Plasma Protein Synthesis in Experimental Cancer Compared to Paraneoplastic Conditions, Including Monokine Administration

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ABSTRACT

During tumor growth, there are characteristic alterations in the concentration and synthesis of various plasma proteins. The purpose of this study was to evaluate whether these changes are unique to a tumor-bearing state, or rather, they represent a generalized response to a paraneoplastic state mediated by the release of monokines or protein-calorie malnutrition. Plasma protein synthesis and concentrations in mice bearing a transplantable fibrosarcoma were compared to animals receiving either a terpentine abscess, Corynebacterium parumum administration, calorie-protein depletion, or administration of the recombinant-derived monokines, murine interleukin 1α or human tumor necrosis factor-α.

Tumor-bearing animals showed a significant increase in total plasma protein synthesis that was similar in magnitude to the increase seen following a terpentine abscess or after administration of interleukin 1 or tumor necrosis factor-α. Similarly, the pattern of protein synthesis and concentration, as determined by isoelectric focusing or sodium dodecyl sulphate-polyacrylamide gel electrophoresis, were similar, albeit not identical, among tumor-bearing animals and those receiving either a terpentine abscess, C. parumum and monokine administration. Serum amyloid P concentrations were markedly elevated in tumor-bearing animals, as they were in animals after a sterile abscess and following interleukin 1 administration, as well as to a lesser extent tumor necrosis factor-α administration.

We can therefore conclude that the majority of changes in plasma protein concentration and synthesis seen in this tumor-bearing model are similar to those seen during an acute inflammation and can be reproduced to a large extent by the administration of the monokines, interleukin 1α or tumor necrosis factor-α.

INTRODUCTION

Changes in plasma protein concentrations and synthetic rates are well known in both clinical and experimental cancer (1–3). However, similar changes in serum protein concentrations and synthetic rates have been reported in nonneoplastic, but chronic and acute inflammatory states (for review, see Ref. 4). Presently, it is unknown to what extent the changes in plasma protein synthesis and concentration during cancer represent a specific host response to the presence of neoplastic tissue. The changes could be explained not by the tumor directly, but rather by other paraneoplastic reactions, such as inflammation associated with tumor necrosis or tumor-specific antigens, cell proliferation and malnutrition. With regards to inflammation, recent evidence has demonstrated that synthesis of many of the acute phase reactant proteins is initiated by the monokine, IL1α (5–7).
PLASMA PROTEIN SYNTHESIS: CANCER VERSUS PARANEoplastIC CONDITIONS

Healthy nontumor-bearing mice (both C57/BL6J and C3H/HeJ) were housed in cages which permitted the analysis of food intake. The animals were allowed to equilibrate in these cages for 5–7 days. Body weight and food intake were monitored daily. After the adaptation period, the animals were randomized to receive the i.p. administration of 940 ng of recombinant murine IL-1α as twice-daily i.p. injections. Additional mice received an isomolar quantity of recombinant human tumor necrosis factor-α (910 ng) or sham injections. The study period lasted either 32 h and the animals received three such injections, or it lasted 5 days. Because administration of these monokines may induce anorexia (13), an additional group of animals receiving sham injections was pair fed equivalent quantities of food as animals receiving the recombinant preparations (PF).

Analytical Procedures. Rates of total plasma protein synthesis were estimated following the i.p. injection of flooding quantities of radiolabeled leucine (14). Either 30 or 10 min prior to sacrifice, each mouse received the i.p. injection of 1 μmol l-leucine/g body weight containing 400 nCi/g body weight l-[1-14C]leucine. At sacrifice, mixed arterial-venous blood was collected by cardiac puncture and plasma separated from cellular constituents by centrifugation. Aliquots of plasma were deproteinized in 165 volumes of ice-cold 10% trichloroacetic acid and the precipitate trapped on glass fiber filters (Whatman GFC; Whatman Ltd., Maidstone, England). Protein concentration was determined using the Lowry technique (15) and radioactivity was quantitated using standard scintillation spectrometry. Thirty min following the injection of l-[1-14C]leucine, greater than 95% of the radioactivity incorporated into tissue proteins was as leucine.6

