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Free DNA in the Serum of Cancer Patients and the Effect of Therapy

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SUMMARY

A radioimmunoassay for ng quantities of DNA was developed. [^{125}I]Iododeoxyuridine-labeled DNA was used as the antigen, and the serum of a lupus erythematosus patient served as the source of antibody. The level of free DNA in the serum of 173 patients with various types of cancer and in 55 healthy individuals was determined by this radioimmunoassay. DNA concentration in the normal controls had a range of 0 to 100 ng/ml with a mean of 13 ± 3 ng/ml (S.E.). For comparison purposes, the range of 0 to 50 ng/ml was designated as normal, and 93% of controls were found in this range. In the cancer patients, the DNA concentration ranged from zero to μg levels with a mean of 180 ± 38 ng/ml. Fifty % of the patients' values were found in the range of 0 to 50 ng/ml; the other 50% were between 50 and 5000 ng/ml. No correlation could be seen between DNA levels and the size or location of the primary tumor. Significantly higher DNA levels, however, were found in the serum of patients with metastatic disease (mean of 209 ± 39 ng/ml), as compared to nonmetastatic patients (mean 100 ± 30, $p <$ 0.02).

After radiation therapy in lymphoma, lung, ovary, uterus, and cervical tumors, the levels decreased in 66 to 90% of the patients, whereas in glioma, breast, colon, and rectal tumors, the DNA levels decreased only in 16 to 33% of the patients. Generally, the decrease in DNA concentration in the serum correlated with improved clinical condition, such as decrease of tumor size and reduction of pain. Conversely, when DNA levels either increased or remained unchanged, a lack of response to the treatment was noted. Of 17 patients who died within a year, 13 showed DNA levels that remained high or unchanged, whereas only 4 showed lower levels during treatment. Persistent high or increasing DNA levels in the circulation, therefore, may signal a relapse and are probably a poor prognostic sign.

The relatively high percentage (50%) of cancer patients with apparently normal DNA levels would suggest that this test may have low diagnostic value. It should be pointed out, however, that all these patients represent a selected group considered for radiation therapy, usually after surgery and/or chemotherapy. It is possible that a better correlation between DNA levels and cancer will be obtained prior to the initiation of treatment. On the other hand, DNA in the serum may be an important tool for the evaluation of therapy or the comparison of different regimens.

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INTRODUCTION

Tissue and cell injury take place under both normal and pathological conditions. It is expected, therefore, that intracellular material such as DNA may be released into the circulation. Indeed, free DNA has been detected in serum and other fluids such as synovial fluid (8). For example, circulating DNA can be found in disorders such as systemic lupus erythematosus, rheumatoid arthritis, pulmonary embolism or infarct, and cancer (5, 10). In addition, therapeutic procedures may also release DNA as after high-dose corticosteroid treatment or surgery (5, 8). Healthy controls, on the other hand, usually showed lower levels of free DNA in the serum. The results for both disease and control groups are highly variable, depending on the methods used for detection of DNA. In some instances, indirect methods such as hemagglutinin inhibition (10), complement fixation (3), and double diffusion in agarose (8) showed μg levels of DNA. In other cases, using counterimmunoelectrophoresis, only fractions of a μg were detected (5, 15). Considerable controversy exists concerning the normal level of DNA in healthy controls, with reported values ranging from barely detectable levels (3, 5) to μg per ml (10). Similarly, a wide range of levels has been reported for a variety of disorders, including leukemia, solid tumors, and chronic inflammatory diseases such as systemic lupus erythematosus and rheumatoid arthritis (10). In addition to the release of DNA, the common trait characterizing these diseases is lymphoid hyperplasia or sustained immunological stress. Most of the results agree in one respect: generally, the levels of DNA are higher than in healthy controls.

