignant lymphoid cells is the simplest example of ecotaxopathy. Cells that normally circulate through blood, spleen, Peyer's patches, and lymph nodes accumulate in large numbers in a compartment well out of the recirculation pathway.

Cells with the characteristics of interdigitating dendritic cells normally present in the thymus and TDA have been observed in the skin of patients with mycosis fungoides.[47] In a series of preliminary experiments, we implanted skin from patients with mycosis fungoides under the skin of nude mice. Cells with Sézary-like morphology were detected in the skin and lymph nodes of one recipient (Figure 6),[48] indicating that the specific interactions between circulating cells and specific tissue sites persist across species barriers. The nature of these interactions in the skin, however, is still unclear. We examined a case of lymphomatoid papillosis and found that the subepidermal area of mononuclear infiltration was very rich in lactoferrin-containing cells. No stainable iron or ferritin- or transferrin-positive cells could be detected in this case.

Discussion

Suggested Models of Ecotaxopathy

The question of T-lymphocyte maldistribution in Hodgkin's disease was first raised in 1972.[49] The search for its possible causes led to the present study of distribution of iron and iron-binding proteins in Hodgkin and non-Hodgkin lymphomas. In the past year, studies of the interaction of

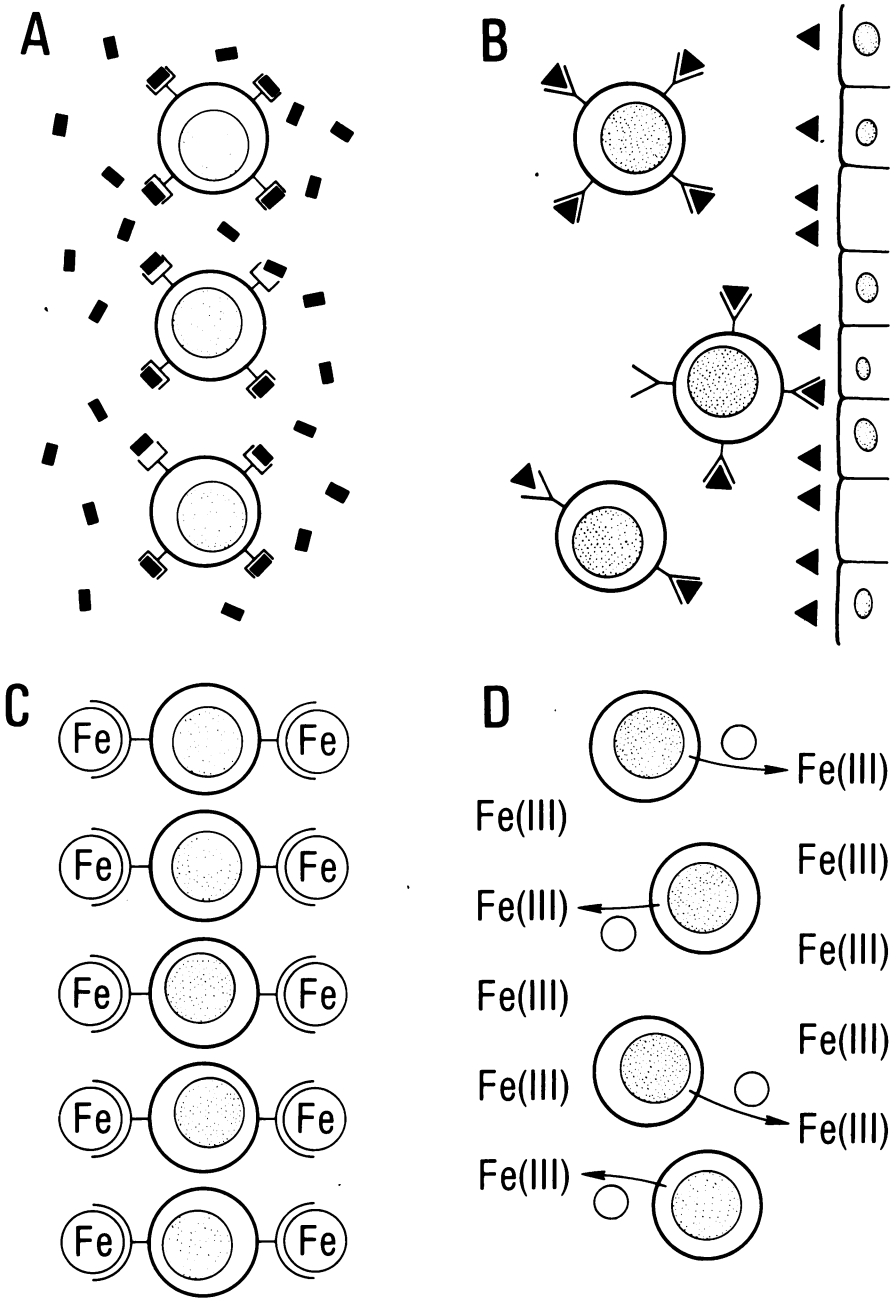
the same or other metalloproteins with lymphocytes [50,51] and macrophages [52] have demonstrated that human lymphocytes have receptors for transferrin,[50] that a subpopulation of T lymphocytes binds ferritin,[53,54] and that mouse peritoneal macrophages and lymphocytes have receptors for lactoferrin.[52]