The plasma-free leucine-specific radioactivity was quantitated following isolation of the free amino acids on a high-performance liquid chromatographic system (Waters; Medford, MA) with a gradient employing sodium phosphate-acetonitrile (5–40%) buffer and a C18 Bondapac reverse-phase column (Waters) (16). Free amino acids were isolated from plasma proteins by extraction into methanol (16). The methanol extract was evaporated to dryness with nitrogen gas and the amino acids reconstituted in the phosphate buffer. The amino acids were treated with o-phthalaldehyde and immediately separated by high-performance liquid chromatography. The individual amino acids were quantitated fluorometrically and the leucine peak was automatically collected after fluorescence detection and radioactivity was subsequently measured with an LKB-Wallace β counter (LKB, Sollentuna, Sweden). Plasma protein synthesis was estimated from the equation (14):

\[ k_s = S_o / S_r \times t \]  

where \( k_s \) is the fractional synthetic rate, in percentage renewed/day; \( S_o \) is the specific radioactivity of acid-precipitated protein, in dpm/nmol leucine, \( S_r \) is the average specific radioactivity of plasma-free l-leucine over the 30-min study period, in dpm/nmol leucine; and \( t \) is the isolation period, 0.0104 days. Because newly synthesized hepatic proteins require 70% of their total nitrogen content in leucine (18) and the average specific radioactivity of acid-precipitated plasma proteins was calculated between 15 and 30 min. It was estimated that total plasma protein is comprised of 8% leucine. The average specific radioactivity was calculated from the linear decline in leucine specific radioactivity between a theoretical time 0 and the 10- and 30-min values (14).

SDS-PAGE and Isoelectric Focusing of Plasma Proteins. To determine the relative circulating concentration of different plasma proteins, plasma from study animals was diluted six times and applied to a 2.5-mm sodium dodecyl sulphate polyacrylamide gel (0.75- or 1.5-mm thickness) and electrophoresed overnight at 80 V using a 25 mM Tris, 192 mM glycin buffer containing 0.1% sodium dodecyl sulphate. The proteins in the gel were either visualized with 0.1% Coomassie brilliant blue or transferred electrophoretically to nylon membranes (Zeta-Probe, BioRad Laboratories, Sunnyvale, CA) at a constant current of 360 mA in a Trans-Blot Cell (BioRad) for 4 h. Carbon-14 radioactivity, contained in the proteins on the nylon membrane, was visualized by autoradiography. Membranes were air dried, sprayed with a commercial scintillant, and exposed at −70° to Kodak X-omat AR film for 14 days. Relative protein concentrations and radioactivity were quantitated by scanning the gels or film with a Shimadzu densitometer at 555 nm. The coefficient of variation for repeated samples assayed by SDS-PAGE and Coomassie staining was around 8%.

In some cases, glycoproteins in plasma samples subjected to SDS-PAGE were visualized directly after electrophoresis to nylon membranes. Membranes were blocked overnight with phosphate-buffered saline (pH 7.4) containing 10% bovine serum albumin at 45–50°C. The membrane was then incubated with free Concanavalin A (10 μg/ml; Pharmacia) in phosphate-buffered saline containing 10 μM Ca2+, Mg2+, and Mn2+, and 0.5% Triton X-100 for 2 h at room temperature. After washing the filter with the same buffer solution free of Concanavalin A, the membrane was incubated in the same buffer containing 50 μg/ml horseradish peroxidase (Sigma Chemicals, St. Louis, MO) for 2 h at room temperature. After repeated washes, the glycoproteins were visualized by incubating the membrane with 4-chloro-l-napthol (0.5 mg/ml dissolved initially in minimal ethanol) in 50 mM sodium phosphate buffer (pH 6.0) containing 0.1% H2O2 until the desired darkness was obtained (18).

Serum amyloid P was quantitated by rocket immunoelectrophoresis (19). Samples were first treated overnight with 2.5 mM calcium lactate to reduce nonspecific precipitation and then applied to 0.8% agarose gels (1-mm thickness) containing 0.75% rabbit antibody to mouse serum amyloid P (Cal-Biochem Laboratories, Lab-Kemi, Göteborg, Sweden). Plasma was electrophoresed for 15 h at 150 V in a Veronal buffer and after washing overnight in 150 mM sodium chloride, protein immunoprecipitates were visualized with 0.2% Coomassie brilliant blue. Protein concentration was quantitated from the height of the rockets using a commercially available serum amyloid P (Cal-Biochem) standard.

