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On The Biology and Immunology of Hodgkin's Disease*

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A. Neoplastic Nature of Hodgkin's Disease

The nature of Hodgkin's disease has been the subject of more than 100 years of intense debate. The occurrence of massive lymphadenopathy, with later spread to the lungs, liver, bone marrow, and other tissues, and the inevitably fatal course of the disease suggested to many scholars that it was a form of malignant neoplasm. Others, however, impressed with its frequently febrile course, with the occasional waxing and waning in size of enlarged lymph nodes, and with the frequent coexistence of tuberculosis or other infectious diseases at autopsy, considered it some form of granulomatous infection or inflammation. Finally, as awareness has grown concerning the curious defect of immune responsiveness which occurs so often in Hodgkin's disease, a third hypothesis has been put forward suggesting that it may stem from a chronic immunologic disorder. Certain similarities to the histologic features seen in immunologic reactions of the graft-vs-host type led Kaplan and Smithers (1959) to suggest that Hodgkin's disease might represent an autoimmune process involving an interaction between neoplastic and normal lymphoid cells, a hypothesis later extended and developed by others (De Vita 1973; Green et al. 1960; Order and Hellman 1972). Definitive evidence that Hodgkin's disease is indeed a malignant neoplasm, albeit a remarkably atypical one, finally emerged during the last two decades from cytogenetic and cell culture studies which demonstrated that the giant cells of Hodgkin's disease satisfy two of the most fundamental attributes of neoplasia: aneuploidy and clonal derivation.

B. Origin and Characteristics of the Giant Cell Population

It was once considered that the giant binucleate or multinucleate Reed-Sternberg cells most closely resembled and were therefore probably derived from the histiocyte (Rappaport 1966). However, histochemical studies (Dorfman 1961) failed to reveal the presence of nonspecific esterase, an enzyme characteristically present in cells of the monocyte-histiocyte-macrophage series. Meanwhile, growing awareness of the remarkable changes in size and morphology which small lymphocytes may undergo during the process of lymphoblastoid transformation in response to lectins and specific antigens led to the hypothesis that the Reed-Sternberg cell might be an unusual form of transformed lymphocyte (Dorfman et al. 1973; Taylor 1976).

There has also been disagreement as to whether Reed-Sternberg cells are capable of DNA synthesis and mitosis. Although giant mitotic figures have been observed by some investigators, cells arrested in mitosis by treatment with vinblastine appeared to be limited to the mononuclear cell population in other studies (Marmont and Damasio 1967). After short-term incubation of cell suspensions of

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fresh lymph node biopsies from ten patients with Hodgkin's disease, autoradiographic evidence of incorporation of tritiated thymidine into DNA was seen only in mononuclear cells (Peckham and Cooper 1969), suggesting that the mononuclear Hodgkin's cells are the actively proliferating neoplastic cells and that the Reed-Sternberg cells are nonproliferating, end-stage, degenerative forms. Later studies, however, were more successful in revealing labeling in Reed-Sternberg cells, as were cell culture studies by Kadin and Asbury (1973) and by Kaplan and Gartner (1977). In the last-cited report, it was observed that 17 (20.7%) of 82 binucleate or multinucleate giant cells were labeled (Fig. 1), a proportion only moderately less than that observed among the mononuclear cell population (334 of 918, or 36.5%). Moreover, binucleate mitotic figures could be seen in some cells of the same culture. Accordingly, it is now clear that Reed-Sternberg cells are indeed capable of DNA synthesis and mitotic division and may thus be considered, together with their mononuclear counterparts, to be the neoplastic cells of Hodgkin's disease.

Chromosome studies have been carried out by the direct method or following short-term incubation of tissues involved by Hodgkin's disease in at least 100 cases from 1962 through 1978 (for review, cf. Kaplan 1980). In addition to cells having a modal chromosome number of 46, believed to represent normal lymphoid cells, another cell population with pseudodiploid or aneuploid chromosome numbers, often in the hypotetraploid range, was detected in 68 cases. For example, Whitelaw (1969) observed near-tetraploids in 31 (16%) of 193 scorable mitoses from four cases of Hodgkin's disease. Aneuploid cells have been detected not only in the more aggressive histopathologic forms but in the paragranuloma or lymphocyte predominance types as well, confirming that even these indolent forms are neoplastic in nature. Marker chromosomes have been observed in 40 of 100 cases, although no single characteristic abnormality has been consistently encountered.

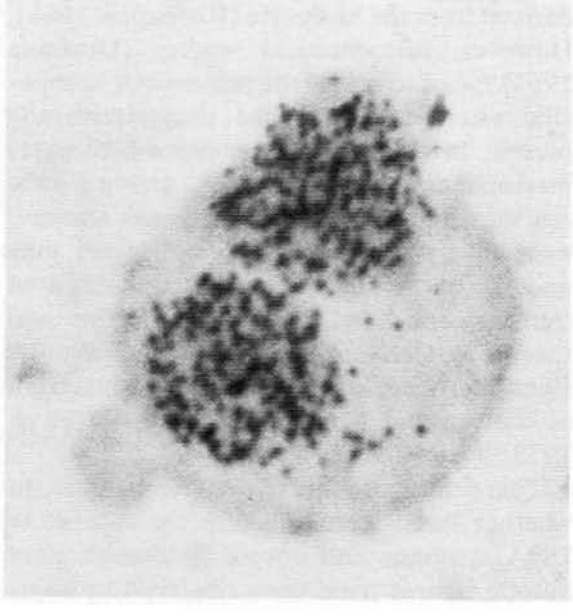

Fig. 1. Autoradiograph of cells from a long-term culture of involved spleen tissue from a patient with Hodgkin's disease. Both nuclei of a binucleate Reed-Sternberg cell are labeled with tritiated thymidine

