QUANTITATIVE ANTIBODY STUDIES IN MAN. III. ANTIBODY RESPONSE IN LEUKEMIA AND OTHER MALIGNANT LYMPHOMATA [1]

By DANIEL L. LARSON [2] AND LOIS J. TOMLINSON

(From the Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital, New York, N. Y.)

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The lymphocyte, the plasma cell and the cells of the reticuloendothelial system have been described as sites of antibody formation in animals (1–5). Since the malignant lymphomata in man are often associated with an increase in the number of these cells, studies were initiated to determine if abnormalities in antibody response occurred in patients with leukemia, lymphosarcoma or Hodgkin's disease.

Several reports have appeared in the literature describing the ability to form antibodies in patients with leukemia and related diseases. Antigens used have included typhoid organisms (6–9), vibrio vaccine (10), and horse serum (11, 12). Antibody responses, measured by methods based on serum dilution techniques, suggested an impairment of antibody formation in patients with chronic leukemia, especially in those with chronic lymphatic leukemia. Heterophile antibody titers have been studied in patients with leukemia and allied diseases, and the data have recently been reviewed (13, 14). The evidence indicates that the incidence of elevated heterophile antibody titers is not increased in the leukemias. The purpose of this communication is to report observations on antibody formation in human leukemia, lymphosarcoma and Hodgkin's disease, using the quantitative precipitin technique developed by Heidelberger and his associates (15).

METHODS

None of the patients who served as subjects in this study received radiotherapy, nitrogen mustard or folic acid antagonists for a period of at least six months before the test period. During the test period a few of the subjects were given supportive blood transfusions. Patients with acute leukemia treated with cortisone were started on the drug several days to several weeks before the test period and were maintained on the drug in dosage of 100 to 300 milligrams by mouth per day throughout the test period. No attempt was made to classify the diagnosis in patients with acute leukemia into the lymphatic and myeloid forms. After obtaining a preliminary blood sample, the patients were given 0.08 milligram each of type-specific pneumococcus capsular polysaccharides I and II subcutaneously. Several post-immunization blood samples were obtained, and the highest post-immunization titer observed is reported as the antibody response. Analyses for C-antibody and antibody to the capsular polysaccharides were carried out according to the method described by Heidelberger and his associates (15). With this method, the de-fatted serum is first treated with crystalline egg albumin and rabbit anti-egg albumin serum to remove the complement. The C-antibody is then removed from the serum by addition of C-polysaccharide derived from a pneumococcus type other than the types used as antigens. The serum is then analyzed for the presence of antibodies to the capsular polysaccharides. Results are expressed as micrograms of precipitable antibody nitrogen per 4 milliliters of serum.

RESULTS

Previous observations have established that the maximal antibody response to type-specific pneumococcus capsular polysaccharides I and II in humans is usually 10 to 60 micrograms of antibody nitrogen per 4 milliliters of serum (15, 16). A few normal individuals have been noted to have post-immunization antibody titers as high as 100 micrograms, and occasionally little or no antibody response is detected.[3] Representative values for antibody response in normal individuals are shown in Table I.

Most of the patients under study had no detectable precipitable antibody to the capsular polysaccharides in the preliminary blood sample. The time of appearance of antibodies following the ad-

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[3] According to the data in (15) the normal means are 30 and 29 micrograms of antibody nitrogen per 4 milliliters of serum, with a S.D. of 37 and 25 for antibody to SI and SII, respectively.

TABLE I

Antibody formation in normal man

Subject	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum: Pre-immunization	Post-immunization
1	C III	46	28
	S I	3	11
	S II	0	11
2	C III	21	18
	S I	1	27
	S II	1	23
3	C III	50	57
	S I	4	30
	S II	1	23
4	C III	7	6
	S I	0	29
	S II	1	45
6	C III	32	25
	S I	0	0
	S II	0	6
9	C III	18	17
	S I	1	4
	S II	0	7
11	C III	60	62
	S I	1	15
	S II	8	24
35	C III	7	8
	S I	0	52
	S II	0	96
39	C III	26	26
	S I	0	15
	S II	0	22
40	C III	39	50
	S I	1	144
	S II	3	61
41	C III	12	8
	S I	0	9
	S II	0	19
52	C III	33	48
	S I	0	22
	S II	2	15