Statistical Analyses. Differences in serum amyloid P concentrations and total plasma protein synthetic rates from freely fed, nontumor-bearing control animals were compared to experimental groups using the two-tailed, Student’s t-test. No additional comparisons were made among experimental groups. Statistical significance was designated at the 95% confidence interval.

RESULTS

Changes in Plasma Protein Concentrations. Plasma obtained from control and tumor-bearing mice, as well as animals given either a sterile terpine abscess, C. parvum administration or a protein-free diet for 3 days were subjected to isoelectric focusing and visualized with 0.2% Coomassie brilliant blue. This was done to compare the concentrations among different circulating plasma proteins between the animal groups. The plasma samples were diluted six times before electrophoresis and corresponded to 42 g/liter (freely fed controls), 37 g/liter (tumor-bearing), 45 g/liter (pair-weighted controls), 51 g/liter (C. parvum) and 50 g/liter (terpine and protein malnutrition). The gels are presented in Fig. 1A and corresponding densitometry scans are presented in Fig. 1B. The protein pattern of plasma obtained from TB, TE, and CP animals was...
PLASMA PROTEIN SYNTHESIS: CANCER VERSUS PARANEOPLASTIC CONDITIONS

Fig. 1. Isoelectric focusing. Left, plasma from tumour-bearing, terpentine, and C. parvum-treated mice were compared to samples from nontumor-bearing, freely fed, pair-weighted, and animals fed a protein-free diet for three days. The circulating plasma was diluted six times before application to the gel at concentrations given in the “Results” section. Samples were subjected to isoelectric focusing and visualized with 0.2% Coomassie brilliant blue, as described in the “Materials and Methods.” Anodal to albumin (Alb) are isoforms of α-1-antitrypsin and haptoglobin located. Ceruloplasmin is located in region a, isoforms of α-2-macroglobulin in regions b and c. Region e, major components of the transferrins; further isoforms of the transferrins are spread over regions b-1 as determined by immunofixation with antimouse transferrin antibodies. C, and fibrinogen are located in regions i-1. Standard proteins: pl = 4.55, soybean trypsin inhibitor; pl = 5.20, β-lactoglobulin A; pl = 5.58, bovine carbonic anhydrase B; pl = 6.55, human carbonic anhydrase B; pl = 6.85, horse myoglobin-acidic band; pl = 7.35, horse myoglobin-basic band; pl = 8.15, lentil lectin-acidic band; pl = 8.45, lentil lectin-middle band; pl = 8.65, lentil lectin-basic band (calibration kit, pH 3–10, from Pharmacia Fine Chemicals).

Right, gels shown in Fig 1, left, were scanned at 555 nm and densitometry curves are presented. Alb and letters a-1 correspond to protein bands identified in Fig. 1, left.

Plasma samples were also evaluated by SDS-PAGE under denaturing conditions (Fig. 2). In addition to the above groups, plasma was obtained from healthy C57/BL6J mice given either 1365 ng (940 ng/day) of murine recombinant IL1α or 1420 ng (910 ng/day) of recombinant human tumor necrosis factor-α as three i.p. injections over the past 32 h. As observed with isoelectric focusing, the protein pattern among tumor-bearing, terpine and C. parvum-treated animals was more similar to each other than to the food-restricted controls (pair weighted and those fed a protein-free diet). In some cases, these “activated” conditions were associated with increased concentrations of certain proteins, especially in the ceruloplasmin, transferrin, albumin, fibrinogen, and orosomucoid regions, as well as some unknown lower molecular weight proteins with molecular weights between 40,000 and 20,000.

Administration of the recombinant monokines, IL1α and tumor necrosis factor-α to healthy mice resulted in a plasma protein pattern in many aspects similar to tumor-bearing, terpentine, and C. parvum-treated animals. Although the protein pattern was similar, it was not identical. In addition, although IL1α and tumor necrosis factor-α administration gave qualitatively similar patterns, the magnitude of the increase in certain proteins was less than seen in tumor-bearing and terpentine-treated mice. In an effort to more closely define the nature of such proteins, plasma glycoproteins were visualized using concanavalin A and horseradish peroxidase (Fig. 3). Many of the similarities among tumor-bearing, terpentine, and C. parvum-treated animals and monokine-injected mice were seen with the glycoprotein stain as reflected by the increased concentration of some bands with a higher molecular weight than 200,000 and in the haptoglobin region.

