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Decline of Natural Nonselective Cell-mediated Cytotoxicity in Patients with Tumor Progression[1]

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SUMMARY

Lymphocytes isolated from the blood of patients and healthy donors include a population of cells that destroy target cells in the direct cell-mediated cytotoxic assay with little indication of specificity. This natural reaction is the dominant feature of most cell-mediated cytotoxic tests and, although it appears to be mostly nonselective, it possesses some selective activity. The observed cytotoxicity from these reactions depends mostly on the reactivity of the effector cell; when several effector cells are tested on different target cells, the relative order of activity is usually maintained on the different target cells. When this natural cytotoxicity was analyzed without regard to the type of cancer of the patient or of the target cells, a weak decline in the average reactivity was observed with increasing tumor involvement.

INTRODUCTION

When a patient with a specific type of cancer reacts selectively against a target cell derived from the same cancer, the reaction is often said to be disease related or tumor associated. This is often supported by findings reporting a higher proportion of patients reacting selectively against the target cell in comparison with control individuals. However, many specificities can be involved in selective reactions between effector and target cells so that the relationship of cytotoxicity to disease or cancer cannot be understood without first determining the exact specificity with which we are concerned. Only after the specificity is identified and its relationship to disease is determined can we discuss tumor-associated reactivity. With our present state of knowledge, it is not possible to predict or diagnose the type of cancer from the pattern of reactions observed.

The dominant aspect of most direct cell-mediated cytotoxic test results is natural cytotoxicity exhibited by effector cells from nearly all individuals whether they have cancer or not (13, 27, 28). The reaction is observed for effector cells isolated from patients as well as from healthy persons. Yet, until recently, this reaction was ignored and virtually excluded as background. Recently, we and others (4, 11, 17, 21) reported that a small population of cells which we called "N"-cells, that bore neither T nor B cell markers but that possessed Fc receptors, was responsible for the effect. Lymphocyte suspensions isolated from different persons vary considerably in N-cell activity so that when many persons are tested a wide range of cytotoxicity is observed.

Past attempts to monitor patients for CMC[2] or to relate CMC and the staging of cancer dealt with specific cytotoxicity for the type of cancer (3, 6, 20, 22). The inadequacies of the method used for demonstrating specificities in these studies have been thoroughly discussed (1, 10, 18), as have been the difficulties involved in monitoring patients (7) for specific immunity. However, several of these studies reported a decline in specific CMC with advancing disease (3, 6). The results from the present study, which tested patients without regard to the type of cancer, suggest that the decline is nonselective or due to an effect on natural cytotoxicity.

MATERIALS AND METHODS

Patients and Controls. Classification of patients was essentially through the TNM system with updating by the attending physician through clinical and laboratory studies. Since the composition of the stages varies with the site of the tumor and a wide variety of cancers was tested, the reader should refer to the TNM system proposed by Union International Contra Cancer (23, 29) for grouping patients into stages. The tests were performed with cells from patients in the UCLA Hospital presenting tumors except those patients who had just undergone surgery. A total of 239 patients were tested in this series: melanoma, 38; bladder, 39; breast, 21; prostate, 19; cervix, 17; gastrointestinal, 13; lung, 8; larynx, 8; ovarian, 7; vaginal, 7; and a variety of other cancers, 62. From these patients 227 were classified into 4 stages as follows: Stage I,41; Stage II,68; Stage III,32; and Stage IV,86. Concurrently, 224 individuals without cancer were also tested; 123 of these had a close association with our laboratory.

Patients were also classified according to the treatments they had received. These groups included 25 untreated patients, 122 patients who had undergone surgery, 6 treated with radiation, 8 treated with drugs, and 72 treated with combinations of such therapies in their histories. Of these, 33 had surgery and radiation, 30 had surgery and chemotherapy, 1 had radiation and drugs, and 8 had all 3 forms of treatment. The patients were divided into groups

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[2] The abbreviations used are: CMC, cell-mediated cytotoxicity; TNM, tumors-nodes-metastasis.

