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Reticuloendothelial System Phagocytic Function in Patients with Hodgkin's Disease *

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Summary. Phagocytic function of the reticuloendothelial system as measured by the clearance of ^{125}I-labeled aggregated human serum albumin (AA) was studied in patients with Hodgkin's disease. Each more advanced stage of the disease was associated with more rapid clearance of the AA. Production of remission by radiation therapy or by chemotherapy was associated in some patients with slowing of the clearance rate, whereas relapse seemed to be associated with acceleration. Thus, impaired phagocytosis cannot be implicated in the several defects in immunity known to be present in Hodgkin's disease. Furthermore, determination of the clearance rate of AA in patients with Hodgkin's disease may have clinical usefulness as another indicator of extent or activity of disease.

Introduction

Hodgkin's disease is known to be associated with an increased incidence of infectious diseases (1, 2). Certain of these may occur throughout the patient's course and are possibly related to deficient mechanisms of delayed hypersensitivity (3–6). However, the majority of infectious episodes occur late in the course of the illness (2), when other factors, such as the extensive use of immunosuppressive agents, especially corticosteroids (2), lymphocytopenia (7), and alterations in circulating antibody (8–10), may be implicated.

One aspect of immunity, the phagocytic function of the reticuloendothelial system, has received scant attention in clinical studies of Hodgkin's disease. This system is known to be important in the removal and destruction of pathogenic organisms from the tissues and blood (11, 12). In addition, recent evidence has suggested that phagocytic cells of the reticuloendothelial system may play a role in the initiation of antibody production (13–15). Studies of reticuloendothelial phagocytosis in man have increased since the development of a clearance technique using aggregated human serum albumin labeled with radioactive iodine (16–18). More recently, Wagner and his co-workers (19–22) have extensively demonstrated the usefulness, safety, and ease of performance of such a technique.

The present studies describe alterations in the phagocytic function of the reticuloendothelial system in patients with Hodgkin's disease and relate such changes to the extent and clinical activity of the disease.

Methods

Preparation of aggregated albumin. Salt-poor, normal human serum albumin [1] was aggregated as described by Iio and Wagner (19). A large quantity of AA was prepared at one time, enabling us to carry out the entire series of studies with the same lot. Radioactive iodine (^{125}I) was used for labeling. After appropriate testing for sterility and pyrogenicity, AA-^{125}I was stored in vials at 4° C.

Performance of the reticuloendothelial clearance tests. Each subject to be evaluated received two doses of AA-

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[1] Courtland Laboratories, Los Angeles, Calif.

^{125}I on each of 2 consecutive days, a 0.03 mg per kg dose to assess changes in perfusion of the reticuloendothelial system, and a 5.0 mg per kg dose to measure actual phagocytic capacity of the system (20). Each dose of AA-^{125}I, whether 0.03 or 5.0 mg per kg, contained 10 μc of ^{125}I. Lugol's solution was administered to the subjects before each day's tests to block uptake of radioactive iodine by the thyroid.

Three ml of heparinized blood was obtained at 5, 6½, 8, and 10 minutes after injection of the 0.03 mg per kg dose and at 5, 9, 13, and 17 minutes after the 5.0 mg per kg dose. Plasma was removed after centrifugation at 3,500 rpm for 10 minutes. Free iodide was removed by passing 1.0-ml aliquots of plasma through 3.0 ml of Amberlite resin (IRA-400 chloride form).[2] Plasma was washed from the resin with 7 ml of iron-free distilled water, and all proteins were precipitated by the addition of 1.25 ml each of 10% sodium tungstate and ⅔ N sulfuric acid. After centrifugation of the precipitated sample at 3,500 rpm for 10 minutes, the entire spun sample was counted in an automatic gamma well counter.[3] Counting error was less than 1%.

