Immune Function of Successfully Treated Lymphoma Patients

GERALD W. KING, BASEL YANES, PAUL E. HURTUBISE, STANLEY P. BALCERZAK, and ALBERT F. LOBUGLIO

From the Division of Hematology and Oncology of the Department of Medicine and The Department of Pathology, Ohio State University College of Medicine, Columbus, Ohio 43210

ABSTRACT Immunologic function was evaluated in 12 patients with Hodgkin's disease and 5 patients with lymphocytic lymphoma who had been successfully treated with either chemotherapy, radiation therapy, or both of these modalities 3–42 mo previously. Only two of the patients were found to have total anergy to a battery of six recall skin test antigens and all were responsive to skin testing with phytohemagglutinin. However, 10 of 16 patients were unable to develop delayed cutaneous hypersensitivity to either of the neoantigens dinitrochlorobenzene or keyhole limpet hemocyanin. Four other patients developed reactivity to only one of these neoantigens for a total of 14 of 16 (88%) of the patients demonstrating some impairment in neoantigen response.

Total lymphocyte, T-lymphocyte, B-lymphocyte, and null cell numbers, as well as serum immunoglobulins were quantitatively normal. Monocyte numbers, chemotaxis, and Fc receptor activity were normal. Monocyte staphylocidal activity at 60 min was modestly depressed and candidacidal activity was depressed in those receiving both chemotherapy and radiation therapy.

Spontaneous (unstimulated) lymphocyte [^{3}H]thymidine incorporation was low in the patients as a group and lymphoblastic transformation to specific antigens was impaired in 11 of 17 patients who had positive skin test reactions to the same antigen. Highly significant suppression of lymphoblastic transformation was noted after stimulation by the mitogens phytohemagglutinin, pokeweed, and concanavalin-A. The greatest impairment of mitogen response was seen in those patients receiving both chemotherapy and radiation therapy.

These data demonstrate specific impairments of neoantigen processing, lymphocyte function, and to a lesser extent monocyte function in successfully treated patients with lymphoma. These impairments may contribute to the increased incidence of infections and second primary malignancies in these patients.

INTRODUCTION

Modern therapy for patients with Hodgkin's and non-Hodgkin's lymphoma has advanced to the point that prolonged unmaintained remissions and possibly even cures are becoming the rule rather than the exception (1). Although immunologic defects have been well characterized in untreated patients with lymphoma (2–12), relatively little is known about the immune status of patients successfully treated with intensive combination chemotherapy and/or radiation therapy who are in unmaintained remission (6).

Patients with untreated Hodgkin's disease have been the most extensively studied and commonly appear to have evidence of impaired cellular immunity. These abnormalities include lymphopenia and impaired response to recall skin tests, neoantigens, and homografts as well as defective in vitro lymphocyte reactivity (2–8). Patients with non-Hodgkin's lymphoma have been studied less extensively, but the described defects primarily involve humoral immune function (5). Defective skin test reactivity and neoantigen processing may occur in non-Hodgkin's lymphoma, but this is usually related to general debility (9). Some investigators have suggested that after successful therapy of Hodgkin's disease, skin test reactivity to recall and neoantigens improves and lymphocyte function may return to normal (10–12). These observations were made in a small number of patients who had not received intensive modern therapy.

Recently, an increased incidence of second malignancies (13, 14) and infections (15, 16) in this treated

This work was presented in part at the meetings of the Central Society of Clinical Research, November, 1975 (*Clin. Res.* 23: 495A and 523A.); and the American Society of Hematology, December, 1975.

Received for publication 18 December 1975.

lymphoma population have been reported and have raised questions regarding the immunocompetence of these successfully treated patients. Canellos et al. have reported a remarkably increased incidence (18-fold) of second malignancies in Hodgkin's disease patients who have received both chemotherapy and radiation therapy (13). Other authors have related an increased incidence of acute leukemia to successful radiation therapy for Hodgkin's disease (14).

The purpose of this study was to evaluate the immune function of successfully treated lymphoma patients and to correlate the findings with diagnosis, type of therapy, and duration of remission. Seventeen patients with lymphoma who had completed successful chemotherapy, radiation therapy, or a combination of the two were utilized in this study. These evaluations were performed at times sufficiently remote from their treatment to avoid monitoring the acute effects of radiation or chemotherapy.

METHODS

Subjects. 17 patients with Hodgkin's disease (HD) [1] or non-Hodgkin's lymphoma were selected from the services of the Ohio State University Hospitals and Riverside Methodist Hospital on the basis of (*a*) having received intensive combination chemotherapy, radiation therapy, or both in "curative" doses and (*b*) having been in complete remission on no maintenance therapy for at least 3 mo before study. Normal values were established in groups of healthy volunteers of similar age and sex distribution and were performed during the same time period as the patient studies.

Skin tests. Recall antigens utilized in skin testing included mumps (V-1059 Eli Lilly and Company, Indianapolis, Ind.), *Candida albicans* (Dermatophyton-0 1:100, Hollister-Stier Laboratories Inc., Spokane, Wash.), Trichophyton (Dermatophytin 1:30, Hollister-Stier Laboratories Inc.), histoplasmin (Parke, Davis and Co., Detroit, Mich.), tuberculin (5-TU, Parke, Davis and Co.) and streptokinase-streptodornase (SK/SD, Varidase SK 100 U/ml. SD 25 U/ml, Lederle Laboratories, Pearl River, N. Y.). Each was injected intradermally in 0.1 ml vol on the forearm and reactions were read at 24 and 48 h. Reactions were considered to be positive if induration of greater than 5 mm was present at 48 h, except for mumps which was considered positive if greater than 5 mm erythema was present. Phytohemagglutinin (PHA[HA 16/17], Burroughs Welcome and Co., Tuckahoe, N. Y.) was administered intradermally in a dose of 2 μg in 0.1 ml. Reactions were read at 24 h and were considered positive if greater than 5 mm of induration was present.