Perhaps the most compelling evidence of the neoplastic character of Hodgkin's disease stems from observations indicating the clonal derivation of these aneuploid cells. One of the most remarkable clones of aneuploid Hodgkin's cells encountered to date is that described by Seif and Spriggs (1967). Of 63 cells 18 had chromosome numbers between 77 and 86. There were two unusually long marker chromosomes (M_1 and M_2); both were present in ten cells, and M_2 alone in an eleventh cell. Clonal distributions of marker chromosomes have been documented in at least half of the 40 instances in which marker chromosomes have been detected to date (cf. Kaplan 1980).

Controversy concerning the cell of origin of the Reed-Sternberg cell has not been resolved by electron micrographic or cytochemical studies. Some investigators (Dorfman et al. 1973) have been impressed by the resemblance of the nuclei of mononuclear and hyperlobated Hodgkin's cells to those of transformed lymphocytes. However, Carr (1975) placed greater emphasis on the presence of elaborate cytoplasmic processes, actin-like cytoplasmic microfibrils, and small lysosomes, some closely resembling those present in macrophages, and concluded that "the ultrastructure of the malignant reticulum cell is such as to make it likely that it is of macrophage lineage". Several investigators have found nonspecific esterase activity to be absent or only very weakly positive in the giant cells of Hodgkin's disease, whereas others have described distinct granular activity in such cells. Using fluoresceinated antisera to human immunoglobins, some investigators have detected surface and/or cyto-

plasmic IgG in a varying proportion of Hodgkin's giant cells. Immunohistochemical staining procedures have revealed both lambda and kappa light chains in the cytoplasm of many of these cells (Garvin et al. 1974; Taylor 1976). Since an individual B-lymphocyte is not capable of synthesizing both types of light chains (Gearhart et al. 1975), the presence of both lambda and kappa suggests that cytoplasmic immunoglobulin was not endogenously synthesized by these cells.

Long-term cultures of the giant cells of Hodgkin's disease were studied by Kadin and Asbury (1973) and by Kaplan and Gartner (1977). Permanent cell lines derived from tissues or pleural effusions involved by Hodgkin's disease have been successfully established by several groups (Gallmeier et al. 1977; Long et al. 1977; Roberts et al. 1978; Schaadt et al. 1979; H. S. Kaplan et al., unpublished work). However, all such efforts confront the dilemma that no definitive criteria exist for the unambiguous identification of Reed-Sternberg cells in vitro. Kaplan and Gartner (1977) observed that the giant cells from involved spleens grew in culture as round or oval adherent cells with diameters ranging from 20 to more than 75 μ, often exhibiting a strong tendency to adhere not only to the surface of the culture vessel but also to each other, leading to the formation of irregular clusters (Fig. 2). When fixed and stained, cells from such cultures exhibited morphologic features entirely consistent with those of Hodgkin's or Reed-Sternberg cells; most were mononuclear, but from 10 to 20% were binucleate, and 1%–2% contained three or more nuclei. In one such culture established from the spleen of a patient with Hodgkin's disease, analysis of 70 countable mitotic figures revealed that all were

