Impaired Lymphocyte Transformation in Hodgkin's Disease

EVIDENCE FOR DEPLETION OF CIRCULATING T-LYMPHOCYTES

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ABSTRACT The kinetics of lymphocyte transformation induced by phytohemagglutinin (PHA) and pokeweed mitogen (PWM) were studied daily, with blood lymphocytes from normal individuals and from untreated patients in all stages of Hodgkin's disease (HD). In addition, spleen lymphocytes and lymph node lymphocytes were studied with similar techniques.

Peripheral blood lymphocyte transformation stimulated by PHA was found to be depressed in all patients with HD (including those with localized disease and no symptoms) when small numbers of lymphocytes were cultured and studied during a 7-day period. Most patients with HD had an increased number of cells circulating in their blood which were actively synthesizing DNA. HD lymphocytes which demonstrated the highest initial rate of spontaneous DNA synthesis usually did not respond to PHA stimulation.

Blood lymphocytes from normal individuals responded equally well to PHA and PWM in our system. HD blood lymphocytes consistently responded better to PWM than to PHA, with the response to PWM frequently within the normal range. Unless the spleen was extensively infiltrated with HD, spleen lymphocytes from patients with HD responded to PHA, even though the blood lymphocyte response was severely reduced. Lymph node lymphocyte response to PHA from patients with HD was variable, but correlated roughly with the blood lymphocyte response.

It is hypothesized from the data presented that in HD, circulating thymus-dependent (T-)lymphocytes are stimulated by the presence of active disease. This stimulation of T-lymphocytes leads to a circulating T-cell depletion and to an increase in the number of cells circulating that are active in DNA synthesis. The degree of impairment of cell-mediated immunity would then depend upon the degree of T-lymphocyte depletion.

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INTRODUCTION

Well-recognized concomitants of advanced Hodgkin's disease (HD) [1] include abnormalities in cell-mediated immune reactions as manifested by increased susceptibility to certain pathogens (1), impaired delayed cutaneous hypersensitivity (2–7), prolonged skin graft rejection (8), and depressed in vitro lymphocyte transformation (9, 10). Reports have varied, however, about the degree and consistency of impaired lymphocyte function in patients with HD, particularly in those patients in the early stages of the illness (11–19). While it appears that defective cell-mediated immunity may not be of etiologic importance in HD, it may be critically important in the pathophysiology of the disease and its complications.

It is now recognized that small lymphocytes in the circulating blood comprise a heterogeneous population of cells which, despite similar morphologic characteristics, have different life spans, cell surface components, receptor sites, and immunologic functions. In man and other animal species, there exists a distinct population of thymus-dependent lymphocytes (T-lymphocytes) that are primarily involved in cell-mediated immune reactions. A thymus-independent population, which in fowls is dependent on the bursa of Fabricius for development, has also been identified and is designated as bursa-equivalent lymphocytes (B-lymphocytes). These cells play a central role in humoral antibody synthesis

[1] *Abbreviations used in this paper:* B, bursa-equivalent; HD, Hodgkin's disease; [^{3}H]TdR, tritiated thymidine; PHA, phytohemagglutinin; PWM, pokeweed mitogen; T, thymus-dependent; TCA, trichloroacetic acid.

(20–22). Recent studies indicate that cell populations may be identified by their response to various mitogens. Although not yet settled, it appears that phytohemagglutinin (PHA) stimulates T-lymphocytes predominantly, if not exclusively (23–35), whereas pokeweed mitogen (PWM) may stimulate both B- and T-lymphocytes (32, 33). There is, however, some data to suggest that the B-lymphocyte response to PWM may be greater than that of the T-lymphocyte (31, 33, 35).

The major objective of the present study was to use a standardized quantitative test of lymphocyte transformation employing both PHA and PWM as the mitogenic stimulus to investigate the response in patients with untreated HD. Emphasis was placed on the time-course as well as the degree of the mitogen-induced transformation. Havemann and Rubin have previously shown the discriminatory value of such a kinetic approach in studies of lymphocyte transformation in chronic lymphocytic leukemia (36). Results presented herein provide evidence for the thesis that there are consistent abnormalities in PHA-induced lymphocyte transformation for all patients with HD. In addition, the data indicate that a mechanism for the abnormal PHA response in HD involves the depletion of a lymphocyte population in the blood capable of responding to PHA stimulation.