Neoantigen sensitization and challenge. Keyhole limpet hemocyanin (KLH) was kindly provided by Dr. Evan Hersh, M.D. Anderson Hospital, Houston, Texas. It was administered by intradermal injection in doses of 100 μg/0.1 ml as a sensitizing dose. Challenge skin tests with KLH in the same dose were administered 3 wk after the sensitization. Reactions of greater than 5 mm induration were considered positive (17).

Dinitrochlorobenzene (DNCB[1-chloro-2,4-dinitrobenzene] Mathieson Coleman Bell, Norwood, Ohio) was diluted to 2 mg/0.1 ml in acetone and applied to the skin surface of the upper arm as a sensitizing dose. Challenge doses of 100, 50, and 25 μg were applied to the volar aspect of the opposite arm 3 wk later. Reactions were considered positive if any erythema, induration, or ulceration was present within any of the areas of challenge at 24 or 48 h. Three normal volunteers developed markedly positive reactions to all challenge doses of this preparation in our laboratory and other authors have shown that 95% of normal subjects develop reactivity utilizing this technique (2).

Mononuclear cell isolation. Mononuclear cells were isolated from venous blood by Ficoll-Hypaque technique as previously described (18). Defibrination with glass beads was performed before the Ficoll-Hypaque separation in those studies in which pure lymphocyte preparations (> 90%) were desired (lymphocyte function and quantitation) and glass adherent monocyte monolayers of > 95% purity were prepared from nondefibrinated suspensions for the Fc receptor assays as previously described (19).

Lymphoblastic transformation (LBT). The response of blood lymphocytes to stimulation by antigens or mitogens was determined by measuring the uptake of [^{3}H]thymidine ([^{3}H]TdR) by 3- and 5-day cultures of lymphocytes as previously described (20). Doses of mitogens were PHA-M (General Biochemicals, Chagrin Falls, Ohio) 25 μl/10^6 cells, pokeweed mitogen (PWM-Grand Island Biological Co., Grand Island, New York) 10 μl/10^6 cells, and concanavalin A (Con-A, Pharmacia Laboratories, Piscataway, N. J.) 25 μg/10^6 cells. Antigen-stimulated LBT was performed with tuberculin, trichophyton, SK/SD, and candida utilizing a dose-response curve for each antigen. The maximum response was used in evaluation of LBT.

Lymphocyte quantitation. The E-rosette assay for identification of T-lymphocytes using sheep erythrocytes was performed according to the method of Jondal et al. (21). The erythrocyte-antibody complement (EAC) assay for complement (C3) receptors of B-lymphocytes was performed according to the method of Bianco et al. (22). Immunofluorescent identification of membrane immunoglobulin on B-lymphocytes (MIg) was performed according to the technique previously reported from our laboratory (23). "Null" cells were determined by subtracting the number of E-rosette cells plus either EAC or MIg cells (whichever was greater) from total lymphocyte numbers.

Monocyte function. Monocyte chemotaxis to the stimuli *Escherichia coli* culture supernates and tuberculin induced lymphokines was determined as previously reported (24). Membrane Fc receptor activity was measured according to the technique described by LoBuglio et al. (25) and in vitro staphylocidal and candidacidal activities were determined as previously reported (26).

Quantitative immunoglobulins. Serum was obtained from each subject before sensitization with the neoantigens. Total immunoglobulins and specific levels of immunoglobulins G,

[1] *Abbreviations used in this paper:* Con-A, concanavalin-A; CT, chemotherapy; CT-RT, chemotherapy and radiation therapy; DNCB, 1-chloro 2,4-dinitrobenzene; EAC, erythrocyte-antibody complement assay for complement receptors of B-lymphocytes; HD, Hodgkin's disease; KLH, Keyhole limpet hemocyanin; LBT, lymphoblastic transformation; LL, lymphocytic lymphoma; MIg, membrane immunoglobulin (immunofluorescence) assay for B-lymphocytes; PHA, phytohemagglutinin; PWM, pokeweed mitogen; RT, radiation therapy; SK/SD, streptokinase/streptodornase; [^{3}H]-TdR, tritiated thymidine.

TABLE I

Successfully Treated Lymphoma Patients

Subject	Sex	Age	Diagnosis/ stage	Treatment	Time since treatment
					mo
P. A.	M	36	HD/II	RT	7
B. B.	M	31	HD/I	RT	30
R. B.	M	33	HD/III	CT-RT	24
C. C.	F	32	HD/III	CT-RT	42
R. C.	M	24	HD/III	RT	42
T. C.	M	17	HD/I	RT	12
J. H.	F	48	LL/III	CT-RT	3
D. K.	M	62	LL/III	CT	11
D. L.	F	28	HD/II	RT	33
L. L.	F	31	HD/II	RT	30
S. P.	F	32	HD/IV	CT-RT	6
B. R.	F	31	HD/I	RT	36
R. R.	M	50	HD/III	CT-RT	5
T. R.	M	57	LL/III	CT	3
C. S.	M	43	HD/III	CT-RT	17
J. S.	F	47	LL/III	CT-RT	7
M. S.	F	64	LL/IV	CT	16

M, and A were determined according to the method of Killingsworth and Savory (27).