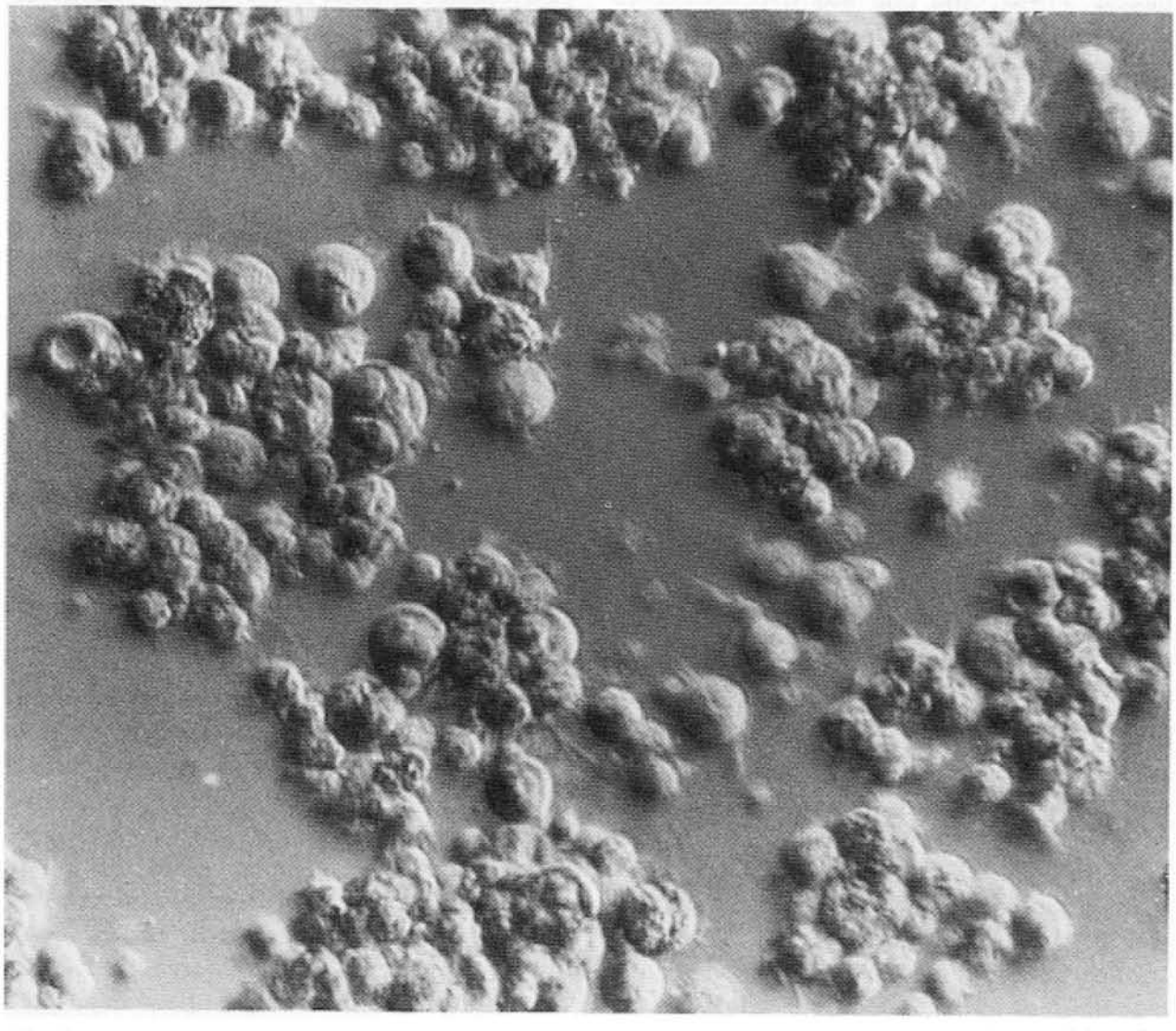

Fig. 2. Long-term culture of cells from the involved spleen of a patient with Hodgkin's disease. Note the clusters of adherent giant cells. The huge size of these cells may be appreciated by comparison with that of the occasional lymphocytes still persisting

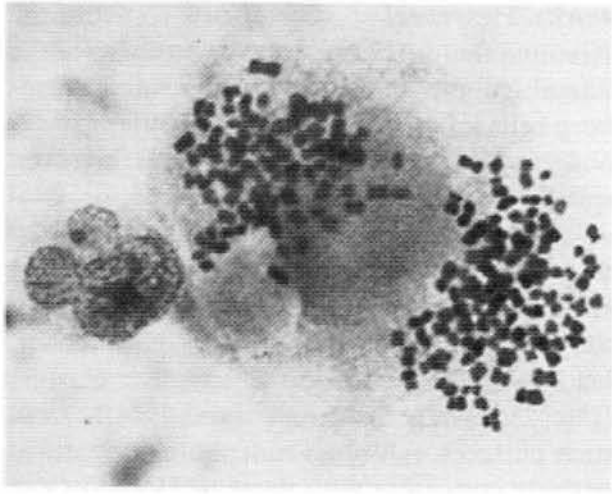

Fig. 3. Binucleate mitosis in an obviously aneuploid giant cell from the involved spleen of a patient with Hodgkin's disease after several weeks in culture

aneuploid; of these 63 were hyperdiploid with a mode of 53 chromosomes, 6 were hypotetraploid with chromosome numbers of approximately 77–91, and one was hyperoctoploid with over 190 chromosomes (Fig. 3).

These cells satisfied another criterion of neoplasia, heterotransplantability, after intracerebral inoculation into congenitally athymic nude mice. The giant cells possessed both Fc and complement receptors as revealed by their capacity for the formation of IgG-EA and IgM-EAC_{3b} rosettes, respectively. In contrast, they lacked T- and B-lymphocyte markers: they failed to form E rosettes and revealed no evidence of surface membrane immunoglobulin. The cultured giant cells exhibited sluggish but definite phagocytic activity for India ink, heat-killed *Candida,* and antibody-coated sheep erythrocytes. Culture supernatants from several cases consistently revealed the presence of elevated concentrations of lysozyme, and in some instances, the cultured giant cells were clearly positive when stained for nonspecific esterase (Kaplan and Gartner 1977).

Kadin et al. (1978), using immunofluorescent reagents for surface and intracellular gamma, alpha, and mu heavy chains and kappa and lambda light chains, examined suspensions of viable Reed-Sternberg cells from 12 patients with Hodgkin's disease. IgG, kappa, and lambda were often detected on the cell surface, whereas IgM and IgA were absent. Whenever surface immunoglobulin (SIg) was detected, cytoplasmic immunoglobulin (CIg) of the same type was also present within the same cell; conversely, CIg was often present in the absence of SIg. Every giant cell that contained CIg contained both kappa and lambda light chains. When viable cells were incubated in medium containing fluorescein-conjugated aggregated human IgG, evidence of both cell surface binding and intracellular uptake of fluorescent aggregates was observed. They concluded that the immunoglobulin found in Reed-Sternberg cells is not synthesized by these cells; instead, it appears to be ingested by them from the extracellular environment.

Collectively, these cell culture and immunofluorescence studies may have resolved the controversy concerning the origin and nature of the Reed-Sternberg and Hodgkin's giant cells. Their capacity for sustained proliferation in vitro, aneuploidy, and heterotransplantability establishes their neoplastic character, whereas the cell marker studies, phagocytic activity, positive staining reactions for nonspecific esterase, and capacity to excrete lysozyme strongly suggest that they are derived from the macrophage or other closely related cells of the mononuclear phagocyte system rather than from the lymphocyte.