* Letter and numeral refer to pneumococcus polysaccharide precipitinogen used, *i.e.*, C III and C VII are the somatic polysaccharides from Type III and Type VII pneumococcus, respectively; S I and S II are the capsular polysaccharides derived from pneumococcus Types I and II, respectively.

ministration of the antigens was similar to that observed in normal individuals. Maximal antibody titers were usually achieved by two weeks following immunization and patients living as long as six months after immunization showed a persistence of these elevated antibody titers. As has been described in normal individuals, there was usually little change in the levels of C-antibody during the test period (15, 16).

Nine of 11 patients with untreated acute leukemia produced unusually large amounts of antibody to at least one of the antigens (Table II). Patient 71 was an adult who entered a spontaneous temporary remission during the test period and formed very little antibody. As is shown in Table III, the seven patients with acute leukemia who

TABLE II

Antibody formation in patients with untreated acute leukemia

Subject	Age	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum: Pre-immunization	Post-immunization
73	39	C VII	29	37
		S I	1	537
		S II	1	669
16	32	C III	39	45
		S I	0	396
		S II	0	563†
104	38	C VII	28	14
		S I	0	624
		S II	1	111
117	38	C VII	30	69
		S I	0	121
		S II	14	604
121	32	C VII	62	36
		S I	0	25
		S II	0	640
97	61	C VII	51	61
		S I	0	122
		S II	9	447
15	19	C III	18	18
		S I	0	78
		S II	0	586
120	15	C VII	20	19
		S I	0	47
		S II	0	428
123	45	C VII	7	19
		S I	0	86
		S II	0	374
118	15	C VII	8	19
		S I	0	14
		S II	2	20
71‡	43	C VII	24	23
		S I	1	4
		S II	3	4

* See footnote, Table I.

† Specimen of serum became contaminated in the course of repeated absorptions and this probably represents a value less than the true anti-S II content.

‡ Patient entered a period of spontaneous temporary remission during the test period.

TABLE III

Antibody formation in patients with acute leukemia treated with cortisone

Subject	Age	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum: Pre-immunization	Post-immunization
85	52	C VII	98	21
		S I	0	284
		S II	0	59
110	61	C VII	43	22
		S I	0	153
		S II	0	116
107	30	C VII	40	35
		S I	0	141
		S II	0	53
112	73	C VII	54	32
		S I	0	45
		S II	0	148
108	55	C VII	15	26
		S I	0	8
		S II	0	139
93	53	C VII	16	189
		S I	0	30
		S II	0	62
111	30	C VII	25	33
		S I	0	10
		S II	0	67

* See footnote, Table I.

were treated with cortisone showed antibody responses which were often well above the normal range but appeared to be considerably lower than in the untreated group.

In Table IV, data on seven patients with chronic myeloid leukemia indicate an antibody response essentially in the normal range. Eight out of nine patients with chronic lymphatic leukemia exhibited little or no detectable antibody response (Table V). Patient No. 113 had an episode of bacterial pneumonia during the test period, accompanied by a normal antibody response to the administered antigens.

Three patients with Hodgkin's disease and four patients with lymphosarcoma presented a low or normal antibody response (Table VI). Previous studies have indicated (17) that patients with multiple myeloma form little or no antibody to pneumococcus capsular polysaccharides I and II.

DISCUSSION

The pneumococcus polysaccharide-anti-polysaccharide system has several features which make it different from many other antigen-antibody systems. The antigen is non-protein and it fails to stimulate antibody formation in the rabbit (18). In man, elevated antibody titers may persist for years following a single antigenic stimulation, and in these individuals, an anamnestic response does not occur (19). For these reasons information on antibody response obtained in man using the pneumococcus polysaccharides does not necessarily apply to other antigen-antibody systems or to other species.