Table 1
Reactivity of patients by stages

Target cell	Controls: Lab normals No.[a]	Controls: Lab normals Av. Score	Controls: Other noncancers No.	Controls: Other noncancers Av. Score	Controls: All Noncancers No.	Controls: All Noncancers Av. Score	Stages: I No.	Stages: I Av. score	Stages: II No.	Stages: II Av. score	Stages: III No.	Stages: III Av. score	Stages: IV No.	Stages: IV Av. score
370	14	54 ± 6	10	38 ± 6	24	48 ± 4	5	43 ± 10	12	37 ± 8	3	61 ± 9	17	24 ± 6[b]
372	43	49 ± 3	43	39 ± 4	86	44 ± 2	25	33 ± 5[c]	41	33 ± 4	18	30 ± 5[c]	41	23 ± 3[b, d]
462	37	63 ± 4	24	38 ± 5	61	54 ± 4	13	51 ± 8	23	41 ± 5[c]	10	41 ± 10	24	38 ± 6[c]
497	18	54 ± 6	13	39 ± 8	31	48 ± 5	11	40 ± 8	21	29 ± 4[b]	7	38 ± 11	16	18 ± 6[b, e]
548	21	55 ± 5	22	50 ± 5	43	52 ± 4	13	41 ± 6	26	36 ± 3[b, e]	14	31 ± 5[b, e]	26	31 ± 4[b, d]
696	36	35 ± 6	31	31 ± 6	67	33 ± 4	18	31 ± 8	23	27 ± 6	12	15 ± 5[b, e]	26	31 ± 6
697	26	48 ± 4	27	34 ± 5	53	41 ± 4	8	38 ± 14	19	34 ± 7	8	39 ± 12	26	34 ± 4
804	29	66 ± 4	22	40 ± 6	51	55 ± 4	3	42 ± 12	19	36 ± 5[b]	9	42 ± 9	16	44 ± 6
917	36	40 ± 4	32	21 ± 4	68	30 ± 3	17	36 ± 5	32	22 ± 4	11	42 ± 9[c]	28	22 ± 4
952	21	39 ± 5	14	43 ± 9	35	41 ± 5	13	22 ± 5[c]	12	20 ± 6[c, e]	7	21 ± 4[b, e]	17	20 ± 4[b, e]
1042	28	61 ± 4	18	41 ± 8	46	53 ± 4	10	50 ± 7	10	50 ± 7	13	32 ± 8[c]	24	42 ± 5
1046	24	49 ± 4	30	30 ± 4	54	38 ± 3	8	44 ± 6	11	41 ± 8	14	28 ± 7	30	35 ± 4

[a] Number tested.
[b] Significant difference ($p < 0.01$) with respect to all noncancer controls (Column 3).
[c] Significant difference ($P < 0.05$) with respect to all noncancer controls (Column 3).
[d] Significant difference ($P < 0.01$) with respect to other noncancer controls (Column 2).
[e] Significant difference ($P < 0.05$) with respect to other noncancer controls (Column 2).

by treatment, retrospectively, as another way of examining the same population of patients.

Microassay for Cell-mediated Immunity. All lymphocyte samples were isolated from heparinized peripheral blood by centrifugation on Ficoll-Hypaque and tested as described previously (28). Target cells used in the study have been described (17, 28) except for Targets 804, 917, 952, 1042, and 1046. Target 804 is a subline of G11, and Target 1046 is a subline of T24, derived from a breast cancer and a bladder cancer, respectively. Targets 952 and 1042 were derived from osteosarcomas and Target 917 is from a melanoma.