C. Natural History and Mode of Spread

Lymphangiography swept away earlier misconceptions concerning the unpredictable, capricious distribution of lymph node involvement in patients with Hodgkin's disease and made possible systematic attempts to map sites of disease. Rosenberg and Kaplan (1966), in a study of 100 consecutive, previously untreated patients with Hodgkin's disease, found that involvement of various chains of lymph nodes was distinctly nonrandom; when a given chain of lymph nodes was affected, other chains known to be directly connected with it via lymphatic channels were likely also to be involved, either concurrently or at the time of first relapse. Even extralymphatic sites such as the lung, liver, and bone marrow were more likely to be involved in association with certain predictable patterns of lymph node and/or spleen involvement. These studies were subsequently extended (Kaplan 1970, 1980) to overlapping series of 340 and 426 consecutive previously untreated cases, with results which strongly confirmed and reinforced the initial conclusions. Similar analyses have been presented by other groups of investigators (Banfi et al. 1969; Han and Stutzman 1967), again with generally similar conclusions.

Two distinctively different theories, the "contiguity" theory of Rosenberg and Kaplan (1966) and the "susceptibility" theory of Smithers (1970, 1973), have been proposed to account for the patterns of spread observed in Hodgkin's disease. The contiguity theory postulates that Hodgkin's disease is a monoclonal neoplasm of unifocal origin which spreads secondarily by metastasis of pre-existing tumor cells, much like other neoplasms, except that the spread is predominantly via lymphatic rather than blood vascular channels. The term *contiguity* refers to the existence of direct connections between pairs of lymph node chains by way of lymphatic channels which do not have to pass through and be filtered by intervening lymph node or other lymphatic tissue barriers.

Smithers (1973) suggested that the giant cells of Hodgkin's disease may move in and out of lymph nodes from the blood stream, following a traffic pattern similar to that known to occur with normal lymphocytes. Emphasis was placed on the concept that Hodgkin's disease is a systemic disorder of the entire lymphatic system. Thus, the possibility was suggested that the disease may have a multifocal origin, perhaps by spread of a causative agent with de novo reinduction in different sites rather than the spread of pre-existing tumor cells. After an initial site had become involved, the theory predicted that each of the remaining lymph node chains would have an independent probability of next becoming involved which was assumed to be proportional to the probabilities of initial involvement of the corresponding lymph node chains in patients with Stage I disease.

Careful mapping of the initial sites of involvement in consecutive, previously untreated patients revealed the occurrence of noncontiguous patterns in only 4 (2%) of 185 patients with Stage II disease (Kaplan 1970). Hutchison (1972) compared the observed distributions in 158 of our Rye Stage II cases whose calculated frequencies were based on the random association of two or more sites with the probabilities given by their respective frequencies in 53 observed Stage I cases. The observed patterns for two or three involved sites departed significantly from random expectation. In particular, there was an apparent deficiency of bilateral cervical node involvement in the absence of associated mediastinal lymphadenopathy, an excess frequency of association between cervical and mediastinal node involvement, and a marked deficiency of all noncontiguous contralateral distributions.

Lillicrap (1973) compared the predictions of the Smithers susceptibility hypothesis with the observed patterns of spread in three different series of patients with Hodgkin's disease. Bilateral cervical lymph node disease was observed significantly less often than predicted, whereas involvement of the neck and mediastinum was more frequent than predicted. There were 46 instances of homolateral cervical-axillary involvement and only two contralateral cases, whereas equal numbers of each would have been predicted by susceptibility theory. Conversely, the observed patterns were consistent with the contiguity theory in all but 8 (4%) of 212 cases. Modifications of the susceptibility theory were subsequently proposed by Smithers et al. (1974) in an attempt to make the theory more consistent with observed distribution frequencies. These modifications, which accept the concept of spread via lymphatic channels, exhibit appreciably better agreement with observed patterns of two and three sites of involvement.

The contiguity theory has also been tested with respect to the sites of first relapse in patients with regionally localized disease treated with limited field radiotherapy. Rosenberg and Kaplan (1966) found that 22 of 26 extensions of disease were to contiguous lymph node chains. Similar findings have been reported by others (Banfi et al. 1969; Han and Stutzman 1967). The most controversial issue is the association between involvement of the lower cervical-supraclavicular lymph nodes and the subsequent occurrence of relapse in the upper lumbar para-aortic nodes. Among 80 such cases at risk, Kaplan (1970) observed para-aortic node extensions in 29 (36%). This was the single most prevalent site of extension in patients treated initially with local or limited field, supradiaphragmatic radiotherapy. Transdiaphragmatic extension was also the first manifestation of relapse in 33 (40%) of 83 patients with clinical Stage I and II disease studied by Rubin et al. (1974). Many para-aortic lymph node relapses occurred several years after initial treatment and frequently involved lymph nodes which were well visualized and appeared normal on the original lymphangiogram. It was suggested (Kaplan 1970; Rosenberg and Kaplan 1966) that spread in these instances had occurred in the retrograde direc-