With the discovery of the existence of such receptors on lymphocytes and the knowledge from earlier work that leukocytes synthesize lactoferrin [55] and that macrophages synthetize ferritin [56] and release intact metal-free transferrin,[57] it is possible to visualize a number of mechanisms that can lead to the accumulation of lymphocytes in the "wrong" place. A diagrammatic representation of such mechanisms is presented in Text-figure 2. This is only a tentative representation because, with the exception of the demonstration of ferritin on the surface of T lymphocytes, no attempt has been made to define whether T and/or B lymphocytes have receptors for transferrin [50] or lactoferrin.[52]

The finding of large numbers of lactoferrin-positive cells in the dermis associated with the mononuclear infiltration in the case of lymphomatoid papillosis may reflect one form of ecotaxopathy mediated by lactoferrin receptors on the surface of macrophages and lymphocytes (Text-figure 2B). The finding of the large numbers of lactoferrin-containing cells in the spleens of patients with HD, which to our knowledge has not been reported before, is of relevance to the T-lymphocyte accumulation in these spleens and to the accumulation of ferritin-containing macrophages. The latter, in turn, may offer a basis for further sequestration in the spleen of T cells with receptors for ferritin (Text-figure 2A). The reasons for the B lymphocytopenias observed in the same spleens remain unclear, however.

It was of interest to find that in the case of the monoclonal B-cell lymphoma, the virtual absence of splenic T lymphocytes concurred with the replacement of the TDA by heavy iron deposits. This observation is of interest because generally it is assumed that iron in the spleen is always present in the red pulp as the result of red cell phagocytosis. This was clearly not the case in this patient, and the replacement by iron-loaded macrophages of these areas normally experiencing traffic of lymphocytes [2] could readily explain the accumulation of the malignant lymphoid cells in the rest of the spleen. The absence of detectable lymphadenopathy in this case [58] may be explained by the cells' failure to circulate from the spleen to lymph nodes.

One alternative must be considered relating lymphocyte receptors for transferrin to accumulation of Fe(III)-transferrin complexes (Text-figure 2C). The blood is the obvious compartment where evidence for such an alternative should be sought, and one is tempted to speculate as to



TEXT-FIGURE 2—Summary of proposed models of lymphocyte ecotaxopathy. Lymphocytes with receptors for ferritin (A: ■), lactoferrin (B: ▲) and Fe(III)-transferrin (C: Fe) are likely to accumulate in sites where the metalloproteins are present in excessive amounts. The alternative, depicted in D, is based on the early observation [46] that human peripheral blood lymphocytes can synthesize transferrin (○).

whether the high serum iron levels and high transferrin saturations frequently present in acute T-cell leukemia could be directly linked to the leukemic process. In the study of the blood in one case of T-cell leukemia, we made the unexpected observation of stainable Fe(III) on red cells in a child with a serum iron of 400 μg and a total iron-binding capacity of 405.[59]

