An Analogy between Fetal Haptoglobin and a Potent Immunosuppressant in Cancer

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ABSTRACT

In spite of the numerous reports indicating the presence of humoral immunosuppressive factors in cancer patients, only a few of these factors have been biochemically identified. Furthermore, their role as effective immunosuppressors in vivo remains to be established. Our laboratory has attempted to isolate and identify the major immunosuppressive factor in the malignant effusions derived from ovarian and lung cancer patients. We have previously demonstrated that the M, 52,000 immunosuppressive factor isolated from the ascites fluid of an ovarian cancer patient inhibited T-dependent immune responses in vitro and in vivo including the induction of E-rosetting. Thus, this immunosuppressive factor was named "suppressive E-receptor" (SER). Our current study demonstrates that this SER factor purified from malignant effusions derived from ovarian, lung, or head and neck cancer patients had a common component which dissociated equally into M, 38,000-42,000 and 17,000-19,000 moieties on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under vigorous reducing conditions. Electrophoretic analysis of these two components followed by a limited amino acid sequence determination revealed these two components to have N-terminal amino acid sequences identical to the \( \beta \) and \( \alpha_2 \) subunits of normal adult haptoglobin. Immunelectrophoresis of SER using a polyclonal antiserum to neonatal cord blood demonstrated that SER, unlike normal haptoglobin, has slower electrophoretic mobility than the normal adult haptoglobin. Western blotting analysis of SER separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under denaturing conditions failed to recognize a monoclonal antibody directed specifically to SER. However, this monoclonal antibody exclusively reacted with the SER separated by an analytical polyacrylamide gel electrophoresis gel under nondenaturing conditions while normal adult haptoglobins or purified but denatured haptoglobin obtained from the same malignant fluid as SER all failed to react with this antibody. Thus, SER appears to bear an additional epitope(s) that is absent in normal adult haptoglobin. Since the SER as well as the neonatal haptoglobin have at least 100 to 1000-fold more potent immunosuppressive activity than the normal adult haptoglobin, this additional epitope(s) present in SER may be responsible for the potent immunosuppressive property of SER.

INTRODUCTION

Immunosuppression can be a major problem in cancer, impairing immune defenses against the tumor, vitiating attempts at immunotherapy, and predisposing the patients to infection. The nature and mechanism of immunosuppression are not fully understood. Specific unresponsiveness to tumor-associated antigens has been reported in a number of experimental systems (1). In addition, nonspecific suppression of a broad spectrum of immune responses may occur. Correlated with the latter phenomenon is the presence of nonspecific immunosuppressive factors in sera and ascites fluids of cancer patients (2). However, these factors are not associated only with cancer. Nonspecific immunosuppressive factors have also been detected in blood and urine of a variety of conditions, including experimental amyloidosis (3), pregnancy (4, 5), liver disease (6), and even in normal human serum (7–10). Therefore, it has been unclear whether immunosuppressive factors associated with cancer are a unique product of tumor cells or whether they resemble substances found in other conditions and perhaps represent a normal feedback mechanism that has been deranged during the development of tumors. To address this question, we have been engaged in the purification and chemical characterization of a nonspecific immunosuppressive material from ascites fluid of patients with metastatic tumors. The mitogen-induced lymphocyte proliferation assay as described by Cooperband et al. (11) was used to detect the serum immunosuppressor molecule. The immunosuppressive factor purified from the ascites fluid of an ovarian cancer patient was an acidic glycoprotein (isoelectric point of 3.6–4.0) of approximate molecular weight of 50,000 on SDS-PAGE under mild reducing conditions. This factor inhibited T-dependent mitogen responses in vitro and T-dependent antibody response in vivo and blocked E-rosetting (12). This immunosuppressive factor, when found to show serological cross-reactivity with a monoclonal anti-E-receptor antibody and to be inhibited by the antibody, was named SER factor. Subsequent work from our laboratory indicated that SER, although biologically and immunochemically similar, may not be identical to the soluble form of E-receptor itself (13, 14). Therefore, its biochemical identity remained to be determined. The current study demonstrates that this SER factor may be biochemically analogous to the neonatal or fetal form of haptoglobin.

MATERIALS AND METHODS

Chemicals, Biochemicals, and Immunochemicals

Normal adult haptoglobin types 1-1, 2-2, and 2-1 and hemoglobin have been purchased from Sigma Chemical Co. AGP was a gift from Dr. K. Schmid from the Department of Biochemistry at Boston University School of Medicine (Boston, MA). Rabbit antiserum to human cord blood was produced in our laboratory by sequential immunization of rabbits with human cord blood emulsified in Freund's complete adjuvant. Rabbit antiserum to normal adult haptoglobin was obtained from Sigma and the goat antiserum to rabbit IgG conjugated with horseradish peroxidase was obtained from Flow Laboratories (McLean, VA). Goat antiserum to mouse IgG + IgM conjugated with horseradish peroxidase was purchased from KPL Laboratories (Gaithersburg, MD). Monoclonal antibody to SER (data to be published elsewhere) has been raised using the procedure of Gefter et al. (15). Monoclonal anti-T3 antibody was a gift from T cell & Sciences Inc. (Cambridge, MA).