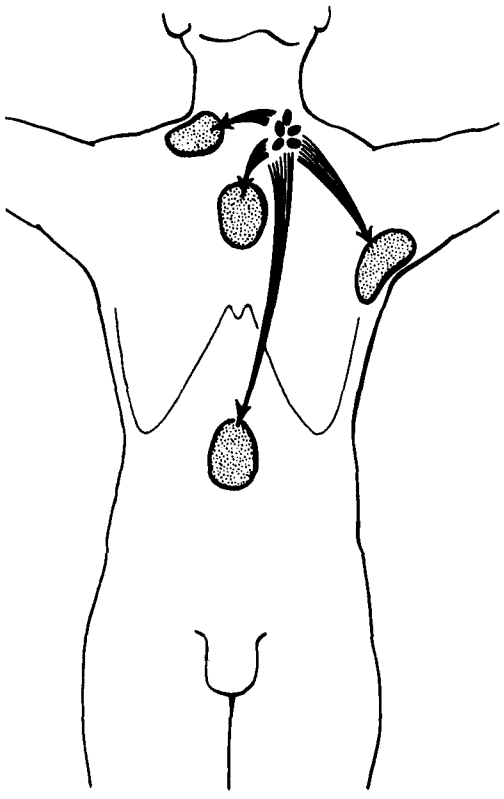

Fig. 4. Schematic diagram of postulated retrograde spread of Hodgkin's disease from low cervical-supraclavicular to para-aortic/celiac nodes via the thoracic duct and of contiguous spread to the mediastinal, ipsilateral axillary, and contralateral cervical-supraclavicular nodes. Reproduced, by permission, from the paper by Kaplan (1970)

tion from the supraclavicular fossa downward along the thoracic duct into the lumbar paraaortic nodes (Fig. 4).

The occasional presence of Reed-Sternberg and Hodgkin's giant cells in the thoracic duct lymph has been documented by Engeset et al. (1968). There is little dispute that these cells may enter the thoracic duct from involved lymph nodes below the diaphragm and travel upward to involve the cervical-supraclavicular lymph nodes. The possibility of retrograde spread from one peripheral lymph node chain to other, more distal chains by way of lymphatic channels lacking valves is also widely accepted. However, the concept of retrograde spread along the thoracic duct has been much more controversial because the duct is equipped with valves which should prevent retrograde flow. Yet, the pressure in the duct is only a few millimeters of water and reversal of flow was readily observed following chronic ligation of the thoracic duct in dogs (Neyazaki et al. 1965). Pressure gradients along the canine thoracic duct were often opposite to those required for antegrade flow (Browse et al. 1971). However, Dumont and Martelli (1973) were able to demonstrate radiopaque material in the para-aortic lymph nodes of only 1 of 16 dogs after ligation and cannulation of the thoracic duct and injection of opaque contrast material in the retrograde direction. Retrograde flow might well occur more often in the thoracic duct of man, which is usually vertical, than in that of dogs, which is horizontal. Rouvière (1932) noted that although the human thoracic duct usually has two competent valves at its upper end, a not infrequent normal variation involves the presence of a single incompetent valve, which is usually compensated by oblique insertion of the duct through the vein wall. Conceivably, prolonged compression and partial occlusion of the duct by enlarged lymph nodes near its insertion into the subclavian vein may cause dilatation of the duct with secondary valvular incompetence and reversal of flow.

The role of vascular invasion (Rappaport and Strum 1970) in the spread of Hodgkin's disease is not fully understood. In a careful review of the original biopsy material in 11 patients with regionally localized Hodgkin's disease who developed extranodal dissemination following primary radiotherapy, Lamoureux et al. (1973) failed to find evidence of vascular invasion. Kirschner et al. (1974) noted that vascular invasion was present in 7 (16%) of 44 spleens involved by Hodgkin's disease and was associated with hepatic and bone marrow metastasis, early relapse, and decreased survival, whereas vascular invasion detected in 4 of 91 lymph node biopsies was not attended by an increased frequency of extranodal dissemination or a decreased survival rate. In a series of patients whose lymph node biopsies showed vascular invasion, Naeim et al. (1974) observed an average survival time of only 21.8 months, significantly less than the 65.8 month average survival of those patients in whom vascular invasion was not demonstrable in the original lymph node biopsies.

D. Nature of the Immunologic Defect

Unresponsiveness to tuberculin was the first immunologic abnormality observed in patients with Hodgkin's disease. Dorothy Reed (1902 reported that tuberculin was given in five

cases but without reaction." However, the immunologic deficiency is not specifically restricted to tuberculosis. Schier et al. (1956) tested the capacity of patients with Hodgkin's disease to mount delayed hypersensitivity reactions to a diversified battery of natural antigens and found that most were unresponsive to all of the antigens tested. Unfortunately, the significance of the early studies cannot be assessed because many patients had been treated, and none had been staged by modern methods.

A series of 50 previously untreated patients with Hodgkin's disease, all staged with the aid of lymphangiography and other modern diagnostic procedures, was studied at the National Cancer Institute by Brown et al. (1967). Responsiveness to the five antigens tested was impaired relative to controls. However, reactions in eight patients with clinical Stage I Hodgkin's disease appeared to be comparable with those of normal controls. With increasing clinical stage, responsiveness decreased sharply. Positive responses to one or more intradermal antigens were noted in seven of eight patients with Stage I disease, 13 of 24 in Stage II, three of seven in Stage III, and 5 of 11 in Stage IV. These studies were later extended to a total of 103 patients with previously untreated disease with generally similar results (Young et al. 1972). Only seven patients, all of whom had constitutional symptoms, were completely anergic (unresponsive to all tests).