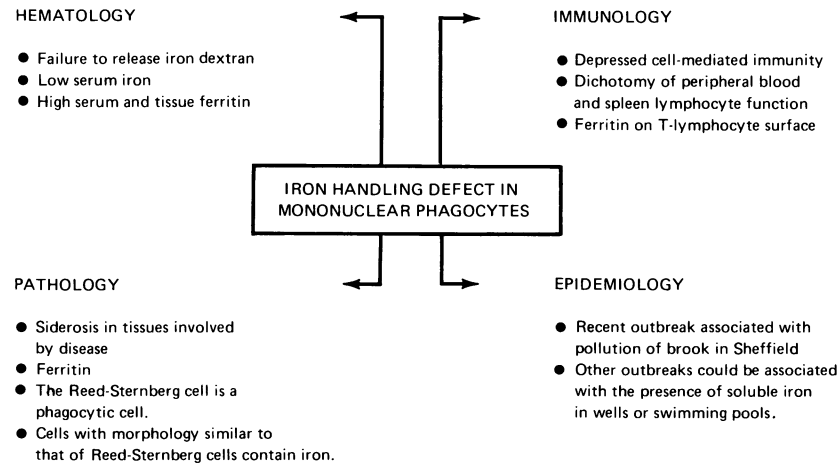
The alternative, illustrated in Text-figure 2C is based on the earlier report of Soltys and Brody [60] and considers the possibility that lymphocytes actively synthetize transferrin and are thus attracted to sites rich in iron.

Suggested Relationship Between Ecotaxopathy in Hodgkin's Disease and Pathogenesis of the Disease

Theoretic models of disease are essentially valueless if not applicable to the understanding of precise clinical situations. In this last section, we shall discuss how the present findings can be linked to other clinical and laboratorial aspects of HD, given the assumption mentioned earlier, ie, that the central defect in these patients is a failure of spleen and lymph node macrophages to handle iron normally (Text-figure 3).

A central defect of a population of phagocytes in lymph nodes and spleen with an abnormally high avidity for iron or a failure to utilize the metal normally could constitute the basis for the multiple hematologic, immunologic, pathologic, and epidemiologic features of HD, as follows:

1. Hematology. The well-established findings of low serum iron,[23,24]



TEXT-FIGURE 3—Summary of clinical and laboratory features of Hodgkin's disease and their possible association with a central monocyte defect. The iron-handling defect in mononuclear phagocytes occurs primarily in lymph nodes and spleen, not in target organs of hemochromatosis or syndromes of intestinal hyperabsorption of iron.

failure to release radiolabeled iron dextran normally from the reticuloendothelial system,[23] and unusually high tissue[45,46] and serum [61] ferritin levels are compatible with the suggested defect.

Further analysis of spleens from patients with HD is needed and a critical comparison with normal spleens must be done before too much importance can be attributed to the present findings. If confirmed, however, the present observation of large numbers of lactoferrin-containing cells in the spleen may be the reason for the frequent finding of low serum iron levels in patients with HD, thus giving further support to the proposal of van Snick, Masson, and Heremans [62] that lactoferrin plays a crucial role in control of sideremia.[63] Fever, another common clinical feature of HD, has also been implicated in the control of sideremia in infection.[64]

2. Immunology. The finding of depressed T-lymphocyte function in the early stages of the disease seems to coincide with the presence of abnormally high numbers of T lymphocytes in the uninvolved spleen.[37] Together with the evidence that lymphocytes have receptors for transferrin [50] and lactoferrin [52] and that T lymphocytes in patients with HD have ferritin on the surface,[53] a possible mechanism for the lymphocyte sequestration can be envisaged as discussed above. As lactoferrin-releasing cells accumulate in the spleen and iron-avid phagocytes are induced to synthesis of ferritin,[63] increased amounts of these proteins become locally available. When lymphocytes with appropriate receptors for the proteins cross the organ, they are likely to be attached to protein-synthetizing cells (Text-figure 2). We have some preliminary evidence for such a mechanism from the analysis of short-term cultures of splenic macrophages. Large clusters of lymphocytes remain attached to macrophages in cultures from spleens rich in transferrin- and ferritin-positive cells.[65]

When the disease progresses and ferritin levels in the serum increase (no analysis of lactoferrin in patients with HD has been done, to our knowledge), such iron-related proteins in serum may act as suppressive factors of E-rosette formation and T-cell function.[54]