After injection of AA, plots of plasma radioactivity versus time were straight lines on semilogarithmic paper, permitting determination of the half-time of plasma clearance. Values of the $t_{\frac{1}{2}}$ for each of the 0.03 mg per kg doses on the 2 successive days were averaged as were those of the 5.0 mg per kg doses. The average value was taken as the measure of reticuloendothelial phagocytic function for that individual at that point in time.[4]

Subjects. The normal $t_{\frac{1}{2}}$ for each dose of AA-^{125}I was obtained by performing clearance studies in ten normal volunteers (two women and eight men) ranging in age from 21 to 35 years. These studies were done on groups of two or three volunteers at a time over the entire duration of the study. Either further aggregation or deaggregation of the preparation over the period of the study would have resulted in progressive changes in the "normal" clearance rate; no such changes were observed.

Patients with stages II, III, and IV of Hodgkin's disease proven by biopsy were available for study. Clinical staging was based on extent of disease as determined by physical examination, roentgenographic studies (including chest X rays, skeletal survey, intravenous pyelography and lymphangiography) and the results of laboratory determinations. Stages of the disease were defined as follows: stage II, lymph node involvement in more than one area but limited to above or below the diaphragm; stage III, lymph node disease above and below the diaphragm; and stage IV, disease involving organs other than or in addition to lymph nodes. The presence or absence of constitutional symptoms such as pruritis, fever, and weight loss was used to divide indi-

[2] Mallinckrodt Chemical Works, St. Louis, Mo.

[3] Model 4223, Nuclear Chicago, Des Plaines, Ill.

[4] Variation between the half-times of clearance of day 1 and day 2, calculated from 20 randomly selected clearances of patients and normal subjects, was small; for the 0.03 mg per kg dose the mean difference was 0.7 minute (SE 0.1 minute), and for the 5.0 mg per kg dose the mean difference was 1.0 minute (SE 0.2 minute).

TABLE I

Clinical data of patients with Hodgkins's disease

Stage	No. of patients	Age		Sex		Previous antitumor therapy
		Range	Mean	Male	Female	
		years				
IIA	7	16–38	25	5	2	6 patients, none; 1, local X ray to neck 1 year before relapse.
IIB	3	22–43	31	2	1	None.
IIIB	5	20–60	35	3	2	3, none; 1, local X ray to neck 7 months before relapse; 1,* chemotherapy and X ray to abdomen 5 months before relapse.
IVB	7	20–61	37	2	5	3, none; 1, local X ray to neck 2 years before relapse; 1,† relapse 6 months after surgical excision of intestinal disease; 2, multiple courses of chemo- and X ray therapy (considered refractory or "end stage").
Complete remission	5	13–48	31	2	3	2, initially stage IIA, radiated 2 and 5 years before study; 1, initially extensive stage IVB, treated with chemotherapy 6 months before study; 1,* initially stage IIIB, treated with chemotherapy and X ray to abdomen 3 months before study; 1,† apparently complete surgical excision of intestinal Hodgkin's.

*† Same patient. Studied first in remission; thus is included in group studied in complete remission then included after relapse in appropriate group with stage based on extent of recurrent disease.

TABLE II

Half-times of plasma clearance of aggregated human serum albumin-^{125}I (AA) in untreated patients with active Hodgkin's disease

Dose of AA	Normal (10)*	Stage of Hodgkin's disease			
		IIA (7)	IIB (3)	IIIB (5)	IVB (7)
mg/kg	*minutes*	*minutes*			
0.03	5.8 ± 0.2†	5.8 ± 0.3	5.3 ± 0.4	6.0 ± 0.3	6.2 ± 0.4
5.0	14.8 ± 0.2	13.4 ± 0.3	10.9 ± 1.0	11.2 ± 0.6	9.6 ± 0.4

* Number of subjects given in parentheses.
† Mean ± standard error.

vidual stages into substages A (symptoms absent) and B (symptoms present). Only the group of patients with stage II disease contained asymptomatic (IIA) as well as symptomatic (IIB) members; all patients with stages III and IV disease were symptomatic. Complete clinical remission was attained when, during or after treatment, both lymph node disease and constitutional symptoms had completely disappeared.

Although some patients were febrile at the time of their clearance study, no patient was known to have active infection; nor was any found to have a hemolytic anemia. None of the patients was receiving antitumor therapy at the time of the initial study of the reticuloendothelial system. Follow-up studies were performed while patients were on therapeutic regimens as outlined in the Results. Pertinent clinical data on the various groups of patients are summarized in Table I.