Among a total of 185 patients studied at Stanford University Medical Center from 1964 through 1968 there were 28 patients with previously untreated Stage I disease, of whom only 12 (43%) responded to mumps antigen and few responded to any other cutaneous antigen (Kaplan 1970). A second study initiated in 1969 accrued 154 previously untreated patients, all staged with the aid of lymphangiography and laparotomy with splenectomy (Eltringham and Kaplan 1973). Only 51 of 151 evaluable patients (34%) responded to one or more intradermal antigens, and a positive reaction to mumps antigen was observed in only 40 (25%) of 151 patients. There was no significant influence of clinical stage on response to mumps antigen. In contrast to the observations of the Bethesda group, unresponsiveness did not occur more frequently among patients with constitutional symptoms. In tests with streptokinase-streptodornase (SK–SD), only 6 (10%) of 58 untreated patients with Hodgkin's disease reacted to 5 units, whereas 93% of age – and sex-matched controls were known to respond to the same dose level (Eltringham and Kaplan 1973).

Clinical investigations using chemical agents known to have the property of inducing delayed cutaneous hypersensitivity reactions essentially indistinguishable from those induced by tuberculin have the advantage that the fact of exposure to the agent and the timing of that exposure are both under the control of the investigator. The most extensively used of these chemicals is 2,4-dinitrochlorobenzene (DNCB). In a series of 50 untreated patients, Brown et al. (1967) observed positive responses in 35 (70%) to sensitization with DNCB at a concentration of 2.0%. Impressed by the fact that all eight of their patients with Stage I disease reacted positively to DNCB and that seven of the eight reacted to at least one intradermal antigen, the Bethesda group concluded that the development of anergy is probably a secondarily acquired manifestation associated with advancing anatomic extent of involvement rather than an intrinsic component of the pathogenesis of Hodgkin's disease.

In an initial study involving 185 previously untreated patients sensitized with 2.0% DNCB at Stanford University Medical Center from 1964 through 1968, an extremely high incidence of anergy was observed, even in patients with Stage I disease (Kaplan 1970). De Gast et al. (1975) also observed negative reactions to challenge after sensitization with the same concentration of DNCB in 20 of 30 patients (67%), including two of five with Stage I disease, and Case et al. (1976) reported negative reactions in 24 of 50 patients (48%), including three of eight with Stage I disease.

In a subsequent Stanford study involving untreated patients staged routinely with lymphangiography and laparotomy with splenectomy, three different sensitizing concentrations of DNCB (0.1, 0.5, and 2.0%) were used (Eltringham and Kaplan 1973). Sensitization and challenge with DNCB occurred prior to the initiation of treatment. At a sensitizing concentration of 0.5%, only 10 (26%) of 39 patients responded as compared with 83% of normal controls. This study was ultimately extended to encompass a total of 531 previously untreated patients of all stages (Kaplan 1980). There were 113 positive responses (36.3%) among 311 patients with Stage I and II disease, a response rate only slightly greater

than that among patients with Stage III and IV disease (56 of 220, or 25.5%). Of a total of 355 asymptomatic patients, 128 (36.1%) responded, a significantly higher response rate than that of patients with constitutional symptoms (41 of 176, or 23.3%). These data support the conclusion that cell-mediated immune reactivity is indeed impaired in patients with Hodgkin's disease. However, the impairment is not an all-or-none phenomenon but a more subtile continuous gradient of immunologic deficit which is present in some degree even in patients with the earliest manifestations of the disease.

A number of in vitro tests are considered analogs of cell-mediated immune responses. These include the capacity of lymphocytes to: (1) undergo lymphoblastoid transformation after stimulation by lectins or antigens and to respond in the mixed lymphocyte reaction, (2) to bind sheep erythrocytes to their surface membranes (E-rosette formation), and (3) to bind and become agglutinated by certain lectins and to mediate the polar migration (capping) and shedding of the bound lectins from the cell membrane. Brown et al. (1967) noted a mean lymphocyte response to phytohemagglutinin (PHA) of 49% in 43 patients with untreated Hodgkin's disease, a highly significant decrease from the 72% mean value observed in their controls. However, responses in patients with Stage I disease were within the normal range. Very similar responses to PHA were noted by De Gast et al. (1975) in a series of 30 patients with Hodgkin's disease. However, these investigators noted that lymphocyte stimulation by α-hemocyanin was impaired in 11 of 15 patients and that the DNCB skin test reaction was also negative in 10 of the 11 nonresponsive individuals. Lymphoblastoid responses to another antigen, tetanus toxoid, were negative in six of nine patients studied by Fuks et al. (1976a). Gaines et al. (1973) observed that lymphocytes from three patients with positive *Toxoplasma* dye test titers as well as those of 20 with negative titers failed to respond to *Toxoplasma* antigen in vitro. Responses to SK-SD were also negative in 22 of 23 untreated patients. Holm et al. (1976) in a study of 31 patients with Hodgkin's disease noted that only 1 of 12 skin test positive patients had an impaired lymphocyte response to the antigen in vitro; conversely, only 1 of 19 patients with a negative skin test reaction had a normal lymphoblastoid response to tuberculin (PPD) in vitro. Deficient responses to PPD were observed in 7 (47%) of 15 patients with Stage I or II disease and in 11 (55%) of 20 patients with Stage III or IV disease.