The development of a direct competitive binding test for ng quantities of DNA (12, 13) in our laboratory provided a sensitive probe for the investigation of DNA release. The method is based on the binding between ^{125}I-labeled DNA as the antigen and the serum of a systemic lupus erythematosus patient containing high-avidity antibody. Extraction and purification of the test sample are not necessary, and the determination can be performed directly on the patient's serum. No interference has been found from either RNA or low-molecular-weight nucleotides (12). The small amount of nonspecific binding by the basic component of complement (C1q) can be eliminated by heat inactivation (1). The conformation of DNA in the serum has been reported as single stranded by some investigators (10) and as double stranded by others (2, 7, 8). The antiserum used in our study contains antibodies against both double- and single-stranded DNA, with a relative avidity of 9.6 and 5.6 × 10^5 liters/mole, respectively (11). This antiserum, therefore, is used for the

determination of total DNA and cannot discriminate between the 2 forms of DNA if present in a mixture.

The purpose of this study was to determine the incidence of elevated DNA levels in cancer patients and whether radiation therapy affects the DNA level.

MATERIALS AND METHODS

Labeling and Preparation of DNA. DNA was labeled by growing *Escherichia coli* in the presence of [^{125}I]iododeoxyuridine as described previously (12, 13). After extraction and purification, the Millipore filtration step (12) was omitted, and the DNA was passed through a methylated albumin-Kieselguhr column (11). With this modification, the nonspecific binding of DNA by serum components was reduced to 2 to 3%.

DNA Antiserum. The serum of a lupus erythematosus patient (L. W.) was used as the antibody in the radioimmunoassay (11, 12).

Radioimmunoassay. The details have been described previously (12). Each sample was tested without antibody, for the determination of nonspecific binding. These counts were subtracted from the counts obtained in the presence of antibody. In the absence of cold DNA (no inhibition control), 45 to 52% of the counts were found in the precipitate. Known amounts of native salmon sperm DNA were added to normal human serum in the range of 0 to 1000 ng/ml, and a standard curve was constructed in the logit transform (12).

Patients and Sera. Blood samples were drawn from patients and normal, healthy controls by venipuncture. The blood was allowed to clot at room temperature within 1 hr, and the serum was separated and frozen until use. Twenty-five μl were taken for the determination of DNA concentration.

Only cancer patients considered for radiation therapy were taken for this study. Samples were drawn before the beginning of the treatment and on biweekly intervals during the treatment. All patients received radiation daily for a minimum of 2 weeks, and some received it for as long as 6 weeks. All patients had confirmed histological diagnosis of cancer. The test was performed without knowledge of their clinical condition, the presence or absence of metastases, or the outcome of surgical procedures. Subsequently, this information was obtained and correlated with the results of the DNA tests.

The normal controls consisted of healthy hospital personnel, matched by age (17 to 61 years) and sex (24 males, 31 females) to the cancer patients.

RESULTS

Normal Values of DNA in the Serum. The concentration of DNA was below detectable levels (0 to 25 ng/ml) in 42 of the 55 sera tested (76%). Another 9 sera contained 25 to 50 ng/ml (16%). Only 4 sera (7%) contained 50 to 100 ng/ml, and none were found above 100 ng/ml (Chart 1). Thus, 93% of the normal sera were found in the range of 0 to 50 ng/ml with a mean of 13 ± 3 ng/ml. For comparison purposes, 0 to 50 ng/ml was designated as the normal range. On repeated tests with subsequent samples from the 4 individuals with a DNA concentration of 50 to 100 ng/ml in the serum, 2 showed lower values in the 0 to 50 ng/ml range. It is unknown at present whether this represents normal fluctuation in DNA concentration in the serum or whether it is due to a latent or subclinical pathological condition. No correlation could be found between the DNA value and the age, sex, and smoking habits of the test subjects. Hopefully, further testing with larger numbers of individuals will clarify these points.