Results

Clearance studies in normal volunteers. As shown in Table II, the mean $t_{\frac{1}{2}}$ for the 0.03 mg per kg dose of AA in ten normal volunteers was 5.8 minutes (range 4.6 to 6.8), and that of the 5.0 mg per kg dose was 14.8 minutes (range 13.8 to 15.9).

Clearance studies in patients with Hodgkin's disease: 0.03 mg per kg dose of AA. Mean clearances ($t_{\frac{1}{2}}$) of the 0.03 mg per kg dose of AA of each group of patients with Hodgkin's disease (Table II and Figure 1) did not differ from one another or from that of the group of normal volunteers ($p > 0.30$, F test, Table II), indicating that blood flow to the tissues of the reticuloendothelial system was the same in all groups.

Clearance of 5.0 mg per kg dose of AA in patients with Hodgkin's disease. Comparison of the mean of the $t_{\frac{1}{2}}$ for the 5.0 mg per kg dose of AA for each group of patients and for the normal volunteer group revealed statistically significant variability ($p < 0.01$, F test, Table II, Figure 1). Individual groups that varied significantly from one another were further delineated by the use of the *t* test, and the following differences were noted: The mean $t_{\frac{1}{2}}$ of each group of patients with Hodgkin's disease differed from normal ($p < 0.01$), and progressively more rapid clearance of AA with each more advanced stage of disease was observed (Figure 1). Patients with stage IIA disease not only differed from normal ($p < 0.01$), but also from patients with more advanced stages of the disease (IIA versus IIIB or IVB, $p < 0.01$) as well as from symptomatic patients of the

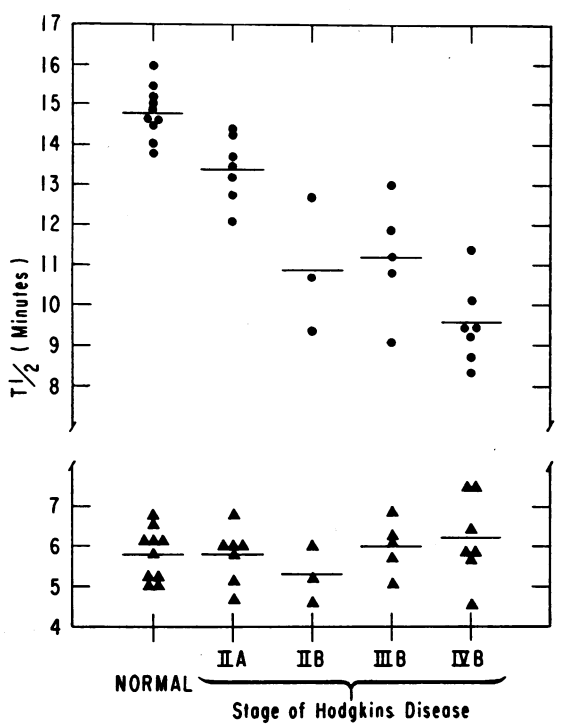

FIG. 1. HALF-TIMES OF CLEARANCE OF TWO DOSES, 0.03 MG PER KG (▲) AND 5.0 MG PER KG (●), OF AGGREGATED HUMAN SERUM ALBUMIN (AA-^{125}I) IN NORMAL VOLUNTEERS AND UNTREATED PATIENTS WITH VARIOUS STAGES OF HODGKIN'S DISEASE. Bars represent means for each group.