METHODS

Patient selection. 26 untreated patients representing all clinical stages and histologic cell types of HD comprised the test group and were compared with 20 healthy laboratory personnel free from recent viral illnesses (Table I). Clinical staging was determined by lymphangiography and bone marrow biopsy in all patients, and exploratory laparotomy when required, according to the Rye classification (37). Pathologic interpretation of the histologic material was performed by the Department of Pathology, Duke University Medical Center, using the Lukes-Butler classification (38); in many instances the tissue sections were also reviewed by the Lymphoma Task Force. With the advent of laparotomies for staging, we were able to obtain adequate lymph node lymphocytes for culture in five patients and spleen lymphocytes in nine patients. In the nine patients undergoing laparotomy, peripheral blood was studied before surgery in five patients, on the day of surgery in three patients and after splenectomy in one patient. None of the patients studied had received steroids, analgesics, or phenothiazines except for one patient, studied after splenectomy, who had received a total of 200 mg of meperidine 48 h before the study of his peripheral blood lymphocytes. Only one normal spleen could be obtained for comparison and that was removed from a 12-yr-old boy with idiopathic portal hypertension. Pathological examination of this spleen revealed no evidence of intrinsic abnormality in the spleen, and the patient had not received corticosteroids or other agents known to affect lymphocyte transformation.

Specimen preparation and culture techniques. Under sterile conditions, "buffy coat" leukocytes were obtained by sedimentation of 50 ml heparinized blood (5 U/ml) to which 10 ml Plasmagel (Laboratoire Roger Bellon, Neville, France) was added. Lymphocytes from fresh surgical specimens of spleens and lymph nodes trimmed of fat were teased in cold TC-199 with fine scissors. When spleen specimens contained nodules of obvious HD, these areas were avoided and only uninvolved portions of the spleen were used to obtain lymphocytes for culture. Particulate tissue was allowed to sediment by gravity and the leukocyte-rich supernate was centrifuged at 1000 *g* for 15 min. The cell button was resuspended in fresh TC-199. From this point on, peripheral blood leukocytes and tissue lymphocyte suspensions were processed in an identical manner. Triplicate cell counts were made on each sample and the cell concentration adjusted with TC-199 to contain 2.0×10^6 lymphocytes/ml. The number of granulocytes present was determined for each peripheral blood culture. 30 individual culture tubes were then prepared with 2 ml of modified McCoy's 5a media (15% fetal calf serum) containing no mitogen, 2% PHA-M or 1% PWM (Grand Island Biological Co., Grand Island, N. Y.). 0.5 ml of the lymphocyte suspension was added to each culture, giving a final lymphocyte concentration of 0.4×10^6 lymphocytes/ml. This concentration of lymphocytes was chosen after a cell concentration and PHA response curve had been determined on the 4th day (Fig. 1). Thymidine incorporation per cell was greatest with lymphocyte concentrations from 0.2 to 0.4×10^6 ml but was

TABLE I
Patient Population Studied

Patient	Age	Sex	Stage	Histology*
P. G.	32	F	IA	mc
C. L.	57	M	IA	lp
M. R.	23	M	IA	mc
E. A. J.	20	M	IA	lp
M. P.	22	M	IIA	ns
B. A.	27	F	IIA	ns
C. D. H.	27	F	IIA	mc
A. C.	29	M	IIA	ns
C. J.	40	M	IIA	mc
C. McF.	18	M	IIA	mc
P. S. S.	21	F	IIA	ns
D. G.	17	F	IIA	ns
T. S.	33	F	IIA	ns
D. D.	32	F	IIA	ns
J. C. P.	20	M	IIB	ns
G. S.	56	F	IIB	lp
B. S.	20	F	IIB	mc
T. G.	21	M	IIIA	mc
J. B.	53	M	IIIB	ns
E. B.	25	F	IIIB	mc
G. H.	37	M	IVB	ld
R. P.	37	M	IVB	mc
A. S.	46	M	IVB	ns
E. W.	18	M	IVB	ld
G. M. G.	32	F	IVB	mc
J. McG.	51	M	IVB	ld

* ld, lymphocyte-depleted; lp, lymphocyte predominance; mc, mixed cellularity; ns, nodular sclerosis.

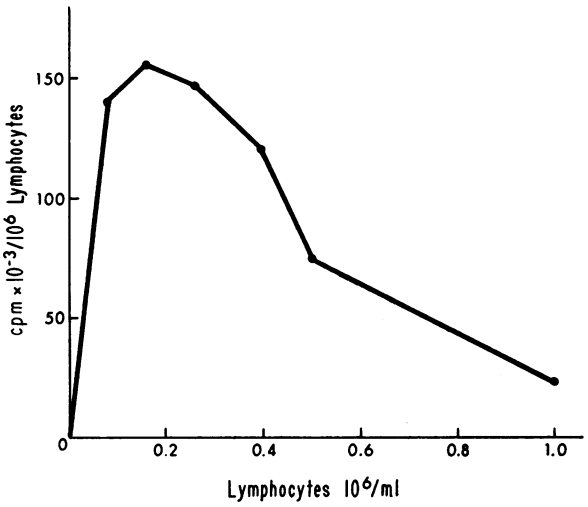

FIGURE 1 Effect of lymphocyte concentration on the uptake of [^{3}H]TdR in PHA-stimulated cultures of normal lymphocytes. Cells were exposed to [^{3}H]TdR for a 2-h labeling period on the 4th day of culture.

depressed with higher cell concentrations. In 10 experiments lymphocytes were also cultured in 20% autologous plasma. Cultures were incubated at 37°C in 5% carbon dioxide in air.