RESULTS

We had observed previously that lymphocytes from healthy individuals associated with our laboratory and used as controls in CMC tests reacted more strongly than did other controls without cancer and that, together, they were slightly greater in cytotoxicity than cancer patients. The reactivity was, however, based on average scores with considerable variability and overlapping so that many patients were stronger reactors than healthy controls were. Table 1 shows a comparison of patients divided into groups by stages with controls consisting of persons associated with our laboratory and others with no indication of cancer. The table also shows results among patients that were significantly different from those of the total control group and the controls after exclusion of results from laboratory personnel. Regardless of the controls used, cancer patients were generally weaker, with more significant differences in the later stages.

In comparison with all noncancer controls, Targets 370, 372, 462, 497, 548, 696, 804, 952, and 1042 showed significant depression in activity at some stage, whereas targets 697, 917, and 1046 showed weaker indications without significance. After exclusion of laboratory personnel from the control group, significant depression was observed with Targets 372, 497, 548, 696, and 952. Results on Target 917 showed significantly higher activity with patients during Stages I and III, but the control value against this target is suspiciously low.

Table 2
Regression analysis of reactivity to staging

Target cell	Slope	Regression coefficient	p
370	−3.88	−0.18	0.285
372	−5.50	−0.21	0.003
462	−3.97	−0.16	0.197
497	−6.30	−0.30	0.029
548	−3.19	−0.18	0.110
696	−1.09	−0.04	0.710
697	−1.84	−0.08	0.547
804	1.89	0.08	0.600
917	−2.77	−0.13	0.224
952	−1.22	−0.08	0.576
1042	−2.97	−0.14	0.272
1046	−4.97	−0.25	0.066

The especially weak cytotoxicity by Stage IV patients indicated a declining reactivity rather than depressed reactivity for all cancer patients. Besides showing the decrease in mean cytotoxicity with progressive stages, we attempted to demonstrate declining reactivity in cancer patients by analysis for correlation of cytotoxicity with stages (Table 2). Although significant correlations were achieved with only 2 target cultures, 11 of the 12 target cells showed negative coefficients. The negative slope in Table 2 shows the decline in cytotoxicity with progressive stages for the best-fitting line.

The difficulties of staging cancer became apparent early in the study of tumor involvement and reactivity. The most objective method of staging was the TNM system but its accuracy decreased with the time interval between staging and testing. Updating by clinical criteria was useful but less

Table 3
Reactivity of patients classified by treatment

Target cell	Other non-cancer controls No.	Other non-cancer controls Av. score	All cancer patients No.	All cancer patients Av. score	Untreated No.	Untreated Av. score	Surgery No.	Surgery Av. score	Surgery and radiation No.	Surgery and radiation Av. score	Surgery and chemotherapy No.	Surgery and chemotherapy Av. score	All patients receiving chemotherapy No.	All patients receiving chemotherapy Av. score
370	10	38 ± 6	39	33 ± 4	7	43 ± 9	16	30 ± 7	8	34 ± 7			5	33 ± 11
372	43	39 ± 4	129	29 ± 2[b]	14	33 ± 7	65	29 ± 3[b]	18	29 ± 6	12	16 ± 5[c]	23	26 ± 5[b]
462	24	38 ± 5	50	45 ± 4	8	36 ± 13	42	43 ± 4	8	60 ± 10	7	27 ± 12	12	29 ± 8
497	13	39 ± 8	58	29 ± 3	9	34 ± 10	30	28 ± 4	7	30 ± 7	6	26 ± 11	9	20 ± 8
548	22	50 ± 5	80	34 ± 2[c]	9	39 ± 7	26	31 ± 3[c]	12	36 ± 7	14	24 ± 3[c]	22	30 ± 4[c]
696	31	31 ± 6	81	27 ± 3	11	23 ± 8	43	23 ± 4	6	53 ± 15	11	28 ± 8	18	34 ± 7
697	27	34 ± 5	61	35 ± 4	6	22 ± 12	33	33 ± 5	4	37 ± 13	6	15 ± 5[c]	14	25 ± 5
804	22	40 ± 6	47	40 ± 4	6	42 ± 10	24	41 ± 5	3	39 ± 6	3	39 ± 6	11	39 ± 9
917	32	21 ± 4	89	27 ± 2	9	39 ± 9	47	25 ± 3	12	41 ± 8[b]	13	16 ± 4	19	18 ± 3
952	14	43 ± 9	49	21 ± 3[b]	5	11 ± 3[c]	29	23 ± 4	4	21 ± 8	6	15 ± 4[c]	9	19 ± 4[b]
1042	18	41 ± 8	70	42 ± 3	8	47 ± 10	36	47 ± 4	10	38 ± 9	8	40 ± 11	14	38 ± 7
1046	30	30 ± 4	62	35 ± 3	7	23 ± 8	32	34 ± 4	8	34 ± 9	7	46 ± 7	14	43 ± 5

