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γG-Globulin Production and Light-Chain Metabolism in Patients with Metastatic Cancer[1]

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SUMMARY

γG-Globulin and excess light-chain metabolism were studied in eight subjects with progressive metastatic malignant disease by determining the plasma radioactivity curves following the administration of appropriately labeled substances. In addition to the plasma die-away curves, which required about 3 weeks for full expression for γ-globulin, but only 3 to 4 days for light-chain, urinary excretion of the label from metabolized protein was determined. The data are compared to similar studies in control individuals. The metabolism of excess light chain was similar to normal in all respects. The total synthesis of γG-globulin was increased with a mean value about twice normal. The mean survival time of a circulating immunoglobulin molecule was short, indicating rapid loss from the system. Other aspects of immunoglobulin metabolism were similar to normal with a normal percentage of the labeled protein appearing in the urine, suggesting no abnormality in the utilization pattern but simply an increased rate of turnover.

The capability of malnourished patients with cancer to produce large quantities of immunoglobulin is not specific for this disease, since similar patterns may be seen in response to infections in protein-depleted individuals. However, there is the possibility that the cancer itself acts as an inciting agent in these subjects. Furthermore, such sustained protein synthesis may place an additional burden on already compromised host metabolism.

INTRODUCTION

The evidence for an immune response to developing tumors is compelling (1, 8). Although the thymus-dependent immune system is more often allocated the primary role (2, 6, 11), recent studies with human bladder carcinoma *in vitro* constitute a possible exception (4). Establishment and spread of malignant disease may be associated with impairment of cellular immunity, although the exact relationship between the 2 phenomena remains undefined. Studies that attempt to assay B-cell thymus-independent immune response during the rapid growth phase of human malignant disease have not been extensive. The following observations are pertinent in this regard. (*a*) The antibody response to specific new antigens may be impaired, although that to bacterial and viral antigens is maintained (10, 17). (*b*) A factor in the sera of patients with malignant disease inhibits cell-mediated lysis by activated lymphocytes, the so-called "blocking antibody" (4, 5). (*c*) γG-Globulin levels are often low in cases of malignant disease primarily involving the lymphatic system, exclusive of elevation of a specific IgG such as in the multiple myeloma, but are nearly always well maintained during the clinical course of most subjects with carcinoma (7, 18). (*d*) Limited studies of production and metabolism of γG-globulin suggest normal or increased turnover (14, 19, 21).

In this study, data are presented on immunoglobulin metabolism in 8 subjects with far-advanced and progressive malignant disease which suggests that the quantity of γG globulin produced during this phase of cancer may well be greater than normal.

MATERIALS AND METHODS

Patient Material. Eight subjects with a wide variety of malignant disease were studied during a phase of rapid progression of the tumor (Table 1). Some evidence of malnutrition was present in most subjects, J. G. being a notable exception. Most of the patients in this study had had some form of chemotherapy or radiation, but were not receiving treatment at the time of this study.

Methods. The experimental protocol, preparation of labeled material for injection as well as the determination of γG-globulin concentrations and serum-free light-chain levels were identical to those used in a series of normal subjects (21). Briefly, serum IgG levels were determined by radial immunodiffusion utilizing commercially available plates specific for an IgG-Fc fragment (Meloy Laboratories, Springfield, Va.). Serum light-chain levels were determined by complement fixation utilizing 2 antisera, broadly reactive with and specific for κ or λ light-chain determinants. Serum-free L-chain pools were isolated from patient sera by exclusion gel chromatography utilizing Sephadex G-100 or G-200 (21) columns equilibrated with phosphate-buffered saline, pH 7.4.

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Table 1

Patient	Diagnosis	Wt (kg)	γG-globulin Level (mg/100 ml)	γG-globulin i.v. pool (g)	γG-globulin IDR[a] mg/day	γG-globulin IDR[a] mg/kg/day	L-chain Concentration (γN/ml)	L-chain IDR[a] mg/day	L-chain IDR[a] mg/kg/day
J. OR.	Epidermoid carcinoma of the pharynx	44	820	15.4	2,870	64	5.63	500	11.3
M. A. #1	Multiple myeloma	54	1385	35.9	5,120	79	5.58	630	11.6
M. A. #2	Multiple myeloma		1605	48.0	5,670	104			
J. G.	Lymphosarcoma	85	725	35.6	10,000	109	4.53	750	9
W. Mc.	Carcinoma of lung	65	605	22.2	4,660	63	11.04	1800	27.7
W. R.	Carcinoma of pancreas	42	985	22.0	8,330	275	6.24	1030	24.5
G. R.	Carcinoma of lung	56	675	15.8	3,141	56	7.32	1320	23
K. N.	Carcinoma of breast	60	1680	61.1	14,900	248			
I. K.	Carcinoma of lung	45	855	19.5	3,080	68			
Control of range[b]			700-1210	20-66		35-62	5.8-8.7		8.1-18.7

[a] IDR, irreversible disposal rate, as determined from plasma pool/$\int_0^\infty$ plasma radioactivity curve.