Determination of relative rate of thymidine incorporation. 1 h after the start of incubation and at the end of each subsequent 24-h period for 7–10 days, triplicate samples from each group were labeled with 5 μCi of tritiated thymidine ([^{3}H]TdR, sp act 20Ci/mmol, New England Nuclear, Boston, Mass.). After the labeled cultures were incubated for an additional 2 h, incorporation of [^{3}H]TdR was stopped by centrifuging the cultures for 10 min at 2000 *g* at 0°C. The supernate was discarded and the cell button solubilized in 1 ml of 1.0 N NaOH and 0.01 N disodium EDTA solution. The samples were then stored at 4°C until all samples in one group had been collected. 0.1 ml of the solubilized material was pipetted onto Whatman 3MM filter paper disks, air-dried, and the DNA precipitated onto the disks by submerging them in ice-cold 5% trichloracetic acid (TCA). The disks were washed twice with cold 5% TCA, twice with cold 95% ethyl alcohol, and once with acetone. Once dried, the radioactivity of each disk was determined by covering it with 3 ml toluene phosphor and counting in a Nuclear-Chicago Scintillation Counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Background activity was determined by incubating triplicate culture tubes in ice with [^{3}H]TdR for 2 h and then processing them in the identical manner described above. Background activity was always less than 50 cpm. After correction for background, the counts per minute of triplicate samples was averaged and specific activity expressed in counts per minute per 10^6 lymphocytes, the total number of lymphocytes in each 2.5-ml culture.

RESULTS

Peripheral blood lymphocytes. None of the patients with HD had an entirely normal response to PHA-induced lymphocyte transformation over the 7-day period. Fig. 2 illustrates the kinetics of PHA-lymphocyte transformation in 26 patients grouped according to the stage of disease, compared to the range of transformation obtained in 20 normal individuals. Although in stage IA patients there was some overlap with the normal response on days 2 and 3 of culture, all patients had an abnormal response to PHA on day 4 and thereafter. The difference in PHA response between the HD patients and the normals was statistically significant from day 4 on when analyzed by Student's *t* test ($P < 0.001$). Lymphocytes from patients that did have some response to PHA, albeit reduced, showed a normal time-course of response. Cultures from patients with HD contained more granulocytes than did the normal controls (Fig. 3A). The increased granulocyte content was seen in cultures from patients in all stages of HD, and did not

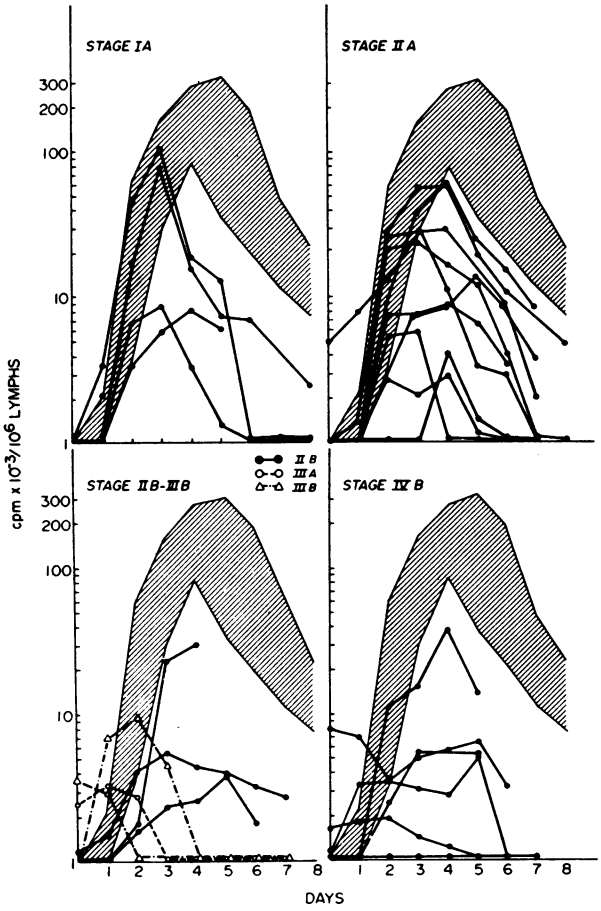

FIGURE 2 Kinetics of PHA-stimulated uptake of [^{3}H]TdR by blood lymphocytes in cultures from 26 patients with untreated HD, compared to the response of those from 20 normal subjects. Patients are grouped according to stage of disease as indicated in each panel. For reference the shaded area depicts the range of the normal lymphocyte response to PHA.