[a] Number tested.
[b] Significant difference ($P < 0.05$) with respect to other noncancer controls (Column 1).
[c] Significant difference ($P < 0.01$) with respect to other noncancer controls (Column 1).

precise. Although, admittedly, staging is weaker than desirable, we felt that it did reflect increasing tumor involvement which correlated with declining cytotoxicity (Tables 1 and 2).

From a different point of view, patients were divided into groups according to the type of treatment received. The average reactivity for each group is presented in Table 3. Division according to treatment left the categories of patients with radiation only, with chemotherapy only, with radiation and chemotherapy combined, and with all 3 forms of treatment, with insufficient numbers for stable comparisons. Untreated patients were, however, among the stronger reactors against Targets 370, 372, 497, 548, 804, 917, and 1042. Most groups with sufficient numbers were weaker than controls although the differences were not striking. The weakest group in Table 3 consisted of those treated by surgery and chemotherapy, suggesting the possible effect of drugs on the cytotoxicity exhibited.

Because immunosuppression during chemotherapy could account for the weak average reactivity of Stage IV patients, we averaged the results for all patients who had received chemotherapy. These patients as a group were less reactive than were controls and most other patients. However, when patients treated with drugs were excluded in calculating the average for Stage IV patients, the mean score was still depressed, indicating that factors other than drugs were responsible for the declining reactivity. These results are shown in Table 4.

Classifying the patient population by staging and by treatment appear to be different approaches to the problem, but a close relationship exists between the 2 methods (Table 5). Information on this relationship also supplied a better understanding of the patient population tested. Table 5 shows the past treatment of the patients divided by stages. The percentage of untreated cases and those treated by surgery alone decreases with progressive stages, whereas the percentage of patients treated by radiation and chemotherapy increases. The percentage of patients tested who had undergone surgery remained rather constant, indicating the

Table 4
Reactivity of Stage IV patients excluding patients receiving chemotherapy

Target cell	No.	Av. score
370	14	26 ± 6[a]
372	25	25 ± 4[a, b]
462	15	45 ± 7
497	9	15 ± 7[a, b]
548	12	33 ± 7[c]
696	16	31 ± 8
697	16	26 ± 6[c]
804	9	51 ± 6
917	19	24 ± 6
952	9	17 ± 7[b]
1042	17	39 ± 6
1046	19	29 ± 4

[a] Significant difference ($P < 0.01$) with respect to all noncancer controls (Table 1, Column 3).
[b] Significant difference ($P < 0.05$) with respect to other noncancer controls (Table 1, Column 2).
[c] Significant difference ($P < 0.05$) with respect to all noncancer controls (Table 1, Column 3).

nature of the UCLA Hospital as one receiving referrals from the community after primary treatment. Since only patients with tumors were included in the study, the analysis by treatment supported the conclusions drawn from staging.

Although we previously examined the relationship between age and cytotoxic reactivity on a larger set of healthy individuals (24) and concluded that little or no relationship existed, the large difference between our laboratory personnel and other normal persons and patients persisted. A reasonable explanation was the difference in ages since the median age for our laboratory workers was younger than for other healthy persons who, in turn, were younger than the patients. As found in the studies on mice (9, 14), we expected to find a decline with age (Table 6). No significant differences were observed on 12 targets when we tested persons without cancer who were divided into 3 age groups. With patients only, a very slight decline in reactivity was observed in tests with those over age 70 (Table 7).