Modifications of technique succeeded in revealing unambiguous abnormalities of the PHA stimulation response even in patients with Stage I disease. Matchett et al. (1973) noted good initial responses during the first 2 days in patients with localized disease, but these responses were not sustained at 4 or 5 days. When the daily uptake of tritiated thymidine (^{3}H-TdR) by limiting concentrations of cells was used as the index of response, all of 26 patients, including those with localized disease and no symptoms, showed a striking degree of abnormality. Levy and Kaplan (1974) measured the uptake of tritiated leucine (^{3}H-Leu) into protein in peripheral blood lymphocytes stimulated with a range of PHA concentrations. This assay requires only 20 h for completion, so that cell viability can be preserved in the absence of serum, thus enhancing precision and reproducibility. They studied 37 normal subjects and 44 consecutive untreated patients with Hodgkin's disease, all staged with lymphangiography, bone marrow biopsy, and in those with negative marrow biopsies, laparotomy with splenectomy. The peak response of normal donor lymphocytes was noted at a PHA concentration of 1 μg/ml. The response of lymphocytes from patients was very significantly below normal at all but the highest PHA concentrations tested. The impairment of response was observed both in patients with limited (Stage I and II) as well as those with advanced (Stage III and IV) disease. These results remained essentially unchanged after this study had been extended (Fuks et al. 1976a) to include 132 patients with untreated Hodgkin's disease (Fig. 5). Stimulation by another lectin, concanavalin A (Con A), revealed impaired responses in a series of 18 patients. Concentration-dependent defects in lymphocyte response to PHA were also observed by Ziegler et al. (1975) and by Faguet (1975) in untreated patients with various stages of Hodgkin's disease.

Negative mixed lymphocyte reactions (MLR) were observed by Lang et al. (1972) in 7 (22%) of 32 patients with untreated Hodgkin's disease. In a study of 30 patients, Rühl et al. (1975) found that the capacity of lymphocytes from patients with Hodgkin's disease to respond to allogeneic cells was significantly

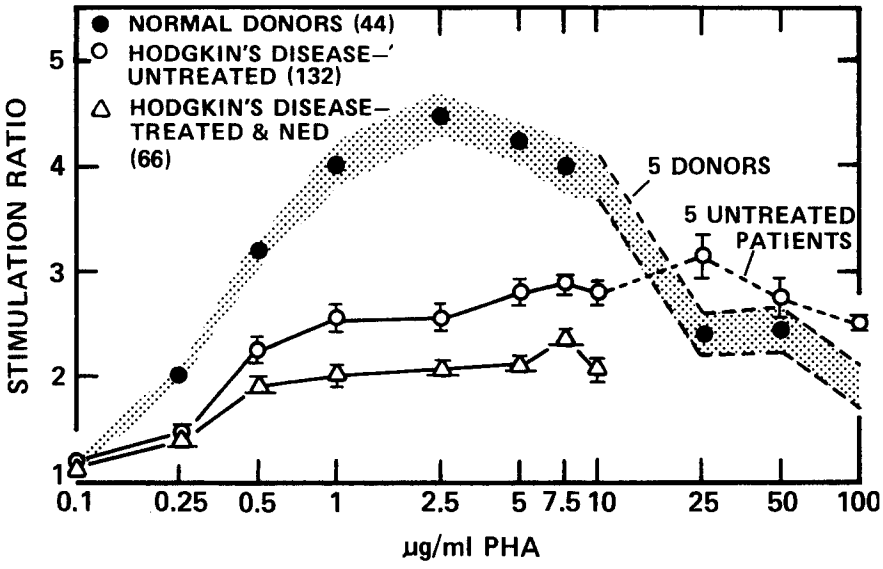

Fig. 5. Impaired lymphoblastoid response to a range of concentrations of phytohemagglutinin (PHA), as measured by a tritiated leucine uptake assay (Levy and Kaplan 1974), by peripheral blood lymphocytes from 132 untreated patients with Hodgkin's disease and 66 other patients in remission following radiotherapy. Reproduced, by permission of the *Journal of Clinical Investigation,* from the paper by Fuks et al. (1976a)

impaired, whereas their capacity to stimulate responses by normal lymphocytes was essentially intact, a result diametrically opposed to that observed by Björkholm et al. (1975) in a study of 39 previously untreated patients. Fuks et al. (1976a) observed positive responses in eight of nine untreated patients whose lymphocytes were used as stimulator cells in one-way allogeneic MLR tests; however, five of these responses were only weakly positive. The lymphocytes of these patients were found to respond adequately to stimulation either by normal donor cells or by cells from other patients with Hodgkin's disease, positive reactions being observed in 20 (95%) of 21 such combinations. A somewhat different test, the autologous mixed lymphocyte reaction, was employed in a recent study (Engleman et al. 1980); the capacity of peripheral blood T-lymphocytes from patients with untreated Hodgkin's disease as well as those previously treated with radiotherapy and in long-term remission to respond was found to be profoundly impaired.

The capacity to form spontaneous E-rosettes with uncoated sheep erythrocytes, a specific property of human T-lymphocytes, was observed by Bobrove et al. (1975) to be impaired in 13 of 15 untreated patients with Hodgkin's disease, whereas the percentage of T-lymphocytes detected by cytotoxic antibody assay was normal. In contrast, the levels of active rosette-forming cells (a subpopulation of T-lymphocytes with high affinity receptors for sheep erythrocytes) were within normal limits in two series of patients with untreated Hodgkin's disease (D.P. King and H.S. Kaplan, cited in Kaplan 1980; Lang et al. 1977).