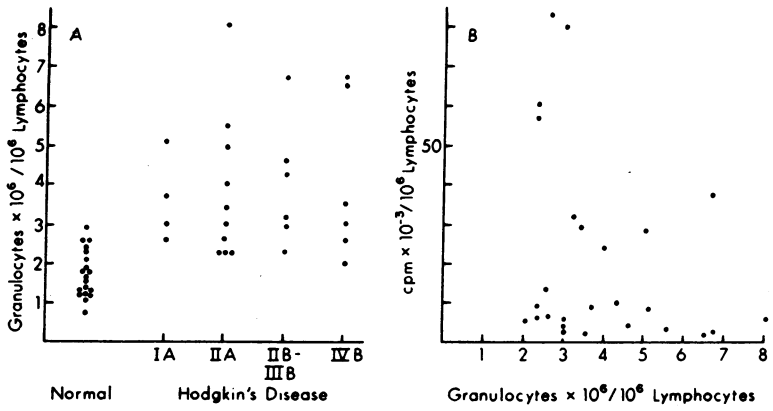

FIGURE 3 Granulocyte content of peripheral blood cultures. A: Concentration of granulocytes per 10^6 lymphocytes in cultures from normals and HD patients grouped according to stage of disease. B: Relationship of peak PHA response by HD lymphocytes to granulocyte content of culture.

correlate with the extent of disease. This was because patients with stage I–II disease usually had an absolute granulocytosis, but those patients with advanced disease had an absolute lymphocytopenia. The degree of PHA unresponsiveness in the patients with HD did not correlate with the number of granulocytes in culture (Fig. 3B). The effect of autologous plasma on PHA-induced transformation was variable in the HD patients (Table II). At times transformation was improved when lymphocytes were incubated free from autologous plasma, but in no instance did the PHA-response curve return to normal. The most common result was no significant change in lymphocyte transformation when cultured with or without autologous plasma.

Another difference apparent in the HD patients' peripheral blood cells was an increased uptake of [^{3}H]TdR on day 0 (Fig. 4). This was spontaneous [^{3}H]TdR uptake and occurred with or without PHA in the culture medium. The differences noted were significant, with a P value of < 0.002. Fig. 4 also illustrates that lymphocytes of patients with HD who had the highest initial spontaneous uptake of [^{3}H]TdR usually did not respond to PHA. In our series there were only four patients with this pattern of response, all of which had clinical stage III or IV disease. Because the number of patients in this study was not sufficiently large, we cannot yet firmly correlate other responses with specific stage or histology

TABLE II
Effect of Autologous Plasma on HD Lymphocyte Resopnse

Patient	Peak PHA response*	
	Autologous plasma	Fetal calf serum
E. W.	6.0	20.0
G. S.	8.4	5.5
P. S. S.	18.7	24.2
M. R.	81.0	62.3
R. P.	25.5	37.2
T. G.	1.1	4.9
M. P.	14.6	13.7
D. G.	62.5	63.9
T. S.	33.0	61.4
D. D.	44.3	29.4
B. S.	4.2	35.0

* cpm × $10^{-3}/10^6$ lymphocytes.

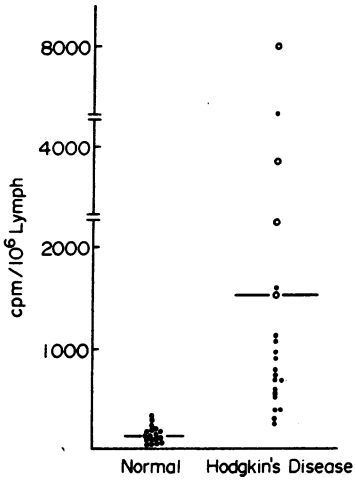

FIGURE 4 Spontaneous DNA synthesis by unstimulated lymphocytes from HD patients and normal subjects. Uptake of [^{3}H]TdR by lymphocytes was determined on the day of isolation (day 0 of incubation) after a 2-h labeling period. Horizontal lines indicate the mean uptake of each group. (●) represents those patients who showed a subsequent response to PHA and (○) indicates those patients who did not respond to PHA.