Significant differences were observed on Targets 462, 917, and 1042 when the over-70 group was compared to those under age 29.

The results in Tables 6 and 7 were also studied for correlations between age and cytotoxicity by regression analysis. These results are shown in the last rows for both tables. The only significant decline with age was observed on Target 1042 with cancer patients. If any relationship does exist in humans between age and CMC, it is extremely weak. When all tests including patients and controls are combined (results not shown), the decline with age becomes more marked. However, this decline can be explained by the difference in reactivity between our laboratory personnel and patients and is probably less related to age.

DISCUSSION

Several investigators have recently reviewed the difficulties in detecting specificities through cell-mediated cytotoxic tests and in monitoring the patient during the course of disease (1, 7, 10, 18, 19). A major problem is the strong natural reactivity exhibited by controls, *i.e.*, persons who supposedly have not experienced immunological contact with the specificities under study. Also, the variability introduced into the test through the preparation of the effector cells, culturing of target cells, or technical aspects of the test makes it difficult to base conclusions on tests with 1 or a few patients. The difficulties arise from variability in re-

Table 5

The relationship between staging and treatment

Therapy	% in Stages I	II	III	IV
Untreated	18	12	11	6
Surgery only	65	64	49	44
Radiation only	0	3	3	4
Chemotherapy only	3	0	0	8
Surgery and radiation	13	12	19	14
Surgery and chemotherapy	0	9	14	17
Surgery, radiation, and chemotherapy	3	0	5	6
Radiation and chemotherapy	0	0	0	1
All surgery	81	85	87	81
All radiation	16	15	27	25
All chemotherapy	6	9	19	32

Table 6

Cytotoxicity by persons without cancer by age

No significant differences were observed with reference to persons aged 0 to 29 years.

	370			372			462			497			548			696		
Age	No.[a]	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.
0–29	13	48	6	46	46	3	38	56	4	17	45	6	28	55	5	38	36	6
30–49	7	56	6	29	46	4	15	55	8	11	55	8	11	44	6	20	30	7
50+	4	32	14	11	32	7	8	41	11	3	34	5	4	58	15	9	30	14
Regression coefficient		−0.32			−0.10			−0.05			0.23			0.18			−0.02	
	697			804			917			952			1042			1046		
0–29	36	44	5	31	55	5	42	30	4	21	38	6	29	52	6	32	38	4
30–49	12	35	6	13	56	8	20	30	5	8	41	8	13	52	7	13	41	5
50+	5	38	13	7	45	14	6	35	14	6	49	16	4	64	12	9	37	9
Regression coefficient		−0.01			−0.12			0.07			0.26			0.27			0.03	

[a] Number tested.

Table 7

Cytotoxicity by cancer patients by age

	370			372			497			462			548			696		
Age	No.[a]	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.	No.	Mean	S.E.
0–29	7	49	8	13	26	6	9	38	10	8	57	9	6	27	9	9	12	4
30–49	4	34	22	27	30	5	9	30	9	16	40	7	16	25	4	19	20	5
50–69	22	31	5	68	27	3	35	23	3	43	45	4	46	37	3	36	33	6
70+	4	46	10	19	29	5	4	52	6	6	25	10[b]	10	35	6	11	31	10
Regression coefficient		−0.26			−0.08			−0.06			−0.16			0.11			0.19	
	697			804			917			952			1042			1046		
0–29	8	37	9				9	38	8	7	28	11	11	51	7	7	32	8
30–49	15	29	7	12	33	6	18	24	6	18	23	5	13	50	7	15	32	6
50–69	26	27	5	26	43	5	46	29	4	20	17	3	35	45	4	27	33	4
70+	8	43	10	7	32	8	15	20	3[b]	4	34	4	10	22	6[c]	12	44	6
Regression coefficient		0.07			−0.21			−0.15			0.02			−0.27[b]			0.11	

[a] Number tested.
[b] Significant difference ($p < 0.05$) with respect to patients aged 0 to 29 years.
[c] Significant difference ($p < 0.01$) with respect to patients aged 0 to 29 years.

sults not only from the patients but also from the controls that are used. We attempted to solve these problems by examining more stable patterns of reactivity obtained from testing larger samples. Classification of patients into different categories, however, tended to decrease sample size and the reliability of the results.