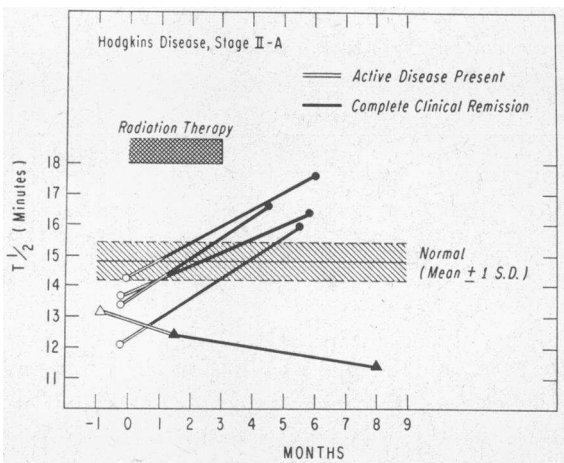

FIG. 2. TIME COURSE OF CHANGES IN THE PLASMA CLEARANCE OF THE 5.0 MG PER KG DOSE OF AA-^{125}I IN PATIENTS WITH STAGE IIA HODGKIN'S DISEASE TREATED WITH RADIATION THERAPY (SHADED BOX). The patient represented by triangles (▲) relapsed 2 months after the last clearance study.

same stage (IIA versus IIB, $p < 0.02$) who cleared the test material significantly more rapidly. The mean clearance rate of the three stage IIB patients was not different from that of patients with stage IIIB or IVB disease ($p > 0.30$). IVB patients were found to have a slightly faster clearance rate ($p < 0.05$) than patients with stage IIIB Hodgkin's disease (Table II).

Correlation of changes in clearance of AA with treatment, onset of remission, and relapse. Patients were treated in the following manner: All stage II patients received 3,500 to 4,000 röntgens (R) radiotherapy to areas of primary lymph node involvement and approximately 3,000 R to adjacent node areas (tumor dose, 1,000 R per week). Some patients received additional extended radiation to the lower abdomen and spleen. All stage III and IV patients were treated with chemotherapy given in six monthly courses (each course consisting of 2 weeks of therapy followed by 2 weeks of rest). Four drugs (prednisone, Ibenzmethazine, nitrogen mustard, and vincristine) were given in doses calculated on the basis of body surface area and in varying combinations, month to month. Details of these methods of therapy and their effectiveness are under evaluation (23).

The effect of radiation therapy on the clearance of the 5.0 mg per kg dose of AA in five patients with stage IIA disease is shown in Figure 2. Four of the five patients, studied from 1 to 2 months after completion of the course of therapy, showed slowing of the clearance of AA. The one patient who did not exhibit slowing of clearance was a 16-year-old male who clinically relapsed 2 months after completion of the last clearance study. The other four patients have continued free of disease for periods of 3 to 12 months.

The effect of chemotherapy on the plasma clearance of AA in patients with stage IIIB disease was not uniform (Figure 3): One patient showed slowing into the normal range on all studies done after the onset of therapy, whereas the other two remained accelerated (Figure 3). All patients with stage IV disease were found to have some slowing of clearance although only one reached the normal range (Figure 4). The one patient in this group who relapsed showed some acceleration of clearance on the last study performed about 3 weeks after the first clear-cut clinical evidence of recurrent disease (Figure 4).

Clearance of AA in the patients studied first while in complete clinical remission (clinical data given in Table I) was variable. The two patients with previous stage IIA disease had only slightly accelerated half-times of clearance (13.0 and 13.2 minutes); they had received radiation therapy 2 and 5 years before the present studies. The patient who initially presented with stage IVB disease was studied 6 months after completion of

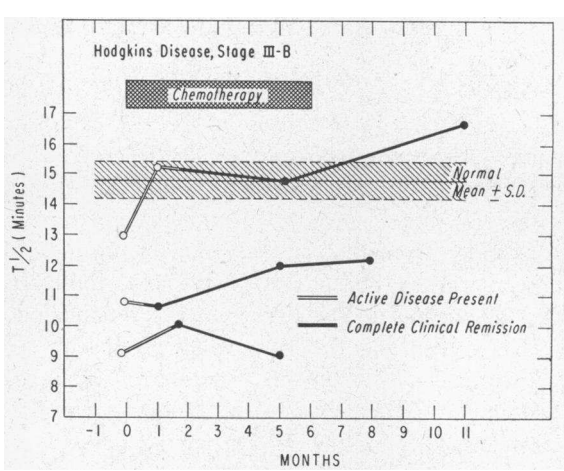

FIG. 3. TIME COURSE OF CHANGES IN THE PLASMA CLEARANCE OF THE 5.0 MG PER KG DOSE OF AA-^{125}I IN PATIENTS WITH STAGE IIIB HODGKIN'S DISEASE. Chemotherapy (shaded box) was given in 2-week courses each month for the 6-month period.