Serum amyloid P concentrations increased 10-fold in both tumor-bearing and terpentine-treated animals (Table 1), and the increases were of similar magnitude to those seen in C3H/HeJ mice given IL1α for 5 days. Serum amyloid P concentrations were increased 3–5-fold by C. parvum, but were unchanged by either food restriction (pair weight) or feeding of a protein-free diet for 3 days. (The amyloid P protein is not detectable in gels stained with Coomassie brilliant blue).

Plasma Protein Synthesis. Total plasma protein synthesis was increased 43% in tumor-bearing animals when compared to freely fed controls (Table 2) and 92% when compared to pair weight controls. In addition to tumor-bearing animals, total
plasma protein synthesis was also increased following a terpentine abscess. The magnitude of increase was comparable to that seen in tumor-bearing animals. Consumption of a protein-free diet for 3 days resulted in a significant decline in total plasma protein synthesis. Administration of murine IL1α and human tumor necrosis factor-α for 5 days stimulated total plasma protein synthesis in both endotoxin sensitive (C57/BL6J) and resistant (C3H/HeJ) strains of mice. The increases in protein synthesis were of similar magnitude to that seen in tumor-bearing animals and following a terpentine abscess.

Plasma samples obtained from mice administered a tracer dose of L-[1-14C]leucine were subjected to SDS-PAGE and autoradiography. The amount of total radioactivity applied to each gel was identical so that the relative proportion each protein band contributed to total protein synthesis is presented (Fig. 4A). Densitometry scans of the autoradiographs are presented in Fig. 4B. Synthesis of plasma proteins from tumor-bearing animals was compared directly to their pair-weighted controls. In addition, the other experimental groups (TE, CP, IL1, and TNF) were all compared to their appropriate controls. None of the inflammatory state animals (TB, TE, CP, IL1, or TNF) showed the same activation of plasma protein synthesis versus control animals. TB, TE, and IL1 animals displayed a particularly increased synthesis of C3, transferrin, α-1-antitrypsin, fibrinogen, and orosomucoid. In contrast, C. parvum and TNF-treated animals displayed decreased incorporation of radiolabeled leucine into the albumin region with transferrin, α-1-antitrypsin, and fibrinogen. A decreased synthesis of two unidentified protein fractions in the low molecular weight region was noticeable in all inflammatory state animals.

**DISCUSSION**

Recent studies have suggested that the transcription and translation of both positive and negative acute phase reactants are under the regulatory control of the monokine IL1 (5–7). In particular, IL1 was shown to down regulate the transcription of the albumin gene (5, 6) and up regulate the transcription of the serum amyloid (6) and metallothionein genes (7). Factors which initiate IL1 synthesis might then be expected to result in the accelerated synthesis of acute phase proteins. Many different afferent stimuli of macrophage activation, such as phagocytosis of bacteria, cell debris, or antigen-antibody complexes would initiate the release of IL1 and the synthesis of acute phase reactants (20). The changes in plasma protein concentration and synthesis in a tumor-bearing state may then not be a specific tumor-induced stimulus, but rather a generalized response to an inflammatory state (21). To evaluate whether the changes in plasma protein synthesis and concentration in a tumor-bearing host are a generalized response to a nonspecific stimulus, we have utilized analytical techniques which resolve most plasma proteins simultaneously. Although we have not tried to identify all the individual proteins, we have used isoelectric focusing or SDS-PAGE to reveal the overall pattern of plasma protein concentrations and synthesis.

The tumor-bearing state was characterized by considerable alterations in the plasma protein concentration and synthetic pattern. These changes cannot be attributed to either anorexia or protein depletion, since the pattern was markedly different than in malnourished control animals. Rather, the changes in both total protein and glycoprotein concentration and synthesis are similar, albeit not identical, to those seen 24 h following a terpentine-induced abscess. However, in several important respects, specifically with regards to total plasma protein synth-
PLASMA PROTEIN SYNTHESIS: CANCER VERSUS PARANEOPlastic CONDITIONS

Table 2 Fractional synthetic rates (percentage renewed/day) of total plasma protein pool