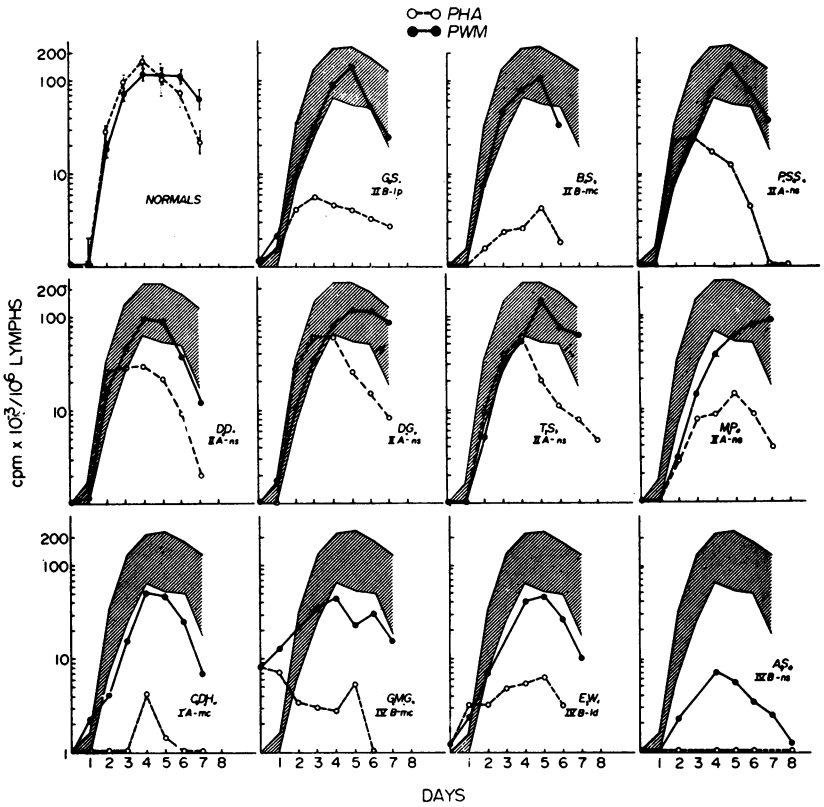

FIGURE 5 Comparison of the kinetic response of [^{3}H]TdR uptake by HD and normal blood lymphocytes stimulated with either PHA or PWM. Upper left, indicated as normals, represents the mean response ±1 SE of lymphocytes from 10 normal individuals. The other panels demonstrate the response of 11 HD patients, identified by their initials, stage of disease, and histologic pattern of HD (lp, lymphocyte predominance; mc, mixed cellularity; ns, nodular sclerosis; ld, lymphocyte depleted). For reference, the shaded area depicts the range of the normal lymphocyte response to PWM. (●), PWM response; (○), PHA response.

except to note that generally the more extensive the disease, the poorer the PHA response.

Blood lymphocytes from normal individuals respond equally well to both PHA and PWM, although the response to PWM may be prolonged (Fig. 5). Fig. 5 compares the PHA and PWM response in 11 patients with HD. It is evident that the PWM response in these patients is normal or approaches normal, even though the PHA response is reduced. In each instance, the PWM response in HD patients was significantly increased over the PHA response, whereas in normals, response to the two mitogens was quite similar.

Spleen lymphocytes. The kinetics of PHA-induced lymphocyte transformation for lymphocytes obtained from spleen and blood of eight patients with HD are illustrated in Fig 6. Control cultures without mitogen had no increase in [^{3}H]TdR uptake over the 7–10-day period. In all patients, except J. McG.[2] (not shown), spleen lymphocytes incubated with or without autologous plasma responded much more than blood lymphocytes, although the response was delayed and frequently showed a heterogeneous pattern, with peaks on the 4th, 5th, or 6th day of culture, and again on the 7th or 8th day of culture. Fig. 7 shows the mean and standard error of the studies performed on lymphocytes obtained from the spleens and peripheral blood of these eight patients with HD, contrasted with the response available from one normal spleen. The PHA response of the normal spleen lymphocytes was delayed, with a broad pattern nearly identical to the mean value of the HD spleen lymphocytes.

Lymph node lymphocytes. Lymphocytes from lymph nodes of patients with untreated HD were available for study in five instances. As depicted in Fig. 8, the re-

[2] Patient J. McG. is the only patient whose spleen lymphocytes did not respond to PHA. He had stage IVB HD with a massively enlarged spleen diffusely infiltrated with HD. It was impossible to find an uninvolved area for study and in this respect the patient differed from all other patients studied.