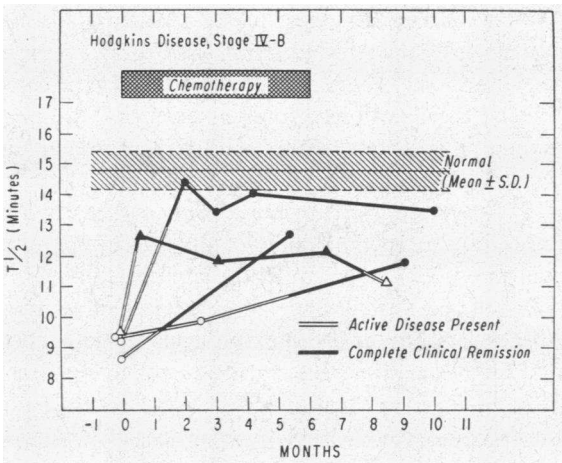

FIG. 4. TIME COURSE OF CHANGES IN THE PLASMA CLEARANCE OF THE 5.0 MG PER KG DOSE OF AA-^{125}I IN PATIENTS WITH STAGE IVB HODGKIN'S DISEASE TREATED WITH CHEMOTHERAPY (SHADED BOX) AS IN FIGURE 3. The patient represented by triangles (▲) relapsed during the eighth month.

the chemotherapeutic regimen, and despite maintenance of complete clinical remission, the $t_{\frac{1}{2}}$ was accelerated (10.1 minutes). The patient with previous IIIB disease had a normal clearance of AA while in remission (14.2 minutes), but when restudied after relapse (again stage IIIB), showed acceleration (11.2 minutes). Similarly, the patient studied after apparently complete operative removal of small intestinal Hodgkin's disease exhibited normal clearance of AA (14.1 minutes), but on relapse 6 months later with stage IVB disease, clearance had accelerated (11.4 minutes).

Discussion

The present studies not only demonstrate enhancement of phagocytosis in patients with Hodgkin's disease but also directly relate its degree to the stage, and therefore to the total extent, of the disease. Several previous studies of reticuloendothelial phagocytic capacity in human neoplastic diseases (all of which also used radioiodinated aggregated human serum albumin as the colloidal test substance) included 11 patients with Hodgkin's disease (18, 24–27); six showed enhanced clearance of AA when compared with control values, and the remaining five were normal. Thus, these previous studies suggest that when phagocytic function of patients with Hodgkin's disease is altered, it is enhanced. More complete interpretation of these earlier data is hampered by the small numbers of patients and the lack of information as to stage of disease and time of performance of the clearance study relative to courses of therapy.

Animal experiments have also shown certain neoplasms to be associated with enhanced clearance of colloidal substances by the reticuloendothelial system. Marked increases have been described in the hepatic uptake of radioactive gold in rats bearing subcutaneously transplanted lymphomas when compared with control animals (28). Other studies have demonstrated progressive increases in the clearance rate of colloidal carbon accompanying the growth of several transplantable solid tumors and certain forms of leukemia in mice (29, 30).

The cause of the enhanced clearance of AA exhibited by our patients with Hodgkin's disease is not apparent. Since none had evidence of coexistent infectious or rheumatic diseases, conditions known to be associated with enhanced reticuloendothelial phagocytic function (20, 31), other explanations must be sought. It is possible that acceleration of reticuloendothelial phagocytic capacity, as measured by the intravascular disappearance of AA, is nonspecific, similar to other secondary manifestations of the disease such as acute phase reactants (sedimentation rate, C-reactive protein, and so on), anemia, and abnormal levels of serum albumin and globulins. Indeed, as might be expected, changes in reticuloendothelial activity during the course of the disease tended to fluctuate in the same direction as alterations in some of these secondary parameters. It should be noted, however, that changes in phagocytic capacity were observed without concomitant changes in the aforementioned nonspecific manifestations. Tumor size and disease activity seemed to be the major variables associated with changes in the clearance rate of the AA. In that regard, then, one of the two following mechanisms could be responsible. First, since acceleration of clearance is proportional to the extent of the disease, the neoplastic tissue itself might take up the AA. Alternatively, increase in function or size of normal reticuloendothelial tissues could be responsible for the more rapid removal of the colloidal substance from the blood [in the case of AA, almost all of the test dose is extracted by the liver and