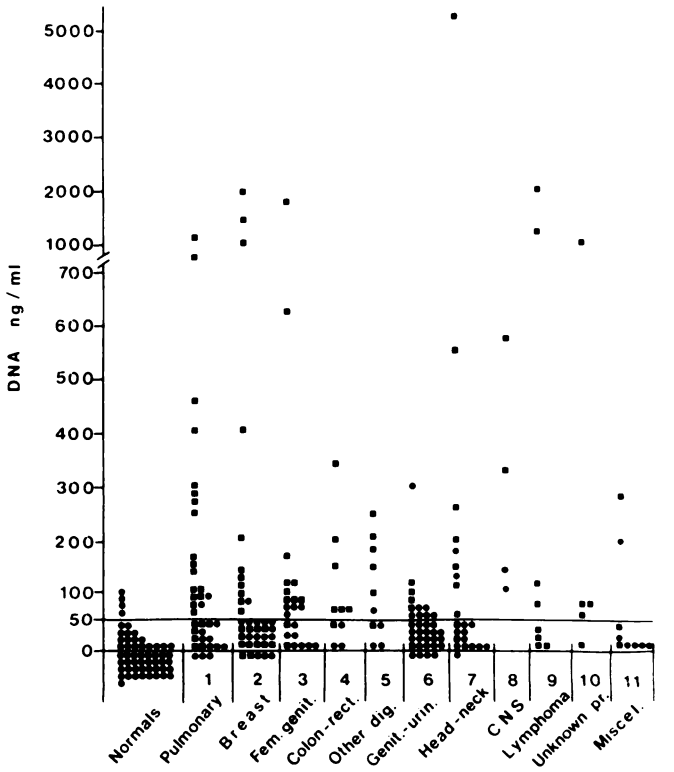

Chart 1. Distribution of DNA levels in the serum of tumor groups (total, 173 patients) and 55 healthy individuals, measured by radioimmunoassay. The values represent the average of duplicate determinations. The reproducibility was within 10 to 15% (12). ■, metastatic; ●, nonmetastatic. *Fem. genit.*, female genital; *Colon-rect.*, colon-rectum; *Other dig.*, other digestive; *Genit.-urin.*, genitourinary; *Unknown pr.*, unknown primary; *Miscel.*, miscellaneous.

DNA Concentration in the Serum of Cancer Patients before Therapy. The 173 patients were classified according to the site of the primary tumor. All types showed very wide ranges, from zero to as high as 5000 ng/ml. The mean for each group (± S.E.) and the p value relative to the mean of the normal controls were: (1) 164 ± 44, $p < 0.002$; (2) 193 ± 79, $p < 0.02$; (3) 182 ± 95, $p < 0.05$; (4) 102 ± 37, $p < 0.01$; (5) 104 ± 28, $p < 0.002$; (6) 41 ± 11, $p < 0.01$; (7) 108 ± 35, $p < 0.005$; (8) 286 ± 106, $p < 0.01$; (9) 493 ± 299, $p = 0.05$; (10) 245 ± 189, p not significant; and (11) 64 ± 39, p not significant. The mean for all cancer patients was 180 ± 38 ng/ml, $p < 0.005$. It can be seen that in some groups of tumors (head-neck, lung, breast, ovary, uterus, lymphosarcoma) very high concentrations of DNA could be found, up to μg levels. In others (digestive tract, genitourinary), the distribution had narrower range (Chart 1). In the central nervous system (glioma of brain or cord), all the patients

showed high values, but the small number of samples (4) precludes further evaluation of this finding. With the possible exception of central nervous system tumors, all groups showed some values in the normal range of 0 to 50 ng/ml. Interestingly, almost all patients with high values (above 100 ng/ml) had metastatic disease. In the low range (0 to 50 mg/ml), on the other hand, both types were found. No correlation could be found between the DNA concentration in the serum and the size, location, or histological composition of the tumor. However, in patients with well-contained, slow-growing tumors, DNA levels tended to be lower than in those with faster-growing, poorly differentiated cells. This correlation was seen clearly when the DNA levels in patients without evidence of metastases were compared to those with proven metastatic disease (Chart 2). The mean DNA concentration in the 1st group was 100 ± 30 ng/ml, whereas the mean calculated for the 2nd group was 209 ± 39 ng/ml ($p < 0.02$). The distribution of normal, healthy individuals is also included in Chart 2 for comparison.