Statistical analyses. Data were analyzed with the *t* test for independent samples (28).

RESULTS

Personal data for the treated lymphoma patients are listed in Table I. The mean age of the patients was 39 yr and there was a relatively equal distribution of men and women in each diagnostic and treatment category. The duration of unmaintained remission varied from 3 to 42 mo with a median of 16 mo. Thus, one-half of the subjects had been off all treatment for almost 1½ yr.

Skin tests. Only two of the patients studied were totally anergic to the battery of recall skin test antigens (Table II) and both of them had HD (L. L. and R. R.). Of the recall antigens tested, mumps, SK/SD, and histoplasmin were the most consistently positive at 82, 47 and 41%, respectively. All the patients had a normal response to skin testing with PHA supporting the evidence for an intact ability to mount a normal mononuclear cell infiltrate.

Neoantigen sensitization. One of the most significant abnormalities noted in these patients was the inability to be sensitized by the neoantigens DNCB and KLH (Table II). 10 of the 16 patients studied were unable to develop skin test reactivity to both these neoantigens and four others (R. B., R. C., J. H., and D. K.) developed reactivity to only one of them. Thus 14 of the 16 patients (88%) demonstrated some impairment in developing delayed hypersensitivity to neoantigens. Five of the patients who did not respond to challenge with DNCB at 21 days did have a spontaneous flare at the site of primary sensitization 10–12 days after the sensitization. Those patients in unmaintained remission for greater than 1 yr demonstrated the same incidence of impairment as those who were studied less than 1 yr from their last treatment.

Lymphoblastic transformation to mitogens. The spontaneous uptake of [^{3}H]TdR by unstimulated lymphocytes

TABLE II

Skin Test Reactivity

			Recall				Neoantigens	
Subject	PPD*	Histo	Mumps	SK/SD	Candida	Trich	DNCB	KLH
P. A.	−	+	−	−	−	−	−	−
B. B.	−	−	+	+	−	−	−	−
R. B.	−	−	+	+	−	−	+	−
C. C.	−	−	+	+	−	−	ND*	ND
R. C.	−	−	+	−	−	−	+	−
T. C.	−	+	+	−	−	+	−	−
J. H.	−	+	+	+	−	−	−	+
D. K.	−	−	+	−	+	+	+	−
D. L.	−	+	+	−	+	−	−	−
L. L.	−	−	−	−	−	−	+	+
S. P.	−	+	+	+	−	−	−	−
B. R.	−	+	+	+	−	−	+	+
R. R.	−	−	−	−	−	−	−	−
T. R.	+	+	+	+	−	−	−	−
C. S.	−	−	+	−	−	+	−	−
J. S.	−	−	+	+	−	−	−	−
M. S.	−	−	+	−	−	−	−	−

* ND, Not done; PPD, purified protein derivative.

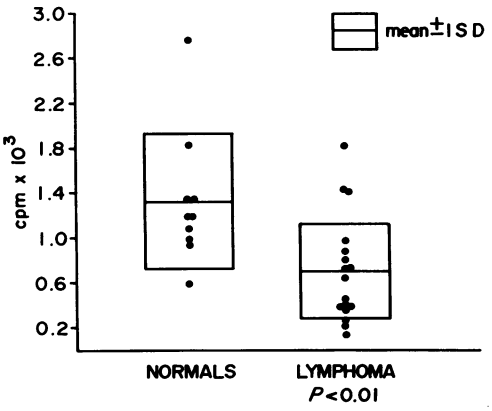


FIGURE 1 The spontaneous (unstimulated) uptake of [^{3}H]-TdR by lymphocytes after 3 days of culture.

is shown in Fig. 1. Compared to lymphocytes of 10 normal subjects, the patients' lymphocytes had a significantly lower [^{3}H]TdR uptake after a 3-day culture period. Although nine of the patients had values within the normal range, differences between the two groups were highly significant at $P < 0.01$. By 5 days culture the [^{3}H]TdR uptakes by patients' cells (947±1,120 cpm) had still not reached normal 3-day levels. In view of these differences in unstimulated cultures, the results of stimulated LBT were evaluated in terms of total counts per minute and increments above the unstimulated values as well as stimulation ratios.

The results of stimulation with the mitogens PHA, Con-A, and PWM are shown in Figs 2A and 2B. Total counts per minute of [^{3}H]TdR taken up by stimulated 3-day lymphocyte cultures were significantly impaired in patients with HD and lymphocytic lymphoma (LL) regardless of the mitogen used, but the responses to Con-A and PWM were more severely impaired than those to PHA. When evaluated on the basis of treatment received (Fig. 2B), those patients who had received both chemotherapy and radiation therapy had the poorest response to all three mitogens. Of course, it should be noted that only three of the patients received chemotherapy alone.

When LBT was evaluated in terms of individual increments above unstimulated [^{3}H]TdR uptake, the same patterns of response were noted in each category with an apparent increase in statistical significance being noted. Analysis of the data using stimulation ratios revealed variable results due to the variability in baseline values, but significant impairment of LBT was identified in the Hodgkin's disease patients with Con-A ($P < 0.001$) and PWM ($P < 0.01$), the radiation therapy (RT) patients with PWM ($P < 0.05$) and chemotherapy and radiation therapy (CT-RT) patients with Con-A ($P < 0.001$) and PWM ($P < 0.001$).