Accurate classification of patients with all types of cancers into stages is a formidable problem (23). Precise staging, even when achieved, loses definition with advancing cancer or treatment. Whether stages of quite different cancers can be grouped together and are comparable needs further investigation. Despite these problems, we felt that the system of classification used in this study did reflect the development of the disease and that as a group each succeeding stage represented tumor progression. The relationship between staging and treatment supported this argument. Since patients who were cured or clinically tumor free were excluded from the study, most errors in staging tended to underestimate tumor involvement. The strongest depression in reactivity was observed with Stage IV patients where such errors were least serious.

A cell in the circulation of patients and healthy individuals that kills target cells rather nonselectively has been described recently (4, 11, 17, 21). A homologous reaction has also been detected in mice and is mediated by a similar type of cell (8, 14). To differentiate this reaction in man and mouse from immune T-cell-mediated cytotoxicity, it was called natural cytotoxicity (8, 9, 14, 15). The specificity of natural cytotoxicity in mice has been reported to be against viral-related antigens (9, 12, 15). Whether natural cytotoxicity in man is also related to viruses or to other factors needs to be demonstrated.

Natural cytotoxicity in the direct cell-mediated cytotoxic test may be divided into selective and nonselective components (26). In testing patients and controls at random on different cultured target cells, most of the cytotoxicity appears to be nonselective. Thus, when a decline in cytotoxicity against most target cells is observed in patients with progressing cancer regardless of its type, it is more probable that the weakening is an effect on nonselective or natural cytotoxicity than on specific immune cytotoxicity.

The decline in reactivity in later stages of cancer may also be explained in different ways. If some patients are treated with immunosuppressive drugs during their chemotherapeutic regimen, patients as a group may be weaker reactors than healthy individuals. With few exceptions, we observed that patients undergoing chemotherapy were generally less reactive. Exclusion of patients undergoing chemotherapy, however, still revealed that cytotoxic activity of lymphocytes from patients in later stages was less than the average reactivity of persons without cancer and weaker than that of most other patients. Also if specific immune reactivity is detected only during the early stages and combines with natural reactivity observed through all stages, a declining total reactivity might also be expected. Pursuing this approach, however, the strongest reactivity by healthy laboratory controls would suggest the greatest specific reactivity for this group. Studies on the strong reactivity by healthy individuals has, however, been shown to be primarily the result of N-cell activity (17), the effector in natural cytotoxicity.

The declining cytotoxicity appears to be closely associated with tumor involvement and has important implications in cancer induction and metastasis. If a role either in surveillance and resistance to tumor induction or in control of metastasis is postulated for N-cells, depressed reactivity would explain the progression and spread of the tumor. On the other hand, several studies have also reported nonresponsiveness or an inability to respond or to react in the presence of the tumor (2, 5, 30). Thus, it is essential to distinguish whether the weaker reactivity allows tumor occurrence and invasion or the presence of the tumor results in weaker reactivity. If the rather nonselective cytotoxicity by N-cells is really the human counterpart of natural cytotoxicity in mice, the first of these alternatives receives support. Kiessling *et al.* (15) have shown a relationship between natural cytotoxicity and resistance to YAC Moloney virus-induced leukemia cells in a semisyngeneic system (16). Moreover, nude mice with stronger than normal natural cytotoxicity (8) are as resistant as or more resistant to the induction of tumors (24, 25). Whether human N-cells serve a similar role needs to be investigated.

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