Significant progress has been made in elucidating the mechanisms underlying the selective impairment of cell-mediated immune responses in Hodgkin's disease. It is now well established that cells capable of specific suppression of immune responses exist in the lymphoid system. Twomey et al. (1975) observed that peripheral blood mononuclear cells from 16 of 30 patients with Hodgkin's disease had an impaired capacity to stimulate responses by lymphocytes from normal donors in the one-way MLR test. Of these 16 patients, all but one had Stage III or IV disease and two had been previously treated. Stimulation was markedly increased when adherent cells were removed by passage through glass wool as well as by preincubation of the cells in a protein synthesis inhibitor, cycloheximide. Similar results were subsequently reported by Goodwin et al. (1977) who found in addition that the inhibitory activity could be counteracted by indomethacin, a known prostaglandin synthetase inhibitor, suggesting that a suppressor cell producing prostaglandin E_2 might be responsible for the hyporesponsiveness to PHA of peripheral blood cells from patients with Hodgkin's disease. This group (Sibbitt et al. 1978) later reported that the PHA response of lymphocytes in patients with Hodgkin's disease could be restored to normal by removal of glass wool adherent suppressor cells and again inhibited by restoration of such cells to the cultures. Suppressor responses have also been reported in a high percentage of patients with Hodgkin's disease by Engleman et al. (1979) and by Hillinger and Herzig (1978). However, the specificity of these suppressor effects remains to be established.

Tests of binding affinity, agglutinability, and capacity for cap formation with lectins such as Con A have provided important new approaches to the study of lymphocyte surface membranes. Ben-Bassat and Goldblum (1975) found that cap formation by peripheral blood lymphocytes from patients with Hodgkin's disease was markedly reduced. Mintz and Sachs (1975) noted a mean of only 2.1% of cap forming cells among 15 patients with active Hodgkin's disease and 10.6% in patients with remission versus 24.9% in normal individuals. Both groups noted an increased agglutinability of patients' peripheral blood lymphocytes by Con A. Aisenberg et al. (1978) found that cap forming cell levels were below normal in 9 of 13 patients with untreated Stage I-A and II-A disease, six of eight in Stage III-A, and eight of eight in Stages III-B and IV. Thus, a subpopulation of lymphocytes in patients with Hodgkin's disease appears to have membrane alterations reflected in enhanced lectin agglutinability and diminished cap formation.

Humoral factors in serum may alter the T-lymphocyte surface membrane, perhaps by masking specific receptors, and thus inhibit or abrogate cell-mediated immune functions. Grifoni et al. (1970) reported that cytotoxic antilymphocyte antibodies are present in the sera of patients with Hodgkin's disease and that such antibodies can inhibit the PHA stimulation response of normal lymphocytes in vitro. Fuks et al. (1976c) discovered that impaired E-rosette formation and PHA responses by lymphocytes from patients with Hodgkin's disease could be consistently restored to normal levels by short-term incubation in fetal calf serum. In a search for direct evidence of an E-rosette inhibitor in the sera of patients with Hodgkin's disease, Fuks et al. (1976b) noted that when lymphocytes were first restored to normal E-rosette forming cell (E-RFC) levels by incubation in fetal calf serum and then reincubated in medium containing 20% Hodgkin's disease serum, E-rosette levels were again significantly reduced in 22 (85%) of 25 patients. In contrast, Hodgkin's disease serum significantly depressed the response of only 1 of 12 patients with non-Hodgkin's lymphomas and failed to depress the E-rosette levels of lymphocytes from any of 34 normal subjects or of 12 patients with various types of carcinomas.

Since the spleen seemed a likely tissue of origin for the serum E-rosette inhibitor, Bieber et al. (1975) prepared extracts from the involved spleens of eight patients with Hodgkin's disease. These extracts consistently showed marked E-rosette inhibiting activity, whereas similarly prepared extracts from the spleens of most patients with non-Hodgkin's lymphomas and from normal spleens of acute trauma victims were devoid of such activity. Rosette levels depressed by the Hodgkin's disease spleen extract could again be restored to normal levels by incubation in fetal calf serum. Lymphocytes from patients with Hodgkin's disease were susceptible to the spleen extracts after they had first been restored to normal responsiveness by incubation in fetal calf serum. Analysis of the active fraction initially indicated the presence of β-lipoprotein, C-reactive protein, and the Clq component of complement. Subsequently, Bieber et al. (1979) fractionated the sera of patients with Hodgkin's disease on sucrose gradients and then on potassium bromide isopyknic gradients, followed by thin-layer chromatography. The active material proved to be a glycolipid, the further chemical characterization of which is still in progress. Similarly fractionated normal sera were devoid of detectable amounts of this inhibitory substance.

By radioiodination of the surface proteins of peripheral blood mononuclear cells from four patients with Hodgkin's disease, Moroz et al. (1977) demonstrated the presence of a blocking protein which could be released from the cell surface by incubation with levamisole, an antihelminthic drug. The blocking protein reacted with antibody to human spleen ferritin but contained no detectable iron and could be dissociated into 18 000-dalton subunits, suggesting that it is an apoferritin rather than ferritin. After release of the blocking protein by treatment with levamisole, the E-rosette response of peripheral blood lymphocytes from patients with Hodgkin's disease rose to normal levels. It is of course possible that apoferritin is merely acting as a carrier for a low molecular weight E-rosette inhibitory substance, perhaps the glycolipid material identified by Bieber et al. (1979).

There is thus abundant evidence that virtually all patients with Hodgkin's disease, including those with localized involvement, suffer from a selective, often subtile, impairment of cell-mediated immunity. In vivo this deficit is expressed by an increased susceptibility to certain types of bacterial, fungal, and viral

infections and by a decreased capacity for delayed hypersensitivity reactions to recall antigens or chemical allergens. A spectrum of in vitro test responses, including lymphoblastoid transformation by lectins and specific antigens, the capacity to form E-rosettes, and the capacity for cap formation after lectin binding, are also impaired. These alterations appear to be due to functional alterations of T-lymphocytes rather than to quantitative depletion of either T- or B-lymphocytes. Humoral inhibitors in the sera of patients with Hodgkin's disease and suppressor cell effects have been implicated.

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