LBT to specific antigens. Table III lists the results of LBT to specific antigens as it relates to skin test reactivity. Impairment of in vitro lymphocyte function can only be implied in those situations with a score of 1, as those patients had positive skin test reactivity to the specific antigen, but their lymphocytes did not respond to stimulation by the same antigen in vitro. 11 of the 17 patients had impaired LBT to at least one specific antigen by this criterion and only 2 of a possible 11 (S. P. and T. R.) had the expected correlation between positive skin tests and specific LBT. Those situations with a score of three suggest normal in vitro lymphocyte response to stimulation by an antigen but some other problem of lymphocyte or nonlymphocyte origin which impaired the development of a skin test reaction to the specific antigen. Of the 17 patients, 12 demonstrated impairment by this criterion and of a possible 64 disparities between negative skin tests and positive LBT, there were 19 occurrences.

Lymphocyte quantitation. Lymphocyte quantitation revealed quite a heterogeneous pattern, (Table IV). Four patients had lymphocytosis and two patients were lymphopenic. Generally, the patients with lymphocytosis had increased numbers of both B and T cells except for patient R. R. whose lymphocytosis appeared to be due largely to cells lacking membrane markers (null cells). Both patients with lymphopenia had reduced numbers of T cells. One patient (C. S.) with a borderline low lymphocyte count and lower limits of normal T cells had almost complete absence of B cells as measured by both

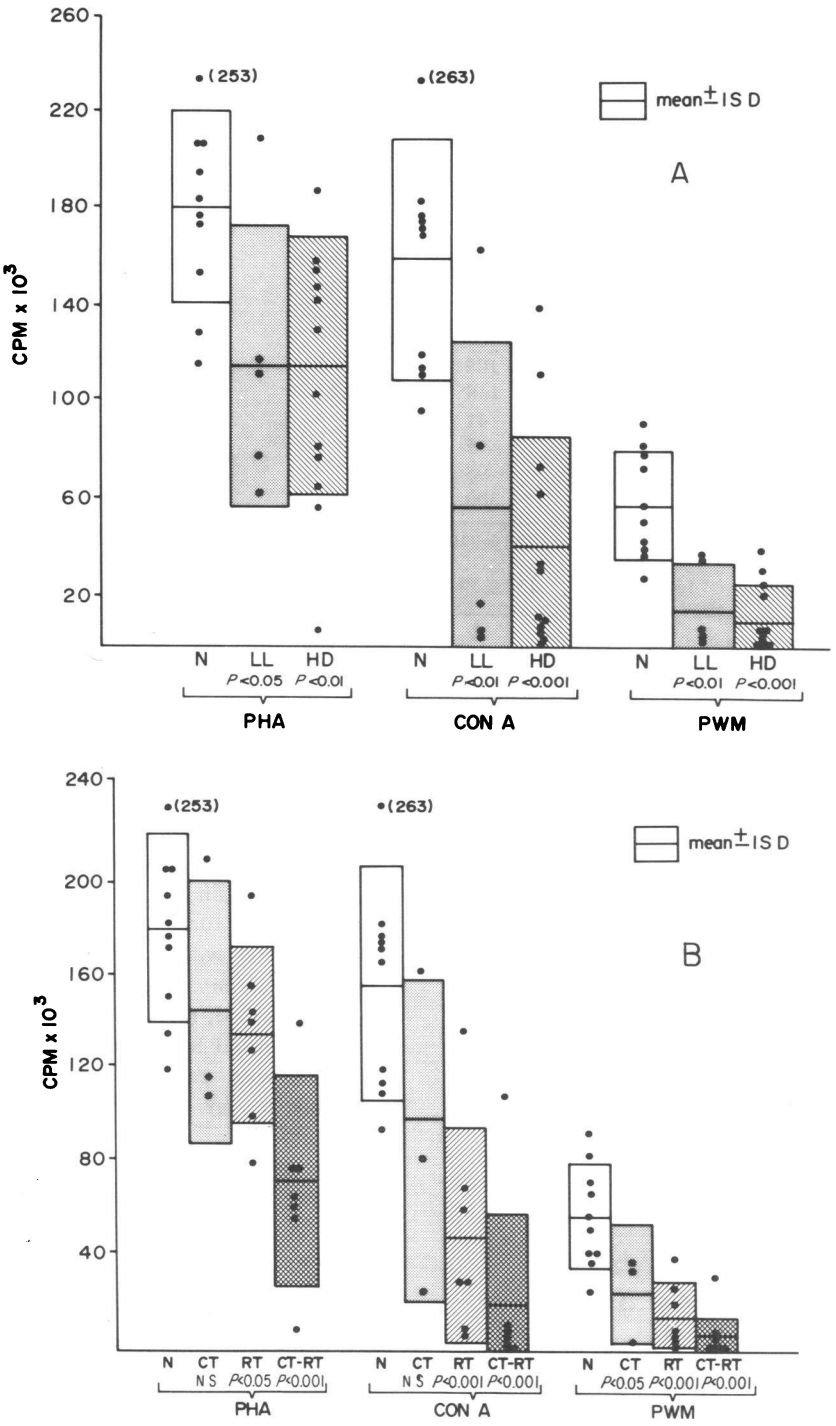
TABLE III
*Correlation of LBT and Skin Tests**

	Candida	PPD‡	SK/SD	Trichophytin	KLH
P. A.	3	2	3	2	2
B. B.	2	3	1	2	2
R. B.	2	3	1	2	2
C. C.	2	2	1	2	ND
R. C.	3	3	2	3	ND
T. C.	2	2	2	1	2
J. H.	2	3	1	2	1
D. K.	1	3	2	1	3
D. L.	1	3	2	2	ND
L. L.	2	3	2	2	1
S. P.	3	3	0	2	ND
B. R.	2	3	1	2	1
R. R.	2	2	2	2	2
T. R.	2	0	0	3	2
C. S.	2	2	2	1	2
J. S.	2	2	1	2	2
M. S.	3	3	3	2	2

* Scored according to following relations to skin test reactivity:

		Skin Test	LBT
0	=	+	+
1	=	+	−
2	=	−	−
3	=	−	+

‡ PPD, purified protein derivative.