[b] Control range, values are from 6 control subjects in whom identical studies were carried out. The subjects ranged in age from 25 to 76, and 2 of the subjects had osteoporosis. Thus, the mean γG-globulin level of this group was 931 mg/100 ml, or slightly lower than the mean value of normal subjects for our laboratory, namely, 1095 ±147 mg/100 ml.

The immunoglobulin utilized was either Cohn Fraction 2 or γG-globulin isolated from the patient's own serum by sodium sulfate precipitation. Both were further purified by DEAE column chromatography and radiolabeled with ^{131}I as previously described (21). Light chains were prepared by reduction and alkylation of purified IgG isolated from fresh pooled human sera and were labeled with ^{125}I. The labeled proteins contained from 0.2 to 0.6 mole of radioiodine per mole of IgG or L-chain, and 1 mg of protein contained a maximum of 50 μCi of specific activity. The labeled preparations were simultaneously administered i.v., and serum samples were obtained at 3, 5, and 10 min, at 4 and 8 hr, and then daily for 21 to 42 days, in order to determine the decay curve of plasma radioactivity. Complete urine collections were done and analyzed for their radioactive content, both precipitable and nonprecipitable in 5% trichloroacetic acid.

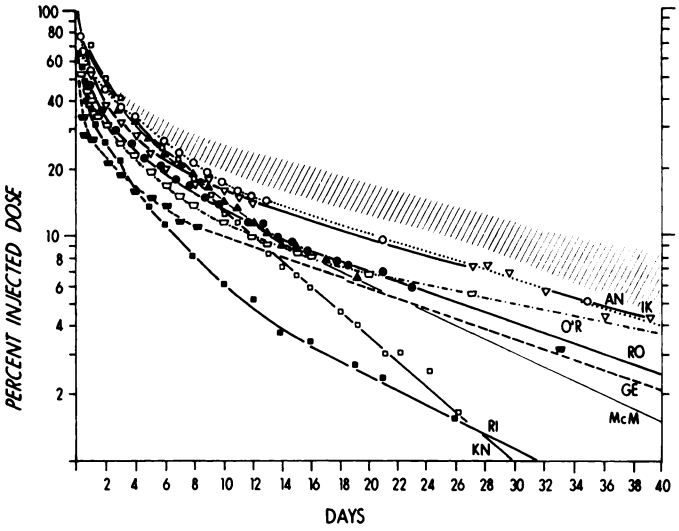

Chart 1. Plasma radioactivity disappearance curves of ^{131}I-labeled γ-globulin of 8 patients with progressive malignant disease. *Shaded area* represents all the data from 6 normal control subjects.

RESULTS

Immunoglobulin Levels. In most instances only 2 γG-globulin levels were obtained during the study period, and the values seen in Table 1 are those determined at the initiation of the tracer study. Two of the subjects had levels that would be considered slightly low for our laboratory (W. Mc. and G. R.), but during the course of the study, 6 of the 8 patients showed small increases in γG-globulin levels. In view of this, 2 patients had serial observations after the tracer studies until the time of death. Both showed increase in γG-globulin levels. The findings in K. N. were of particular interest since, in the month immediately preceding his death, the IgG level rose 16%, from 1740 to 2010 mg/100 ml.

γG-Globulin Turnover. The plasma decay curves obtained for the 8 patients studied are shown in Chart 1 and may be contrasted with the normal decay curve. The percentage of injected radioactivity remaining in the plasma at 14 days was about 10%, while normal subjects have nearly 20% at this time. For the most part, this was the result of a final rapid decay rate, although an occasional subject also showed a more rapid early distribution phase. The $t_{½}$ of the final decay rate was 15 days or less in all but one subject (J. O.), with a mean of 13 days as contrasted with our normal mean $t_{½}$ of 18 days.

The intravascular pool of γG-globulin calculated from the plasma volume determined from the 3- to 10-min radioactive samples and the γG-globulin levels differed little from that of normal subjects (Table 1). The production rate, calculated from the integrated specific activity curve (ID/$\int_0^\infty$ radioactivity per mass) varied from 56 to 275 mg/kg/day with an average of 118 mg/kg/day, while the normal production rate averaged 47 mg/kg/day (range, 35 to 62 mg/kg/day).

In all studies of γG-globulin metabolism done thus far by us, we have never been able to recover all the radioactivity injected in carefully collected, continuous urine samples. Furthermore, the final decay rate of urinary excretion values has always been greater than the final decay rate in plasma. In these subjects we again failed to recover quantitatively the injected radioactivity in urine collections. The total urinary excretion of iodine, derived from the metabolized γG-globulin, ranged from 57 to 86% of the injected dose, quite comparable to values seen in the normal group. Thus, long-term extravascular storage appears to be uninfluenced by the presence of cancer. However, the urinary excretion of iodine as related to the plasma radioactivity (fractional catabolic rate) was increased, ranging from 11 to 17%/day, and was consonant with the rapid turnover described above.