DNA Concentration in the Serum after Radiation Therapy. In some tumors, such as lung, ovary, uterus, cervix, and lymphoma, the majority of the patients (66 to 90%) responded with lower DNA levels after radiation. In others, such as breast, colon, rectum, and glioma, this effect was observed only in a few of the patients treated (16 to 33%) (Table 1). Generally, when the treatment was beneficial, as determined by decrease in tumor size or reduction of pain, a decrease in serum DNA concentration also was observed. Conversely, when the treatment was unsuccessful, DNA levels tended to remain unchanged or even increased. Some representative examples for each type of response are illustrated in Table 2. The results for all types of cancer tested are summarized in Table 3. Clearly, in patients with metastatic disease, the DNA levels are generally higher (see above) and tend to stay high during and after treatment. Relapses occurred more frequently in this group, although this observation has not been evaluated critically because of the small number of patients. Of the 17 patients who died within 1 year, 13 showed DNA levels that increased or remained unchanged during the treatment, whereas the DNA decreased in only 4 cases.

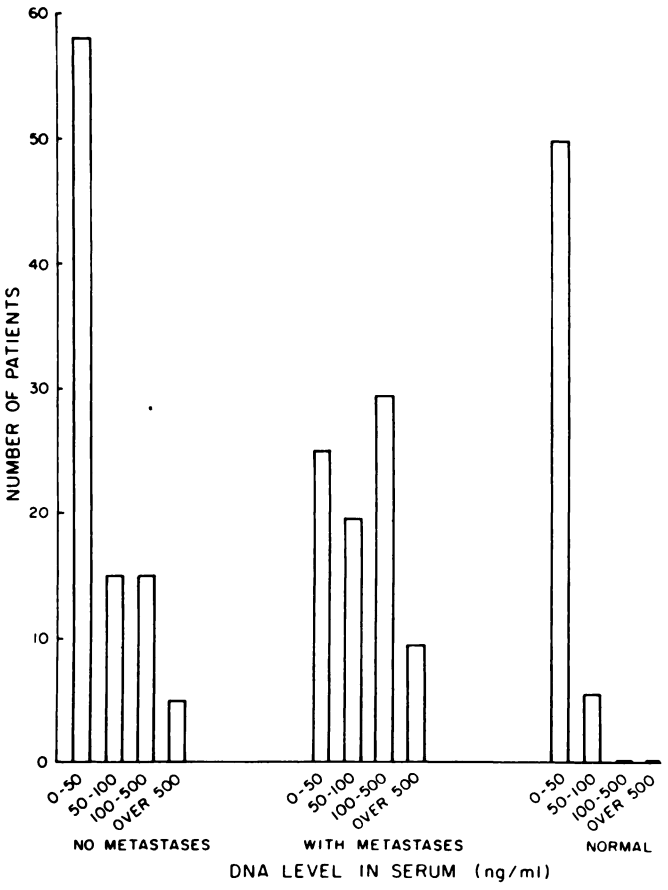

Chart 2. DNA levels in metastatic and nonmetastatic patients, compared to normal controls. The highest concentration in each group ranged between 500 and 5000 ng/ml.