FIGURES 2A and 2B. The response of lymphocytes to mitogen stimulation for a 3-day culture period. N = normal controls; LL = lymphocytic lymphoma; HD = Hodgkin's disease; CT = chemotherapy; RT = radiotherapy; CT-RT = chemotherapy and radiotherapy. The *P* values refer to significance of differences between patient groups and normal controls with that mitogen. □ = mean±1 SD.

TABLE IV
Lymphocyte Quantitation

Subject	Diagnosis	MIg*	Eac‡	E§	Null	Total
P. A.	HD	109	122	1,590	164	1,876
B. B.	HD	226	287	1,304	148	1,739
R. B.	HD	322	306	2,608	472	3,402
C. C.	HD	161	126	1,333	81	1,575
R. C.	HD	146	372	1,847	395	2,614
T. C.	HD	792	1,094	3,263	67	4,424
J. H.	LL	84	154	995	80	1,229
D. K.	LL	1,654	376	4,807	385	5,568
D. L.	HD	245	294	1,489	114	1,897
L. L.	HD	88	163	1,262	173	1,598
S. P.	HD	72	60	578	61	711
B. R.	HD	97	108	815	160	1,083
R. R.	HD	247	148	2,164	1,157	3,568
T. R.	LL	21	41	863	52	956
C. S.	HD	9	5	865	50	924
J. S.	LL	58	95	479	186	750
M. S.	LL	217	300	1,114	126	1,540
		268±400	238±249	1,610±1,099	227±271	2,085±1,388
Normals (13)		151±57	154±39	1,697±505	105±67	1,977±522

* Membrane immunoglobulin cells/mm³.
‡ Complement receptor cells/mm³.
§ Sheep erythrocyte rosette cells/mm³.

B cell markers. Although mean levels of B cells (EAC and MIg) and null cells were increased in the patients as a group compared to normals, these differences were not statistically significant. T-lymphocyte numbers, as determined by the E-rosette assay were essentially the same for patients and normals. When broken down into diagnostic and treatment categories, the only significant difference from normal was an increased number of EAC cells in patients treated with radiotherapy (349± 343, $P < 0.05$). Thus, no consistent pattern or change in lymphocyte quantitation was found with the majority of patients falling within the normal range.

TABLE V
Monocyte Function Studies

	Normals	Patients
Chemotaxis		
lymphokines	10.3±2.0* (15)	11.9±5.1 (10)
E. Coli supernates	45±5.4* (15)	49±11.9 (11)
Fc Receptor activity	51.6±9.5‡ (18)	55.2±14.0 (11)
Microbicidal activity		
60 min staphylocidal activity	7.6±1.2§ (21)	6.7±0.9 (15) {$P < 0.02$}
4 h candidacidal	8.4±0.8§ (11)	7.2±2.0 (15)

* Number of migrated cells per high power field.
‡ Number of anti-D coated erythrocytes bound per 10^4 μg monocyte DNA.
§ Number of organisms killed × 10^6.

Quantitative immunoglobulins. Quantitation of immunoglobulins G, M, and A as well as total immunoglobulins were normal for all 11 patients who were studied. Of special note, patient C. S. who had essentially no B-lymphocytes by EAC and MIg assays had normal immunoglobulins.

Monocyte function studies. The patients as a group had normal absolute numbers of blood monocytes (441± 144/mm³). Monocyte chemotaxis to two stimuli and membrane Fc receptor activity were normal (Table V). Although a modest decrease in monocyte staphylocidal activity was noted at 60 min ($P < 0.02$), all but two of the patients fell within the normal range. No significant differences from normal were noted when the data were evaluated on the basis of diagnosis or therapy. Candidacidal activity was not significantly impaired in the patient group as a whole. However, four of the patients (3 HD, 1 LL) were clearly below the normal range. When evaluated on the basis of diagnosis and

treatment, significant differences in candidacidal activity were found between normals and those patients who had received both CT and RT ($6.2 \pm 2.8 \times 10^6$ organisms killed, $P < 0.05$).

Correlations. Attempts to correlate the observed alterations in lymphocyte function and neoantigen response with other characteristics of the patients were for the most part disappointing. Although most of the patients had LBT to all mitogens below the normal range (PHA 10/17, Con-A 14/17, and PWM 12/17), no correlation could be made between these functional impairments and total lymphocyte quantitation or number of B cells, T cells, or null cells. Also, no significant correlation was found between impaired LBT or neoantigen response and initial stage of disease or duration of unmaintained remission.

The most significant correlations were found in the relationship of lymphocyte function to type of therapy with the majority of the patients with impaired LBT having received CT/RT. Although statistically significant differences between the RT and CT/RT could only be defined with PHA stimulation ($P < 0.02$), the distribution (Fig. 2B) would suggest that except for a few individuals, CT/RT had a greater suppressive effect on the lymphocyte response to all the mitogens than did RT. CT alone appears to have the least long term effect of the three therapeutic modalities, but only three of our patients received CT alone making this point difficult to support. HD also would appear to be associated with a greater suppression of LBT than LL as far as distribution of individual values is concerned, but this difference is not statistically significant.