spleen (22)]. There have been only suggestive data available in the literature to refute or support either hypothesis. As regards the first possibility, it has been recently observed that the abnormal tissues of Hodgkin's disease seem to concentrate L-methionine-^{75}Se rather selectively (32). However, this substance is not colloidal, and it seems more probable that it is incorporated into the tumor proteins than taken up by phagocytosis. We have been unable to find reports that the abnormal cells in Hodgkin's disease have any phagocytic capabilities for colloidal substances.

The alternative possibility of hyperfunction of normal reticuloendothelial tissues in Hodgkin's disease, perhaps through the presence or increased concentration of a humoral factor (for example an opsonin), seems more likely. Indeed, such a mechanism appears to explain the enhanced reticuloendothelial phagocytosis associated with certain experimental tumors. It has been reported that rats bearing lymphomas showed an increased hepatic deposition of colloidal radioactive gold despite the extrahepatic location of the transplanted tumors, and it was felt that the neoplasms probably released substances that affected phagocytic function in distant reticuloendothelial tissues (28, 33). Old, Clark, Benacerraf, and Goldsmith (30) noted that not all experimental neoplasms in mice were associated with reticuloendothelial stimulation; but, when present, enhanced reticuloendothelial phagocytosis seemed best correlated with the degree of "foreignness of the inoculated tissue." Thus, these authors feel that reticuloendothelial stimulation accompanies the general homograft reaction of the host to the transplanted neoplastic tissue. Indeed, other models of graft–host interaction are clearly associated with enhanced reticuloendothelial phagocytosis (34). The reticuloendothelial stimulation associated with Hodgkin's disease could be secondarily related to such factors.

On the other hand, the long term presence of incompatible tissues could lead to prolonged reticuloendothelial stimulation and secondary lymphomatous transformation (35). Such considerations are supported by the recent experimental observations of Schwartz and Beldotti (36), who reported the development of lymphomas in long term survivors of graft–versus–host reactions in mice, a model similar to that used by Howard (34) in his elucidation of the relationship between reticuloendothelial stimulation and graft–versus–host reaction. Similarly, Walford (37) injected mice with cells differing only at a very weak histocompatibility locus. Such animals eventually developed lymphomas even though no overt evidence of graft–versus–host reaction was present. The status of the reticuloendothelial phagocytic function was not assessed in these animal studies.

Prolonged stimulation with antigens other than those related to histocompatibility is also associated with an increased incidence of lymphomas (38) as are experimental (39) and clinical "autoimmune" diseases (40, 41), disorders recently associated with enhanced reticuloendothelial phagocytic function (31).

It seems evident, then, that enhancement of reticuloendothelial phagocytic function is regularly associated with certain neoplastic diseases of animals and man. As pertains to Hodgkin's disease, the finding of enhanced phagocytosis seems paradoxical in view of the increased incidence of infectious complications and the several immunologic defects that might have been more readily explained by the finding of impaired reticuloendothelial phagocytic function. Whether the observed alteration plays any role in the pathogenesis of Hodgkin's disease itself, its infectious complications, or its immunologic deficits remains to be demonstrated. Nonetheless, the determination of the clearance of AA in such patients may offer another measure of extent or activity of disease and be of prognostic value. Further, serial measurements of AA clearance may permit earlier diagnosis of relapse. In our studies, clinical remission was generally associated with slowed clearance of AA (towards normal), whereas relapse was accompanied by acceleration. Instances occurred, however, even in this small series of patients, when the clearance of AA remained rapid despite apparently complete remission. Elucidation of the clinical significance of these findings will require long term studies.

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