DISCUSSION

The source of free DNA in solution in the serum is unknown at present. In addition to viral DNA, other sources have been reported such as normal lymphocytes (2) and tumor cells (14). The release of DNA from lymphocytes *in vitro* may also take place *in vivo*, although evidence for such a phenomenon is not available. Autolysis of cells after the collection of blood samples and release of DNA can be ruled out on the basis of control experiments. Repeated freezing and thawing of the white cell layer suspended in normal serum did not result in detectable DNA concentration in the supernatant fraction (S. A. Leon and B. Shapiro, unpublished results). The fact that DNA levels in normal individuals in our study are usually zero or very low (25 to 50 ng/ml), in contrast to other reports (3, 8, 10), is probably due to the method used. The choice of [^{125}I]iododeoxyuridine-labeled DNA, with its low affinity for normal globulin, as well as an antiserum containing high-avidity antibody for DNA, results in a very specific and sensitive determination of DNA (12). For example, we had shown previously that low-molecular-weight nucleotides and RNA do not interfere in this direct competitive binding radioimmunoassay, indicating that the antibody is highly specific for DNA (12). For these reasons, we conclude that normal serum contains very little free DNA, in the range of 0 to 50 ng/ml and, in a small number of sera, up to 100 ng/ml. Further study will be required to establish whether fluctuation in DNA levels within this range is a normal physiological phenomenon or due to a latent or subclinical pathological condition.

In cancer patients, higher concentrations of DNA in the serum may be expected if we assume that the source of this material is proliferating tumor cells (14). Another possibility is that the DNA is released from normal cells (lymphocytes) during their interaction with virus or tumor cells (16, 18). Isolation and characterization of the DNA in serum may be required to distinguish between these possibilities.

The persistence of DNA in the circulation raises another question, namely, the function of DNAse I and II in the serum (17). In malignant disease, lower activity of these enzymes has been reported. This may be due to the release of an inhibitor which may be present in both tumors (4) and normal cells (thrombocytes) (6, 9). We are presently investigating this question in our laboratory.

The elevated concentration of DNA in the serum of cancer

Table 1

Radiation therapy and DNA levels in serum of cancer patients

Pulmonary tumors included bronchogenic and alveolar adenocarcinoma; female reproductive tract: ovary, uterus and cervix; colorectal tumors were examined separately from other digestive types: esophagus, pancreas, and gallbladder. The genitourinary types included bladder, prostate, and testicular carcinomas. In the head and neck group, 8 tumors are represented: palate, epiglottis, gingiva, nasopharynx, tonsil, larynx, maxillary sinus, and submaxillary gland. The central nervous system group included glioma of the brain and cord. In the miscellaneous group were included bone giant cell carcinoma, epithelioma, melanoma, kidney, and hepatocarcinoma. Samples during and after treatment were not available for some patients. Levels were considered increased or decreased only if the change was greater than 25%.

	Before therapy					
	No. of patients			Effect of therapy (% no. of patients)		
Type	Total no. of patients	0–50 ng/ml	Over 50 ng/ml	Over 50 ng/ml (%)	Decreased	Increased	No change
1. Pulmonary	33	13	20	61	75	25	0
2. Breast	32	20	12	38	22	66	11
3. Female genitals	19	7	12	63	90	10	0
4. Colon-rectum	9	4	5	56	16	50	33
5. Other digestive	10	4	6	60	50	0	50
6. Genitourinary	29	19	10	34	55	27	18
7. Head-neck	17	9	8	47	55	33	11
8. Central nervous system	4	0	4	100	33	66	0
9. Lymphoma	7	3	4	57	66	33	0
10. Unknown primary	5	1	4	80	0	100	0
11. Miscellaneous	8	6	2	25	0	0	100
Total	173	86	87	50			
12. Normals	55	51	4	7			

Table 2

DNA levels in individual patients

The 1st number in each sequence represents the DNA level before radiotherapy. Subsequent values represent the levels after 2, 4, or more weeks of treatment.