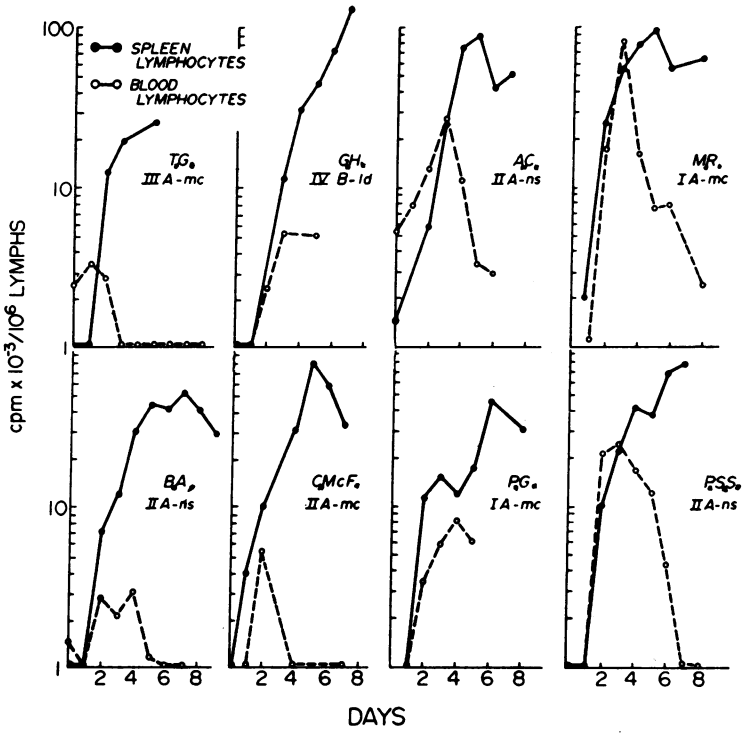

FIGURE 6 Kinetics of PHA-stimulated uptake of [^{3}H]TdR by blood and spleen lymphocytes in eight patients with HD. Each panel represents eight patients, identified by their initials, stage of disease, and histologic pattern of HD. (mc, mixed cellularity; ld, lymphocyte-depleted; ns, nodular sclerosis).

sponse of lymph node lymphocytes to PHA was variable, but was usually poor and correlated roughly with the blood lymphocyte response. Patients T. G. and E. B. had a poor PHA-blood lymphocyte response with a similar lymph node lymphocyte response. Occasionally a single peak of PHA response occurred, as with patients G. H. and D. P. Four of the patients had HD involving the lymph node, from which the lymphocytes were taken for study. C. J.'s lymph node contained very few lymphocytes, histologically was not diagnostic for HD, and did not respond to PHA.

DISCUSSION

All of the HD patients in this series had abnormal PHA-induced blood lymphocyte transformation when the daily uptake of [^{3}H]TdR was used as an index of transformation. Aside from the fact that all of the patients in this study were previously untreated, this study differs in two important aspects from published reports that have concluded that lymphocyte transformation in HD is variable and often normal in the early stages of the disease (11, 15–19). First, we observed the entire time-course of the PHA course. The deficient transformation

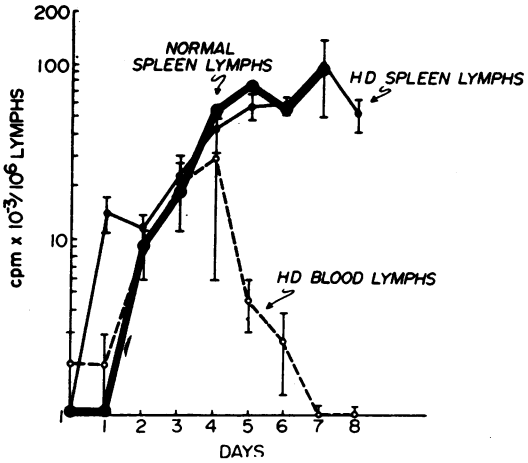

FIGURE 7 Kinetics of PHA-stimulated uptake of [^{3}H]TdR by HD spleen and blood lymphocytes, compared with the response by normal spleen lymphocytes. (●), mean response ±1 SE by spleen lymphocytes from seven HD patients; (○), mean response ±1 SE by blood lymphocytes from the same seven patients with HD; dark line represents the response by normal spleen lymphocytes from one individual.

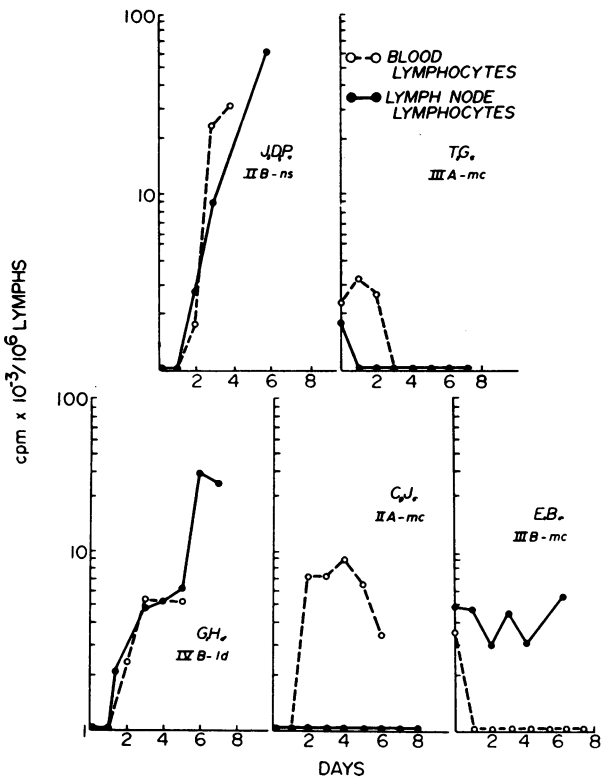

FIGURE 8 Kinetics of PHA-stimulated uptake of [^{3}H]TdR by blood and lymph node lymphocytes in five patients with HD. Each patient is identified by initials, stage of disease, and histologic pattern of HD. (ns, nodular sclerosis; mc, mixed cellularity; ld, lymphocyte-depleted).