3. Pathology. The classic pathologic feature of HD is the presence of large numbers of Reed–Sternberg cells. Studies of tissues from patients with HD *in vitro* have demonstrated conclusively that the malignant cell is a monocyte.[66] We suggest that it is a phagocyte whose excessive avidity for iron or inability to utilize the metal in a normal manner leads to its malignant transformation. The possible role of iron in carcinogenesis has been extensively discussed by Haddow and Horning.[67] Linder and Munro [68] have observed a direct relationship between increased iron:ferritin ratios and rate of tumor growth in hepatoma cell lines. The

question of the carcinogenic action of iron in humans has been raised again by the development of a poorly differentiated spindle cell fibrosarcoma at the site of injection of intramuscular iron.[69] If iron plays a role in carcinogenesis, its mechanism of action is unclear. Iron, however, has been shown to play a crucial role in control of cell division [70,71] and to be injurious to DNA.[72-74]

We are not certain whether the bizarre binucleated cells with intracytoplasmic and intranuclear iron represent the progeny of the malignant cell in HD, but their finding is of sufficient interest to warrant further investigation.

4. Epidemiology. The epidemiologic features of HD need further clarification in the light of the defect we are presently proposing. It is of interest to recall that Thomas Hodgkin, in the original description of the disease,[20] remarked on the fact that one child had a brother, "his constant companion with whom he had habitually slept, who died of phthisis a few months previously." The other child had been healthy "til about thirteen months ago, when his strength, flesh, and healthy appearance began to fail. He was at that time living in the west of England."

A more recent report from England has described the finding of a small cluster of 9 cases of HD in an area of less than 1 sq km, associated with a polluted water reservoir.[75] Differences in iron content of water should also be considered in the analysis of urban vs rural incidence of the disease.[28] We are investigating geographic distribution and eating, drinking, and swimming habits of the pediatric patients. The data are only in a preliminary form, but they suggest that many of the patients use water from wells located in geologic areas rich in iron. Although the defect may be present all the time, the disease may become apparent only if a patient is exposed to excessive iron intake. This could occur when a patient moves into a geologic area rich in soluble iron, takes a drug,[76] has an infection or a surgical operation that may cause macrophages to be exposed to regional or systemic disturbances of storage iron, ie, following tonsillectomy [26] or during anticonvulsant therapy.[76]

Conclusion

We have suggested that iron-binding proteins play a role in directing abnormal lymphocyte migration. Moreover, we suggested that in HD, abnormal handling of iron by populations of macrophages in lymph nodes and spleen may be directly linked to the pathogenesis of the disease, thus helping to explain its clinical, laboratorial, and epidemiologic features. The association of abnormalities of iron metabolism with malignancy is generally believed to be secondary to the malignant process. We are

raising the question that in some instances the link between the two may be more complex than hitherto anticipated. Weinberg has raised essentially the same question in an elegant discussion of the role played by iron in the balance of host–parasite interactions occurring during infection.[64] The finding of numerous lactoferrin-positive cells in the spleens of patients with HD (Figure 2) indicates that at least in this form of malignancy, the mechanism of hyposideremia may be similar to that proposed by van Snick, Masson, and Heremans [51] for the hyposideremia of infection. It seems worthwhile to conclude by quoting again Hodgkin's reference [20] to the boy with the disease, whose brother died of "phthisis a few months previously," wondering whether the same defect that made one brother susceptible to *Mycobacterium tuberculosis* made the other develop Hodgkin's disease.

Direct evidence of immunodeficiency in siblings of HD patients has been recently found by Björkholm et al [77] in a study of mitogen responses of healthy twins whose monozygotic or dizygotic same-sexed twin partner had died from HD.