		DNA (ng/ml)		
Patient	Tumor	Increase	Decrease	No change
H. M.	Lung (M)[a]		400–360–110	
G. D.	Lung (M)		250–140–75	
A. M.	Lung (M)		280–95–30	
J. G.	Lung (M)	140–1000–250		
H. S.	Lung (M)	30–190–160		
V. B.	Lung	25–115–120		
F. S.	Lung (M)			35–50–50
J. G. A.	Lung			80–60–80
I. B.	Breast (M)	25–120–120		
I. R.	Breast (M)	125–160–320–300		
R. J.	Breast	40–80–100		
B. M.	Breast (M)	115–210–200		
D. H.	Breast			90–95–60
H. P.	Breast (M)		140–90–80	
A. T.	Cervix (M)		170–100–50	
F. O.	Ovary (M)		1800–0–0	
D. Y.	Ovary (M)		650–50–50	
P. M.	Uterus			75–40–75
J. F.	Rectum (M)			175–200–150
A. G.	Colon (M)	150–1600		
J. G. L.	Rectum		340–40–40	
H. G.	Pancreas (M)			145–60–120
C. B.	Larynx		550–2000–110	
J. A.	Tonsil (M)	260–330–360		
J. M.	Glioma	110–55–350		
E. K.	Lymphosarcoma		2000–90–340	

[a] M, metastatic.

Table 3

DNA levels after radiotherapy in metastatic and nonmetastatic patients

DNA levels were compared before therapy and at 2 weekly intervals during the treatment in 81 metastatic and 92 nonmetastatic patients.

DNA Level	Patients (%)	
	Metastatic	Nonmetastatic
Increased	61	58
No change	13	3
Decreased	26	39

patients seems to correlate with the presence of tumor and/or metastatic disease. In preliminary studies, the DNA levels in patients with malignant lesions of the gastrointestinal tract were significantly higher than in those with inflammatory conditions (carcinoma of the pancreas *versus* pancreatitis; Hodgkin's gastric ulcer *versus* duodenal ulcer) (B. Shapiro, S. A. Leon, E. Cohn, and M. Desai, DNA in the Serum of Patients with Tumors of the Gastrointestinal Tract, in preparation). The diagnostic value of this finding, either for screening of suspected patients or for predicting a relapse, may be questionable at present. The reason for this conclusion is the relatively high number of cancer patients (50%) with DNA levels in the normal range (0 to 50 ng/ml). It should be pointed out, however, that the patients reported in this study by no means represent a random sample of malignant diseases. These patients represent a selected group considered for radiation therapy, in many cases after surgery and/or chemotherapy. It is possible that a prospective study of patients suspected for cancer, before, during, and after treatment, will yield results with better correlation between DNA levels and neoplastic disease. We conclude for the present that high levels of DNA in the serum accompany a pathological condition, but normal levels do not rule out cancer and, probably, other diseases.

After radiation therapy, a surprisingly high percentage of the patients (about 40%) showed decreased or unchanged DNA levels. This was especially obvious in patients who remained in the normal range of 0 to 50 ng/ml. Although radiation should have induced cell death and release of DNA in the circulation, it is equally possible that the arrest of tumor cell proliferation also reduced DNA release, on the assumption that they are the source of this DNA. Another explanation may be that the release of DNAse inhibitor from the tumor cells was reduced. In either case (and possibly both), lower levels of DNA in the serum would result. Although the mechanism is not clear at present, our findings suggest that persistence of DNA in the circulation after therapy correlates with poor response to treatment. In these patients, X-ray visualization or palpation (when possible) did not show a decrease in tumor size; persistence of pain and relapses were observed. Conversely, decrease in DNA levels during treatment seem to be a better prognostic sign, with reduction in tumor size, pain, and other signs of remission. We hope, therefore, that sequential measurements of DNA concentration may be a useful tool for monitoring the effects of therapy. Preliminary studies indicate that similar results are obtained with chemotherapy (R. Bornstein, B. Shapiro, and S. A. Leon, Effect of Chemotherapy on DNA Levels in Cancer Patients, in preparation). This test may be useful, therefore, to compare and evaluate objectively the effectiveness of different treatments or their combinations.

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