<table>
<thead>
<tr>
<th>Treatment of C57/BL6J mice (n)</th>
<th>IL1 (10)</th>
<th>TNF (10)</th>
<th>FF (10)</th>
<th>PW (10)</th>
<th>PF (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of C3H/ HeJ mice (n)</td>
<td>IL1 (10)</td>
<td>TNF (10)</td>
<td>FF (10)</td>
<td>PF (5)</td>
<td></td>
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<tr>
<td>TB (10)</td>
<td>10.9 ± 0.5</td>
<td>13.9 ± 0.9</td>
<td>6.8 ± 0.2</td>
<td>7.6 ± 0.5</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>TE (5)</td>
<td>18.2 ± 1.3</td>
<td>16.4 ± 1.0</td>
<td>11.7 ± 0.7</td>
<td>4.7 ± 0.2</td>
<td></td>
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</tbody>
</table>

* Fed a protein-free diet for 5 days.
* *P < 0.05 versus FF; values, mean ± SE.
* *P < 0.01 versus FF; values, mean ± SE.
* *P < 0.001 versus FF; values, mean ± SE.
* Pair-fed equivalent quantities of food as in IL1 group.
* IL1 = murine interleukin-1 α for 5 days, human tumor necrosis factor-α for 5 days.
* PF = pair-fed equivalent quantities of food as IL1 group.

Fig. 4. Left, autoradiograph of [1-14C]leucine-labeled proteins subjected to SDS-PAGE. Plasma samples as described in Fig. 2 were transferred to nylon membranes following SDS-PAGE and subjected to autoradiography (see "Materials and Methods"). The quantity of radioactivity applied to each lane was identical (640 dpm) so that the darkness of each band represents a relative proportion of total protein synthesis. PF = pair-fed equivalent quantities of food as IL1 group. The abbreviations of protein regions are the same as in Fig. 2. Right, densitometry scans. Autoradiographs in Fig. 4, left, were scanned at 555 nm, as described in "Materials and Methods." The plasma protein-synthetic pattern from the different experimental groups were compared to their respective controls. The region where albumin is located is highlighted to accentuate the differences in synthesis between TB mice and the other groups. PW, equivalent quantities of food to be weight-paired to the carcass of tumour-bearing animals: PF = pair-fed equivalent quantities of food as IL1 group. The abbreviations for protein regions are the same as in Fig. 4, left.

sion and serum amyloid P concentrations, the response between tumor-bearing and C. parvum-treated animals differed significantly (Tables 1 and 2). The greater similarity between tumor-bearing animals and mice given a terpentine abscess than C. parvum administration implies that the host response to cancer significantly (Tables 1 and 2). The greater similarity between tumor-bearing animals and mice given a terpentine abscess than C. parvum administration implies that the host response to cancer is more similar to acute inflammation rather than to cell proliferation and immune cell activation. Such a conclusion is also supported by the observation that the response from tumor-bearing animals was also comparable to that seen when healthy animals were administered recombinant IL1α or tumor necrosis factor-α. Such animals showed a similar increase in total plasma protein synthesis as well as a corresponding change in the pattern of synthesis of plasma proteins.

It was frequently observed that the plasma protein response to tumor necrosis factor-α was similar but less in magnitude than that seen when isomolar quantities of IL1 were administered. It may well be that human tumor necrosis factor-α is a less potent inducer of the hepatic acute phase response in the mouse than murine IL1α. However, we cannot exclude the possibility that the results observed with tumor necrosis factor-α may be secondary to an endogenous IL1 production, since recent studies have suggested that tumor necrosis factor-α stimulates IL1 biosynthesis in peripheral blood monocytes (22). There may well be specific protein responses in a tumor-bearing animal that cannot be reproduced exactly by either a terpentine abscess or monokine administration.

However, the great bulk of protein changes in a tumor-bearing state, especially those involving increased synthesis and concentration of plasma proteins, were similar to an acute inflammatory stress and IL1 administration. In conclusion, we suggest that the changes in plasma protein concentration and synthesis seen in tumor disease are generally consistent not with a tumor-specific induction, but rather reflect a host's generalized response to inflammation, communicated in a significant part by IL1. Whether the exogenous signal behind this inflammatory response is necrotic tumor or phagocytosis of tumor antigen-antibody complexes remains to be determined.

REFERENCES

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