DISCUSSION

This study clearly demonstrates impaired cellular immunity in patients with lymphoma who are in prolonged, unmaintained remission after successful treatment with CT and/or RT. This impairment is documented in several ways. First, and most striking, is the observed impairment in developing delayed hypersensitivity to the neoantigens DNCB and KLH. Immunization with these antigens is almost always successful in normal subjects (2) and yet 11 of 16 (69%) of our patients failed to develop delayed hypersensitivity to the neoantigen DNCB and 13 of 16 (81%) failed to respond to KLH. The incidence of failure to respond to DNCB sensitization has been reported to be 30–100% (2, 10) in untreated HD patients but the response to KLH in lymphoma patients is unclear. This impairment in developing delayed hypersensitivity to neoantigens occurred despite a normal response to skin testing with the mitogen PHA and relatively normal skin testing with a battery of recall antigens. All of the patients developed erythema and induration 24 h after primary injection of PHA and only two of the patients failed to respond to any of the recall antigens. Neither of the anergic patients had any suppression of total lymphocyte counts or circulating T-cells and monocyte chemotaxis was normal. In view of these observations we interpret the failure to respond to DNCB and KLH as a defect in initiating a primary immune response since (*a*) lymphocyte and monocyte numbers were intact, (*b*) immunologic memory was relatively intact (recall skin testing), and (*c*) the effector arm of the delayed hypersensitivity response was normal (PHA skin testing and monocyte chemotaxis).

Another grossly abnormal function was the response of the patients' lymphocytes to in vitro stimulation with mitogens. Impaired responses to PHA occurred at least as frequently as reported by others in untreated patients (3, 4, 6, 7, 12, 28) but no correlation could be made with T-cell or total lymphocyte numbers, a point in contrast to previous reports of untreated patients (29, 30). Impaired response to Con-A and PWM was even more striking. This pattern is quite different from that reported for untreated HD patients where impairments in PHA responsiveness appear to be more severe than PWM (3). The impaired response to PWM was not associated with any decrease in blood lymphocytes or B-cell levels, suggesting a qualitative disturbance of B-cell response to mitogen stimulation. Other studies have reported chronic depression of B-cell numbers and impaired PWM response in radiated cancer patients (31) or increased B-cell numbers (32, 33) and normal PWM response (33) after RT of lymphoma.

Finally, a more difficult area to interpret is the noted disparity between LBT to antigens and skin test response to the same antigens. Churchill et al. have reported almost unanimous correlation of skin test reactivity and LBT to antigens in normal subjects and a frequent occurrence of positive LBT to an antigen with negative skin test to the same antigen in patients with untreated HD. They did not observe the converse, i.e., positive skin test and negative LBT and concluded that there was a nonlymphocyte defect in the immune response of their lymphoma patients (34). Although we did observe findings similar to those of Churchill et al. in 12 of our patients, 11 had positive skin tests to at least one antigen that did not stimulate LBT in vitro. This latter observation can be interpreted as a lymphocyte defect since it is clear that the patients had delayed hypersensitivity to the antigens in question. This disparity has been noted in congenital and acquired immune deficiency states (35). Of course, it might be argued that skin test reactivity and LBT to antigens do not always correlate in normal individuals (36) and that this is not a valid means of assessing cellular function in individual patients. However, the incidence of such

disparities in our treated lymphoma patients seems inordinately high.

Although the question of impaired monocyte function is raised by the foregoing studies of skin test reactivity and LBT to antigens, we were unable to demonstrate any significant impairment of monocyte chemotaxis by our in vitro assays utilizing two stimuli. This suggests that movement of the monocytes into sites of skin tests should have been intact and is further supported by the normal response to PHA skin testing which is thought to depend on mononuclear cell migration into the site of nonspecifically stimulated lymphocytes. The normal to slightly decreased microbicidal activity is probably more significant than is readily apparent in that untreated lymphoma patients have enhanced monocyte microbicidal activity against staphylococcus organisms (26). Apparently, eradication of the disease and/or therapy results in a decrease in microbicidal activity as compared to untreated lymphoma patients. Although staphylocidal activity was significantly lower than normal at 60 min, this modest change is probably not biologically significant since only two of the patients fell outside the normal range. Candidacidal activity was only impaired in those patients who had received both CT and RT, a point of possible significance since it would seem that this group had the greatest impairment of lymphocyte function.

These observations on the immune status of successfully treated lymphoma patients can be interpreted in several ways. First, the skin test anergy, impaired response to neoantigens, and in vitro lymphocyte abnormalities are similar to those of untreated patients and may indicate that the immune deficits associated with acute disease are not reversible with successful therapy. Second, therapy may have successfully eradicated disease and replaced the immune deficits due to disease with deficits due to irradiation and/or CT (37, 38). Third, these deficits may represent a combination of some residual disease related abnormalities and the addition of therapy-induced defects. We would suggest that therapy is at least contributing to the in vitro abnormalities noted in lymphocyte function. Studies of a large number of successfully treated lymphoma patients are underway in the Southwest Oncology Group (Kansas City, Kansas) to allow correlation of immune status with stage of disease, histologic subtypes, type of therapy, incidence of infection or second malignancy and ultimate prognosis.