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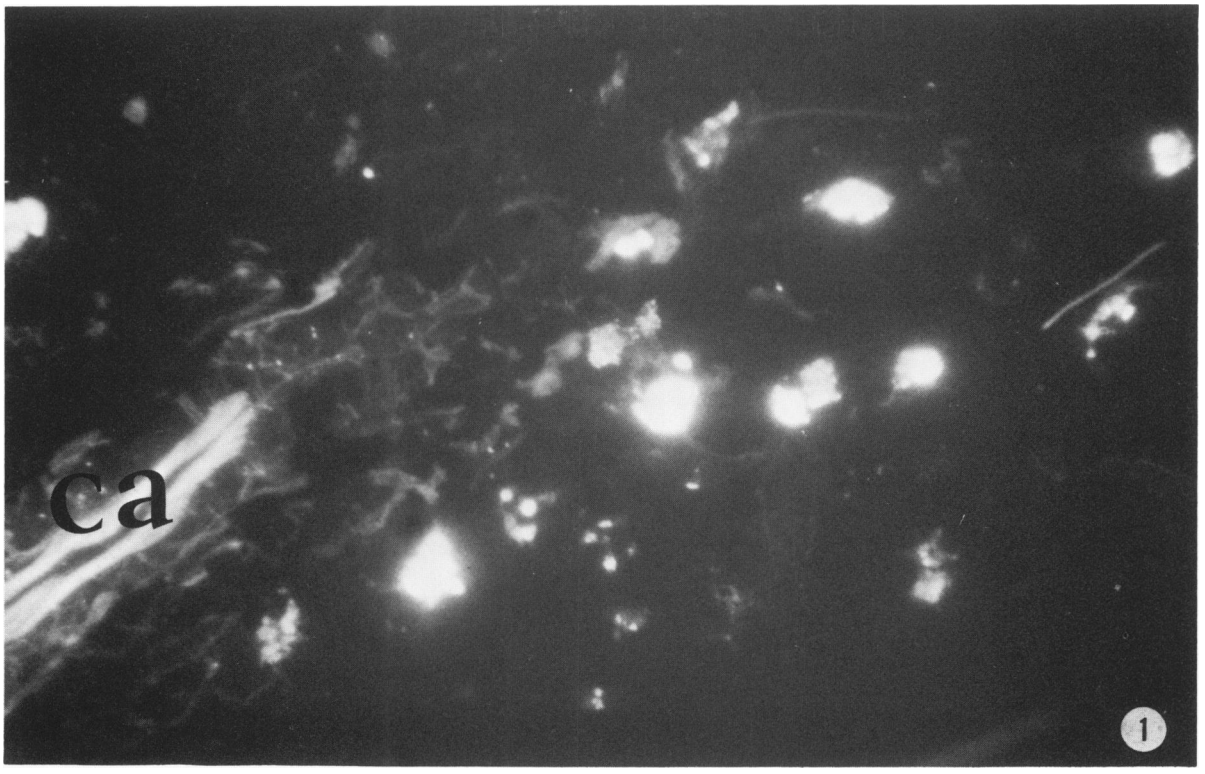
[*Illustrations follow*]