in HD patients' blood lymphocytes would not have been as apparent if only one point in time, e.g., day 3, had been taken to represent the entire PHA response. The deficiency is striking, and statistically significant when the kinetics of the complete PHA response are compared with those of the normal PHA response. Second, our in vitro cultures contained limiting concentrations of lymphocytes. The small number of lymphocytes per culture allowed for a maximum PHA-induced [^{3}H]TdR uptake per lymphocyte, and perhaps allowed this system to be more sensitive to small defects in transformation that might be missed if an excess of lymphocytes capable of responding to PHA were present. The number of stage IA and IIA patients in this series was not sufficiently large to conclude that *all* HD patients, no matter how minimal the extent of involvement, have detectable abnormalities in lymphocyte transformation. However, the demonstration of impaired PHA response in all patients in this series, including those with localized disease and no symptoms, indicates that impaired lymphocyte function is inherent to HD and is not a complication of far-advanced and extensive disease only.

In this study, peripheral blood cultures from HD patients contained more granulocytes than did the normal cultures. In one study, increasing concentrations of granulocytes appeared to inhibit PHA-induced lymphocyte blastogenesis (39), and there are data to indicate that high granulocyte concentrations have an inhibitory effect in mixed leukocyte cultures (40–42). Although the inhibitory effect of granulocytes must be considered as a possible explanation for the impaired PHA response by HD lymphocytes, the lack of correlation between PHA unresponsiveness and granulocyte content, and the normal PWM response in the face of depressed PHA response argues against this possibility.

In addition to the fact that all of the HD patients had a reduced PHA response, most HD patients had cells circulating in their blood active in DNA synthesis. Those patients who showed no transformation to PHA had the highest spontaneous uptake of [^{3}H]TdR. The difference between HD patients and normal subjects in this regard was statistically significant, and as can be seen from Fig. 4, there was little overlap in values. Similar observations of increased spontaneous DNA synthesis by the leukocytes of the HD patients have been made by Crowther, Fairley, and Sewall (43, 44). Utilizing autoradiographic techniques, they showed that the cells actively engaged in DNA synthesis were large pyroninophilic lymphocytes, and that similarly increased numbers of these lymphocytes also occurred after various infections and after immunizations. Recently, Hersh, Butler, Rossen, Morgan, and Suki (45) have also shown an increased [^{3}H]TdR uptake by blood lymphocytes from patients in the early phase of renal transplant rejection. Several previous reports on lymphocyte transformation in HD have noted that in a small number of patients, no PHA-induced lymphocyte transformation occurred, and that the cells "died" by the 3rd or 4th day of culture (11, 13, 17). The reciprocal relationship shown in this report between spontaneous [^{3}H]TdR uptake at the initiation of culture and the subsequent absence of PHA-induced transformation by HD lymphocytes is similar to the in vitro behavior of lymphocytes from patients with renal transplant rejection and chronic uremia (45, 46). This relationship indicates that lymphocytes already transformed in vivo may be refractory to or may not survive for in vitro stimulation. More direct evidence for this type of relationship is seen with spleen cells from tumor-bearing mice or immunized with membrane antigens. Spleen cells recovered from these animals had undergone in vivo transformation, as judged by increased unstimulated DNA synthesis and a shift of the cell population to a less dense fraction on density gradients, and they also had an impaired blastogenic response to PHA in vitro (47). However, an earlier study using rabbit spleen cells demonstrated increased

in vitro PHA response in animals previously immunized as compared to control animals (48). Whether the four patients with no PHA response and high spontaneous DNA synthesis represent a distinct group or whether they represent the end of a spectrum of patients with a gradually increasing number of blood lymphocytes active in DNA synthesis and a depletion of cells capable of responding to PHA is not clear. The latter seems more likely since most of the patients with some PHA response also had a significant increase in spontaneous [^{3}H]TdR uptake.

The PWM-induced response of HD blood lymphocytes were normal or approached normal, but the PHA response in the same patients was distinctly abnormal. In normal individuals the two responses in our system were nearly identical, whereas in HD the PWM response was always greater than the response to PHA. All patients with HD responded to PWM, including one patient who had no response to PHA. From these data it appears that the abnormality in lymphocyte transformation in HD is relatively specific for the PHA-stimulated lymphocyte. If in fact the PWM-induced response reflects to some degree B-lymphocyte function in man, as has been suggested (31), these data correlate well with the observations that humoral immunity remains relatively intact in HD (7, 11, 49).