The observed defects in these patients may well contribute to an increased incidence of infectious complications but the role of these defects in the development of second malignancies is unclear. Canellos et al. (13) observed the greatest incidence of second primary malignancies in those HD patients who had received both CT and RT. It is tempting, therefore, to speculate that the immune functional abnormalities noted in our study are responsible for the development of these malignancies since there is at least a suggested greater impairment of lymphocyte function and monocyte function in this treatment group. However, one might also ask why the incidence of second malignancies does not more closely parallel the incidence of immunologic abnormalities if immunocompetence is so important in the resistence of these second cancers. Indeed, the entire concept of immune surveillance being important in the resistence of cancer is now being debated (39).

Another consideration in reference to the increased incidence of cancer in treated lymphoma patients is whether or not the therapy itself is oncogenic. There is good evidence that both ionizing irradiation (40) and CT with alkylating agents (41) are associated with an increased incidence of primary malignancies in man, and, of course, the treatment of lymphoma involves these modalities. None of these questions can be answered by our study but expansion of these studies to larger numbers of patients with a more prolonged period of observation may clarify these important relationships. It may well be that as radiation and CT of other tumors becomes more successful, the problem of second malignancies will be seen in increasing numbers of these patients as well.

ACKNOWLEDGMENTS

The authors appreciate the assistance of Dr. Thomas Stevenson of the Department of Pathology and Riverside Hospital, Columbus, Ohio; Dr. William Knospe of Rush-Presbyterian-St. Luke's Hospital, Chicago, Ill. and members of the Division of Hematology who provided patient material for this study.

This work was supported by the following grants: 1) Clinical Research Center of the College of Medicine RR 34; 2) Interdisciplinary Oncology Unit of the Cancer Research Center 5PO1-CA-16-58-02; 3) Leukemia-Lymphoma Program of the Cancer Research Center 1 PO1 CA-15147-01, and 4) National Cancer Institute contract NCT-CB-53936. Dr. Yanes is a post-doctoral fellow of the Division of Hematology and Oncology and is supported by the Clinical Cancer Education Program of the National Cancer Institute 1 R25 CA 18016. Dr. LoBuglio is an American Cancer Society Clinical Professor of Oncology.

REFERENCES

1. Fairley, G. H. 1974. Introduction: Leukemia and Lymphoma I. *Semin. Hematol.* **11**: 1–3.
2. Young, R. C., M. P. Corder, H. A. Haynes, and V. T. DeVita. 1972. Delayed hypersensitivity in Hodgkin's disease. A study of 103 untreated patients. *Am. J. Med.* **52**: 63–72.
3. Matchett, K. M., A. T. Huang, and W. B. Kremer. 1973. Impaired lymphocyte transformation in Hodgkin's disease. Evidence for depletion of circulating T-lymphocytes. *J. Clin. Invest.* **52**: 1908–1917.