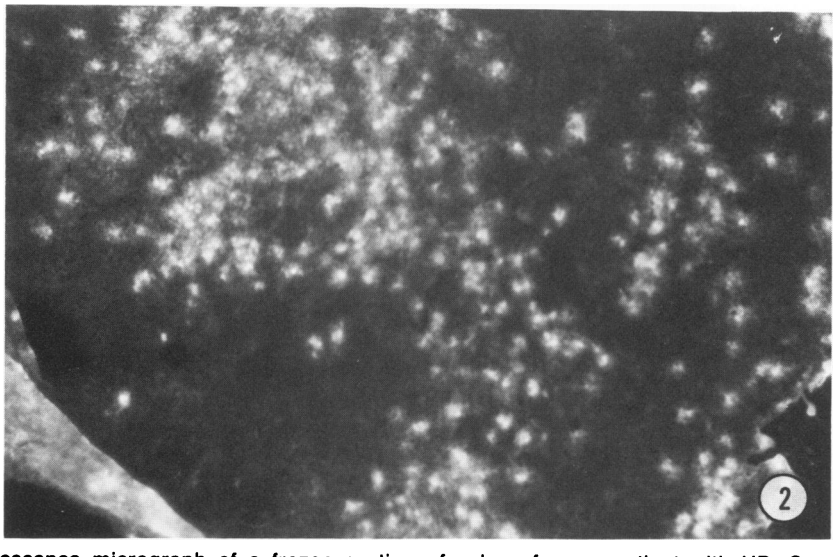


Figure 1—Fluorescence micrograph of a frozen section of spleen from a patient with HD. Several large, bright, granulous autofluorescent cells are visible in the thymus-dependent area (TDA) surrounding a small central arteriole (*ca*) (seen at lower left enmeshed in a web of collagenous fibers). Under the conditions of illumination used (wide-band blue light, 490 to 510 mm, Leitz Filterpak H), the cells appear brilliant yellow. The same cells also autofluoresce deep red with green light illumination (546 to 580 nm, Leitz Filterpak M). (× 1250) **Figure 2**—Fluorescence micrograph of frozen spleen section from patient with HD. The section has been stained for human lactoferrin by the "indirect" method using rabbit antihuman lactoferrin followed by FITC-conjugated goat antirabbit immunoglobulin. The lactoferrin-containing cells occupy the red pulp and marginal zone regions with occasional scattered cells in the white pulp. (× 300)

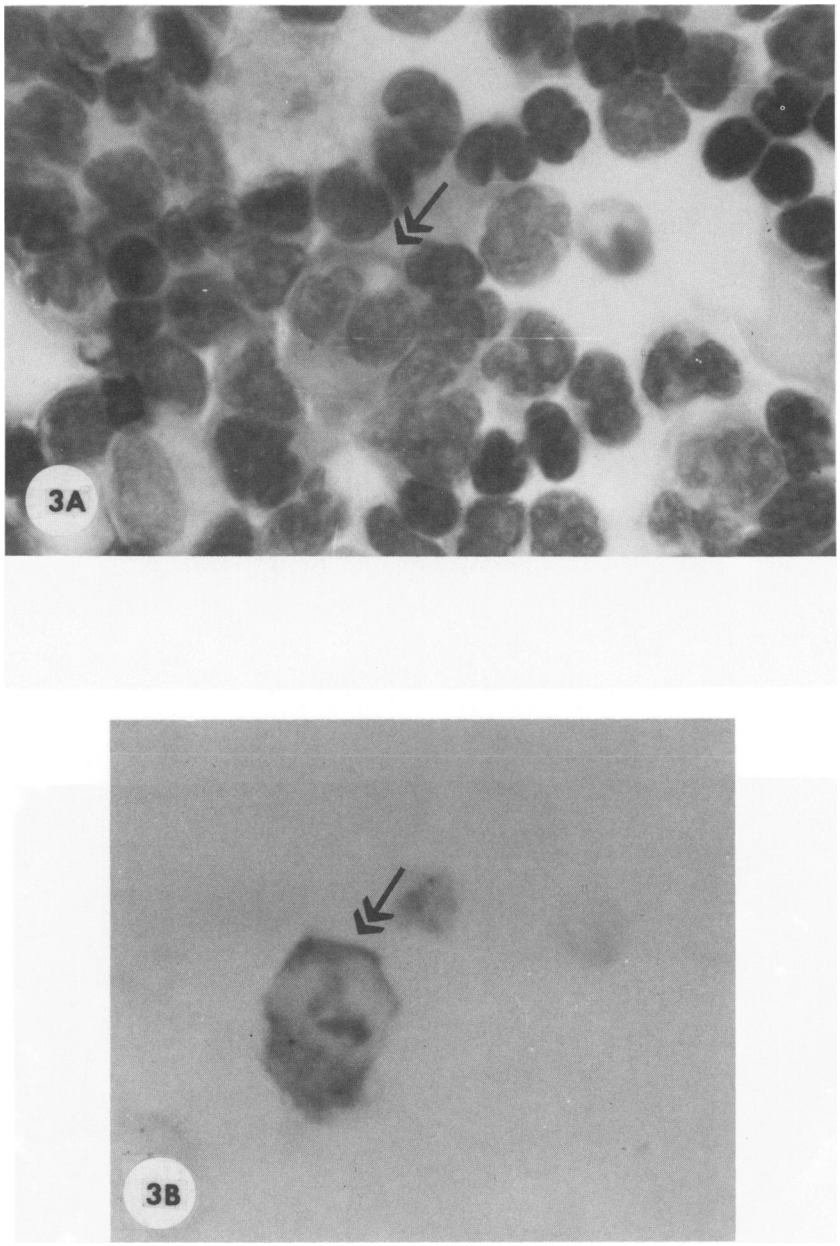
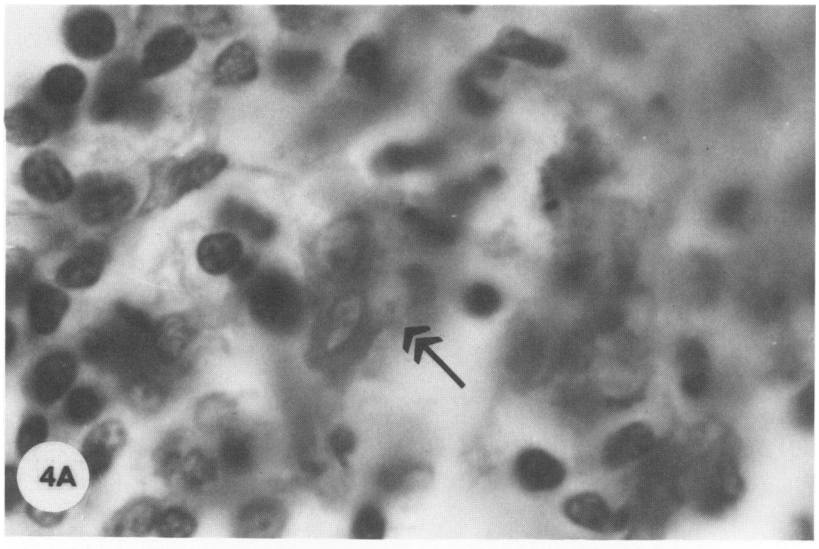