The ability to form antibodies to the pneumococcus capsular polysaccharides in these studies appeared to be influenced by the type of leukemia present. Individuals with acute leukemia had the largest number of immature white blood cells in their peripheral blood and bone marrow, and were also noted to have the largest antibody responses. There was no apparent relation between the total white blood cell count in the peripheral blood and the amount of antibody produced. The data do raise the possibility that, in man, the immature white blood cell may play a role in the formation

TABLE IV

Antibody formation in patients with chronic myeloid leukemia

Subject	Age	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum: Pre-immunization	Post-immunization
61	69	C III	25	71
		S I	0	144
		S II	0	38
70	51	C VII	10	15
		S I	0	211
		S II	9	0
22	51	C VII	6	0
		S I	0	45
		S II	0	67
23	60	C III	26	21
		S I	4	53
		S II	0	57
82	55	C VII	24	51
		S I	0	44
		S II	0	63
83	45	C VII	21	33
		S I	0	26
		S II	0	46
96	62	C VII	82	86
		S I	0	24
		S II	0	10

* See footnote, Table I.

of antibody to the pneumococcus polysaccharides. Other studies (15, 16) have indicated that a large antibody response to one antigen is not necessarily accompanied by a similar response to another antigen. This observation is confirmed by the present studies and remains without an explanation.

It has been shown that the administration of cortisone to patients with rheumatoid arthritis has no detectable effect on the level of circulating antibody to pneumococcus polysaccharides when cortisone is given either before or during the period of active immunization (16). In the present studies, cortisone administration to patients with acute leukemia was associated with the production of smaller amounts of antibody than was observed in the group of patients with acute leukemia who did not receive cortisone. However, almost half of the values were well above the normal range of antibody response. The dosage of cortisone used in treating patients with acute leukemia was considerably larger than the dosage used in the treatment of rheumatoid arthritis and this may have influenced the results.

TABLE V

Antibody formation in patients with chronic lymphatic leukemia

Subject	Age	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum: Pre-immunization	Post-immunization
19	72	C III	26	16
		S I	0	0
		S II	0	0
21	49	C III	39	36
		S I	0	0
		S II	0	0
72	52	C VII	35	42
		S I	0	0
		S II	0	0
81	73	C VII	12	12
		S I	0	3
		S II	0	0
86	65	C VII	49	18
		S I	0	0
		S II	0	0
94	63	C VII	8	18
		S I	0	3
		S II	0	3
116	47	C VII	0	0
		S I	0	3
		S II	1	3
119	57	C VII	12	10
		S I	0	0
		S II	2	0
113†	48	C VII	74	35
		S I	0	43
		S II	0	32

* See footnote, Table I.

† Patient had an episode of acute bacterial pneumonia during the test period.

TABLE VI

Antibody formation in patients with Hodgkin's disease or lymphosarcoma

Subject	Age	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum: Pre-immunization	Post-immunization
20	20	C III	52	28
Hodgkin's		S I	0	0
disease		S II	0	7
31	48	C III	68	70
Hodgkin's		S I	1	32
disease		S II	0	39
76	37	C VII	64	64
Hodgkin's		S I	1	0
disease		S II	1	1
18	55	C III	25	22
Lympho-		S I	0	0
sarcoma		S II	0	17
74	42	C VII	61	61
Lympho-		S I	0	25
sarcoma		S II	8	27
80	34	C VII	48	50
Lympho-		S I	0	1
sarcoma		S II	1	0
50	74	C VII	5	13
Lympho-		S I	0	3
sarcoma		S II	0	40

* See footnote, Table I.

SUMMARY

Antibody formation to pneumococcus capsular polysaccharides I and II has been studied in a group of human subjects with leukemia and other lymphomatous disease, using quantitative immunochemical techniques. Nine of 11 patients with untreated acute leukemia had unusually large antibody responses. These responses were impaired in seven patients with acute leukemia receiving cortisone. Eight of nine patients with chronic lymphatic leukemia exhibited little or no antibody response. Seven patients with chronic myeloid leukemia, three patients with Hodgkin's disease, and four patients with lymphosarcoma had antibody responses essentially within the normal range.

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