4. Hersh, E. M., and J. J. Oppenheim. 1965. Impaired *in vitro* lymphocyte transformation in Hodgkin's disease. *N. Engl. J. Med.* **273**: 1006–1012.
5. Aisenberg, A. C. 1973. Malignant lymphoma. *N. Engl. J. Med.* **288**: 883–890.
6. Levy, R., and H. S. Kaplan. 1974. Impaired lymphocyte function in untreated Hodgkin's disease. *N. Engl. J. Med.* **290**: 181–186.
7. Papac, R. J. 1970. Lymphocyte transformation in malignant lymphomas. *Cancer.* **26**: 279–286.
8. Kelley, W. D., D. L. Lamb, R. L. Varco, and R. A. Good. 1960. An investigation of Hodgkin's disease with respect to the problem of homotransplantation. *Ann. N. Y. Acad. Sci.* **87**: 187–202.
9. Miller, D. G. 1971. Immunological disturbances in lymphomas and leukemia. *In* Immunological Disease. M. Samter, editor. Little, Brown and Co. Inc., Boston. 2nd edition. **1**: 548–570.
10. Aisenberg, A. C. 1962. Studies on delayed hypersensitivity in Hodgkin's disease. *J. Clin. Invest.* **41**: 1964–1970.
11. Sokal, J. E., and N. Primikirios. 1961. The delayed skin test response in Hodgkin's disease and lymphosarcoma: effect of disease activity. *Cancer.* **14**: 597–607.
12. Han, T., and J. E. Sokal. 1970. Lymphocyte response to phytohemagglutinin in Hodgkin's disease. *Am. J. Med.* **48**: 728–734.
13. Canellos, G. P., V. T. DeVita, J. C. Arseneau, J. Whang-Peng, and R. E. C. Johnson. 1975. Second malignancies complicating Hodgkin's disease in remission. *Lancet.* **1**: 947–949.
14. Rosner, F., and H. Grunwald. 1975. Hodgkin's disease and acute leukemia. Report of eight cases and review of the literature. *Am. J. Med.* **58**: 339–353.
15. Casazza, A. R., C. P. Duvall, and P. P. Carbone. 1966. Infection in lymphoma. Histology, treatment and duration in relation to incidence and survival. *J. Amer. Med. Assoc.* **197**: 710–716.
16. Schimpff, S. C., M. J. O'Connell, W. H. Greene, and P. H. Wiernik. 1975. Infection in 92 splenectomized patients with Hodgkin's disease. A clinical review. *Am. J. Med.* **59**: 695–701.
17. Curtis, J. E., E. M. Hersh, W. T. Butler, and R. D. Rossen. 1971. Antigen dose in the human immune response. Dose-response relationships in the human immune response to keyhole limpet hemocyanin. *J. Lab. Clin. Med.* **78**: 61–69.
18. Sagone, A. L., A. F. LoBuglio, and S. P. Balcerzak. 1974. Alterations in hexose monophosphate shunt during lymphoblastic transformation. *Cell. Immunol.* **14**: 443–452.
19. Koller, C. A., G. W. King, P. E. Hurtubise, A. L. Sagone, and A. F. LoBuglio. 1973. Characterization of glass adherent mononuclear cells. *J. Immunol.* **111**: 1610–1612.
20. Sagone, A. L., and S. Murphy. 1975. Chronic lymphocytic leukemia lymphocyte: correlation of functional, metabolic, and surface immunoglobulin characteristics. *Cell Immunol.* **18**: 1–8.
21. Jondal, M., G. Holm, and H. Wigzel. 1972. Surface markers on human T and B lymphocytes. I. A large population of immune lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* **136**: 207–215.
22. Bianco, C., R. Patrick, and V. J. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. *J. Exp. Med.* **132**: 702–720.
23. King, G. W., P. E. Hurtubise, A. L. Sagone, A. F. LoBuglio, and E. N. Metz. 1975. Leukemic reticuloendotheliosis. A study of the origin of the malignant cell. *Am. J. Med.* **59**: 411–416.
24. Rinehart, J. J., A. L. Sagone, S. P. Balcerzak, G. A. Ackerman, and A. F. LoBuglio. 1975. Effects of corticosteroid therapy on human monocyte function. *N. Engl. J. Med.* **292**: 236–241.
25. LoBuglio, A. F., R. S. Cotran, and J. H. Jandl. 1967. Red cells coated with immunoglobulin G. Binding and sphering by mononuclear cells in man. *Science (Wash. D. C.)* **158**: 1582–1585.
26. King, G. W., G. Bain, and A. F. LoBuglio. 1975. The effect of tuberculosis and neoplasia on human monocyte staphylocidal activity. *Cell Immunol.* **16**: 389–395.
27. Killingsworth, L. M., and J. Savory. 1971. Automated immunochemical procedures for the measurement of the immunoglobulins IgG, IgA and IgM in human serum. *Clin. Chem.* **17**: 936–940.
28. Worcester, J. 1966. The statistical method. *N. Engl. J. Med.* **274**: 27–36.
29. Falletta, J. M., N. Ramanujam, K. A. Starling, and D. J. Fernbach. 1973. Ig-positive lymphocytes in Hodgkin's disease. *N. Engl. J. Med.* **288**: 581–582.
30. Bobrove, A. M., Z. Fuks, S. Strober, and H. S. Kaplan. 1975. Quantitation of T and B lymphocytes and cellular immune function in Hodgkin's disease. *Cancer.* **36**: 169–179.
31. Young, R. C., M. P. Corder, C. W. Berard, and V. T. DeVita. 1973. Immune alterations in Hodgkin's disease. Effect of delayed hypersensitivity and lymphocyte transformation of course and survival. *Arch. Int. Med.* **131**: 446–454.
32. Stratton, J. A., P. E. Byfield, J. E. Byfield, R. C. Small, J. Benfield, and Y. Pilch. 1975. A comparison of the acute effects of radiation therapy, including or excluding thymus, on the lymphocyte subpopulations of cancer patients *J. Clin. Invest.* **56**: 88–97.
33. Engeset, A., S. S. Frölaud, K. Bremer, and H. Host. 1973. Blood lymphocytes in Hodgkin's disease. Increase of B-lymphocytes following extended field irradiation. *Scand. J. Haematol.* **11**: 195–200.
34 Churchill, W. H., R. R. Rocklin, W. C. Maloney, and J. R. David. 1973. *In vitro* evidence of normal lymphocyte function in some patients with Hodgkin's disease and negative delayed cutaneous hypersensitivity. *Natl. Cancer Inst. Monogr.* **36**: 99–106.
35. Spitler, L. E., A. S. Levin, D. P. Stites, H. H. Fudenberg, B. Pirofsby, C. S. August, E. R. Steihm, W. H. Hitzig, and R. A. Gatti. 1972. The Wiscott-Aldrich Syndrome. Results of transfer factor therapy. *J. Clin. Invest.* **51**: 3216–3224.
36. Chaparas, S. D., J. N. Sheagren, A. DeMeo, and S. Hedrick. 1970. Correlation of human skin reactivity with lymphocyte transformation induced by mycobacterial antigens and histoplasmins. *Amer. Rev. Respir. Dis.* **101**: 67–73.

37. Stjernsward, J., M. Jondal, F. Vanky, H. Wigzell, and R. Sealy. 1972. Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma. *Lancet.* **1**: 1352–1356.
38. Winkelstein, A. 1973. Differential effects of immunosuppressants on lymphocyte function. *J. Clin. Invest.* **52**: 2293–2299.
39. Schwartz, R. 1975. Another look at immunologic surveillance. *N. Engl. J. Med.* **293**: 181–184.
40. Bizzozero, O. J., K. G. Johnson, and A. Ciocco. 1966. Radiation-related leukemia in Hiroshima and Nagasaki, 1946–64. I. Distribution, incidence, and appearance time. *N. Engl. J. Med.* **274**: 1095–1101.
41. Rosner, F., and H. Grunwald. 1974. Multiple myloma terminating in acute leukemia. Report of 12 cases and review of the literature. *Am. J. Med.* **57**: 927–939.