Figure 3—Spleen cell from patient with HD, stained for ferric iron (Prussian blue). **A**—*Arrows* indicate large binucleate cell containing intracytoplasmic iron. Taken with green interference filter. **B**—Same as **A** but taken with red filter. (× 1800)



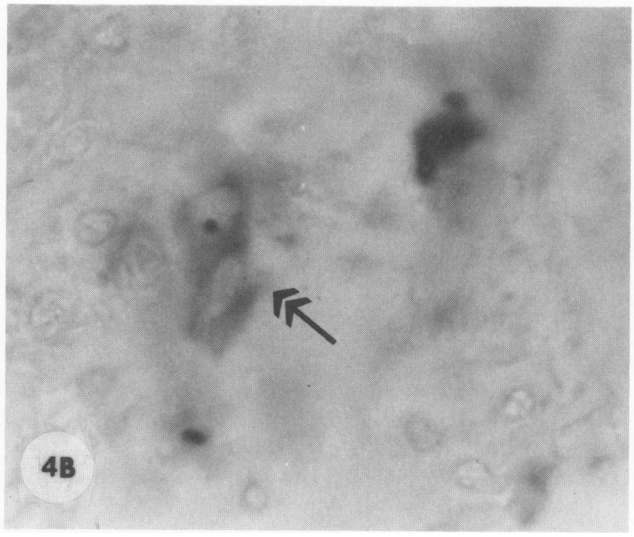
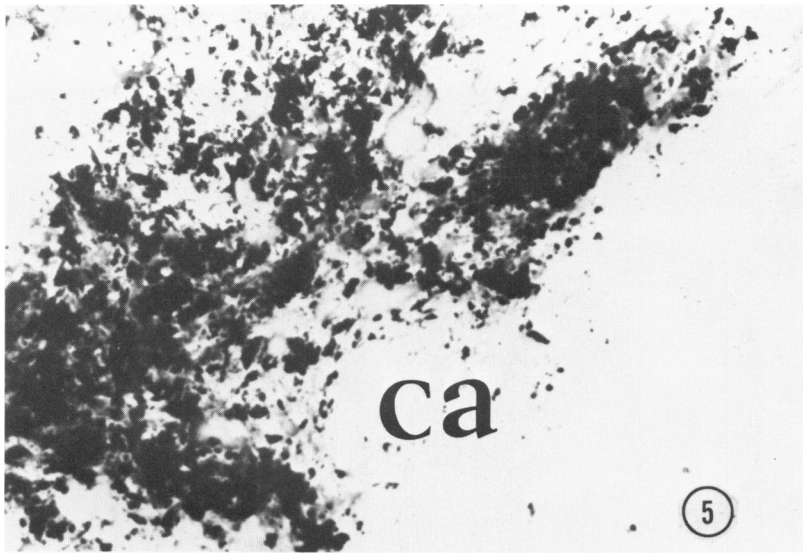