L-Chain Levels and Metabolism. The serum light-chain levels and quantities in the intravascular pool for these subjects are shown in Table 1. All free light-chain levels were within normal limits and exhibited a normal ratio of κ to λ chains. Furthermore, as seen in normal individuals, the κ/λ ratios of free light chains were similar to the κ/λ ratios of intact immunoglobulins.

The metabolism of excess light chains is very rapid, with 1 to 5% of the radioactivity remaining in the serum at the end of 24 hr. All injected radioactivity was recovered in 4 to 5 days. These data are not different from those seen in normal individuals, although a tendency toward increased turnover is manifest in some of the subjects.

DISCUSSION

The evidence for increased γG-globulin production is clear in this study group. The mean survival time in plasma is shortened and the plasma levels are well maintained, implying an increased rate of destruction, balanced by an increased rate of production.

Other studies of γG-globulin turnover in patients with neoplasia have been done. Spiegelberg *et al.* (18) and Tee and Watkins reported a shortened half-life of γG-globulin in subjects with carcinoma. However, Morrell *et al.* (14), studying the subclasses of γG-globulin, felt that the turnover of at least γ_1G and γ_2G fell within normal limits. Since the greatest percentage of γG-globulin is comprised of heavy chains derived from these 2 γ-chain subgroups, this could be construed as evidence for a normal production. It seems possible that patient selection might be a factor in the discrepancies seen by various investigators. In this study, patients with rapidly progressive malignant disease have been purposely chosen.

Many observations are in accord with our findings of nearly normal serum IgG levels in carcinoma (7). The meaning of such preservation of levels bears some comment. Although it has been suggested that very low serum IgG levels are usually associated with prolonged survival rates and high serum levels with shortened survival rates (16), there is considerable evidence that the correlation is not a strict one (18), including the data from some of our subjects. However, from a teleological point of view, such an association might be expected to help maintain stable levels. The rise of γG-globulin levels to a new steady state concentration, as was seen in 2 of our subjects terminally, must result from factors other than change in degradation rate, even though the ultimate level attained may be modified by the capability for destruction. We would suggest that an increased γG-globulin level in these subjects might signify production of new molecules not participating in any general increase in turnover and that the absolute level reached represents the capability for biological escape from normal control mechanisms.

Six of the 8 subjects of this study were in the terminal phase of malignant disease. Their p.o. intake was small and i.v. supplementation was given only to I. K. during the experimental period. Thus, the factor of malnutrition must be considered in the interpretation of our data. Recent studies in malnourished children have shown impaired thymus-dependent immune functions and impaired humoral antibody response to specific antigens (12, 13), but others (9) have found normal serum immunoglobulin levels. There are a few data concerning immunoglobulin production in malnutrition. One study, however, shows nearly normal production in kwashiorkor and increased production when infection was present (3). Thus, the capability to synthesize γG-globulin probably remains normal in malnutrition, even though specific antibody response may be depressed.

In view of rapidly advancing knowledge in the area of immunology in patients with cancer, some thought has been given to the interpretation of our data. The presence of surface antigens on some human tumor cells is now established (5). Sensitized T-cell lymphocytes can destroy such cells, at least *in vitro*. More recent evidence suggests a role for antibodies themselves in inducing lymphocyte cytotoxicity (15). Finally, sera from some patients in certain stages is known to block the cytotoxicity of lymphocytes; the latter reaction is thought to be related to surface antigen-antibody complexes not allowing access of lymphocyte to the target cell (6). Our data, which imply increased antibody production in the presence of rapid tumor growth, are consistent with the theory of blocking antibody production. It is conceivable that antibody production to tumor antigen may at one stage of carcinoma act as the inducer of lymphocyte cytotoxicity while, at another stage, perhaps dependent on the amount of antigen present, it may allow enhancement of growth by serving as a blocking antibody.

Other fates of the demonstrated increased production of antibodies must be considered. Waldmann *et al.* (20) have suggested gastrointestinal excretion of immunoglobulins in certain patients with cancer, particularly gastrointestinal and pancreatic carcinomas. Such loss is usually associated with lymphopenia. In spite of the fact that lymphopenia is rather commonly seen in patients with carcinoma, this was not a consistent finding in this group of subjects, since all but 3 had lymphocyte counts of over 1000/cu mm.

Such antibody production as noted in our subjects should be considered in terms of total body economy. From 3 to 15 g of protein were synthesized daily for immunoglob-

ulin production. While this might not constitute any real metabolic burden in a normal person, such obligatory production would seem to constitute a significant drain in host tissues already compromised by a caloric deficit. We suggest that the stimulus for such synthesis must override normal stoichiometric metabolism and thus add to the problems caused by the growth of the tumor.

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