Unless the spleen was completely infiltrated with HD, spleen lymphocytes responded to PHA with increased [^{3}H]TdR uptake, even though the blood lymphocytes from the same patient either failed to respond or responded poorly to PHA. The spleen lymphocyte response differed from the peripheral blood response in that it was delayed and sustained, but the magnitude of the spleen lymphocyte PHA-induced response was in the same range as the normal blood lymphocyte response. In the one normal spleen available, a similar delayed and sustained response was seen, which was nearly identical to the mean response of the eight HD spleens studied. Although it is not yet possible to explain the kinetic pattern of the spleen lymphocyte response to PHA, it is apparent that in HD, spleen lymphocytes undergo blast transformation, even when the blood lymphocytes are severely deficient in PHA responsiveness. This does not appear to be a plasma effect, since incubating spleen cells in autologous plasma did not inhibit the spleen lymphocyte PHA response. The observation that spleen lymphocytes can still respond to PHA when blood lymphocytes do not suggests that the spleen may be an important storage compartment for these cells. Indeed T-lymphocytes, identified by characteristic membrane receptors, have been found to be diffusely distributed throughout the human spleen but have not been found in the follicular areas of the white pulp (50). Whether this distribution is altered in HD and interferes with the normal transit of T-lymphocytes is not known. Lymph node lymphocytes responded variably to PHA, but in general the response of these cells paralleled that of the peripheral blood. Unfortunately, the number of lymph nodes studied to date is too small to correlate the type of response with the histologic pattern.

There are at least four possible mechanisms for an abnormal PHA lymphocyte response in vitro: first, one or more serum factors inhibiting PHA-induced lymphocyte transformation, second, an intrinsic abnormality of the circulating lymphocyte, third, a central defect preventing the development of PHA-responsive lymphocytes, and fourth, a decreased number of PHA-responsive cells present to respond to the stimulus.

Although serum factors have been implicated in the diminished PHA-induced lymphocyte transformation in HD (13), most studies demonstrate either no consistent inhibition by HD plasma (10, 14), or a persistent defect in lymphocyte transformation when cultured in normal serum (15, 17, 51). In a recent study, Han was unable to demonstrate any inhibitory effect of plasma from a large number of patients with active HD on the PHA response by normal lymphocytes (52). In the present study, both the failure of plasma removal to significantly improve the PHA response of HD blood lymphocytes and the ability of HD spleen lymphocytes cultured in autologous plasma to respond to PHA argue against serum factors being an important cause of the abnormal PHA response in HD. In view of the PWM response by HD lymphocytes, any inhibitory serum factor postulated would have to have a differential effect, inhibiting PHA-induced transformation more than the PWM-induced transformation.

The normal kinetic but diminished HD blood lymphocyte response to PHA, the differential response seen when these lymphocytes are stimulated with PWM, and the response of spleen lymphocytes to PHA in the face of a diminished blood lymphocytes response support the concept that impaired PHA lymphocyte transformation in HD is due to a depletion of circulating cells capable of responding to PHA, rather than to an intrinsic defect of the lymphocyte, or a failure of development of PHA-responsive lymphocytes. The observation that PHA transformation can return to normal in patients achieving a remission of their disease after treatment (16, 17) indicates that this depletion of PHA-responsive lymphocytes is a reversible phenomenon.

As indicated earlier, there is suggestive evidence from experimental animal studies, as well as observations in patients with immunodeficient diseases, to consider PHA responsiveness a function of the T-lymphocyte system, and PWM responsiveness a measure of the B-lymphocyte system (although not exclusively) (20–35, 53). Our data may indicate that in HD a depletion of circulating

T-lymphocytes occurs, probably in all stages of disease, but most evident in advanced HD, while B-lymphocytes appear to be present in relatively normal amounts. Along with the depletion of T-lymphocytes, a new population of cells appear in the blood actively synthesizing DNA. These cells might be abnormal (or even malignant) but because a similar increase in DNA-synthesizing lymphocytes has been shown to occur after in vivo antigenic stimulation, these cells may very well represent lymphocytes already stimulated and committed.

From the data, we hypothesize that in HD, circulating T-lymphocytes are stimulated by the presence of HD in the involved lymph node. The finding of HD tumor-associated antigens may indicate the reason for this stimulation (54). Whether this is a response to the presence of a malignant reticulum cell, or, as hypothesized by Order and Hellman (55), a response of T-lymphocytes to virus-infected lymphocytes, which then leads to the development of neoplastic reticulum cells is not known. This stimulation of T-lymphocytes leads to a circulating T-cell depletion and to an increase in the number of cells circulating which are active in DNA synthesis. The degree of impairment of cell-mediated immune reactions seen in HD will then depend upon the degree of T-lymphocyte depletion and the sensitivity of the tests used to detect these reactions.

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