Figure 4—Lymph node section from patient with HD, stained for ferric iron. **A**—Bizarre, large binucleate cell containing intracytoplasmic and nucleolar iron. **B**—Same as **A** but taken with red filter. (× 1800)



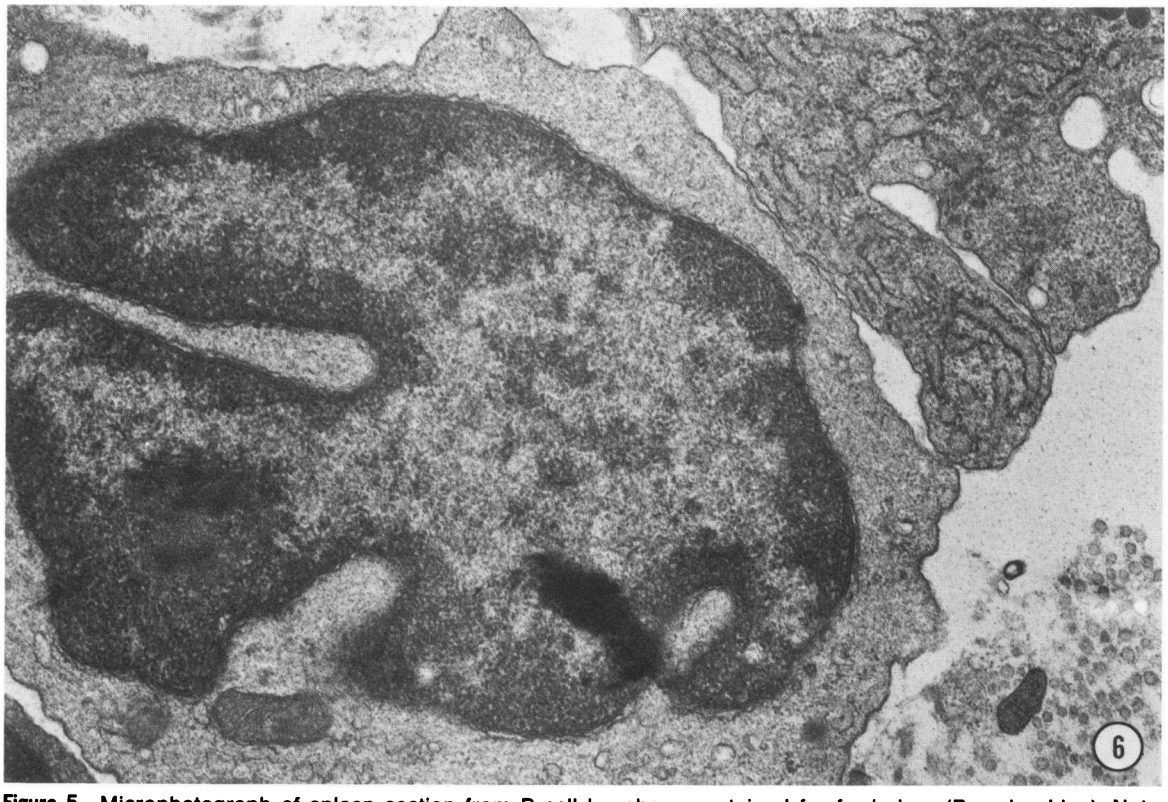


Figure 5—Microphotograph of spleen section from B-cell lymphomas, stained for ferric iron (Prussian blue). Note replacement of the thymus-dependent area around central arteriole (*ca*) by macrophages loaded with heavy clumps of iron. Taken with red filter. (× 180) **Figure 6**—Electron micrograph showing a cell with the morphology of a Sézary cell in lymph node of nude mouse killed 2 weeks after receiving a subcutaneous implant of skin from a patient with mycosis fungoides. A cell with similar morphology was found in the mouse's own skin. (× 8000) (Photograph taken by M. Vitale, from work in collaboration with Drs. B. Safai and E. de Harven.)