PHA was obtained from Burroughs-Wellcome Laboratories (Research Triangle Park, NC) and Seakem agarose was purchased from FMC Corp. (Rockland, ME). Prestained molecular weight standards were purchased from Signa Chemical Co. AGP was a gift from Dr. K. Schmid from the Department of Biochemistry at Boston University School of Medicine (Boston, MA).

The immunosuppressive factor purified from the ascites fluid of an ovarian cancer patient was an acidic glycoprotein (isoelectric point of 3.6–4.0) of approximate molecular weight of 50,000 on SDS-PAGE under mild reducing conditions. This factor inhibited T-dependent mitogen responses in vitro and T-dependent antibody response in vivo and blocked E-rosetting (12). This immunosuppressive factor, when found to show serological cross-reactivity with a monoclonal anti-E-receptor antibody and to be inhibited by the antibody, was named SER factor. Subsequent work from our laboratory indicated that SER, although biologically and immunochemically similar, may not be identical to the soluble form of E-receptor itself (13, 14). Therefore, its biochemical identity remained to be determined. The current study demonstrates that this SER factor may be biochemically analogous to the neonatal or fetal form of haptoglobin.

Materials and Methods

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3 Fellowship recipient from the Karin Grunebaum Cancer Research Foundation.

4 The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; AGP, \( \alpha_2 \)-acid glycoprotein; PHA, phytohemaglutinin; SER, suppressive E-receptor factor.
were obtained from Bethesda Research Laboratories (Gaithersburg, MD). SDS, acrylamide, bisacrylamide, and protein-assay reagent were purchased from BioRad Laboratories (Richmond, CA). N,N,N',N'-Tetramethylenediamine was purchased from Kodak Chemicals and trichloroacetic acid, 4-chloro-1-naphthol and 2-mercaptoethanol were Sigma chemicals. All other chemicals were reagent grade Fisher chemicals.

Neonatal Blood and Malignant Effusions

Malignant effusions derived from ovarian and lung cancer patients were obtained from the Oncology Department at the University Hospital (Boston, MA) and from the Cytology Laboratory at the Mallory Institute of Pathology Foundation (Boston, MA). Neonatal blood was obtained from the Obstetrics Department at the Boston City Hospital (Boston, MA). Malignant effusions and pooled neonatal blood were centrifuged at 2000 x g for 10 min at 4°C to remove the cellular elements and were kept frozen at —20°C until used.

Purification of SER from Malignant Effusions and Cord Blood

The previously published procedure for the purification of SER (12) has been used with an additional final purification step involving hydrophobic phenyl-Sepharose column chromatography in 1.7 M ammonium sulfate containing 0.1 M sodium phosphate buffer (pH 7.4). The immunosuppressive fraction from cord blood has been isolated using the same procedure as was used in the purification of SER from malignant effusions.

Purification of Haptoglobin by Affinity Media

Haptoglobin was isolated from malignant effusion using hemoglobin-bound Sepharose affinity media as described by Javid and Lian (16). Conjugation of hemoglobin (Sigma) to Sepharose beads was performed utilizing the cyanogen bromide activation procedure of Cuatrecasas (17).

Immunosuppressive Activity Assay in Vitro

Inhibition of PHA-induced Lymphocyte Proliferation Assay. The original assay method has been described in detail (12) and the current method included the modifications as described in our recent publication (18), i.e., purified PHA (Burroughs-Wellcome) was used at 1 μg of PHA/5 x 10⁶ cells/ml of culture medium and the culture was incubated for a total of 48 h at 37°C. Cultured lymphocytes were pulsed with 0.5 μCi of [3H]thyminde/0.25 x 10⁶ cells/well for the final 6 h prior to harvesting.

Inhibition of Anti-T3-induced Mitogenesis Assay. Lymphocyte mitogenesis was initiated with anti-T3 antibody as described by Van Wauwe et al. (19) using monoclonal anti-T3 antibody at a concentration of 5 ng/1 x 10⁶ cells. Culture was carried out in a manner analogous to that of PHA-induced mitogenesis.

Radial Immunodiffusion and Immunoelectrophoresis

Ouchterlony radial immunodiffusion was performed in 1% Seakem agarose gel dissolved in 0.05 M sodium barbitol buffer (pH 8.6). Immunoelectrophoresis was performed in 1% agarose gel after separating the proteins at 10 V/cm length of gel for 1.5 h at room temperature until the tracking dye moved three-fourths of the entire gel length.

Analytical and SDS-PAGE

SER preparations and various types of normal adult haptoglobin (types 1-1, 2-2, and 2-1), as well as purified haptoglobin obtained from the same ascites fluid used to purify the SER, were separated on an analytical 4% acrylamide resolving slab gel as described by Raam et al. (20). Electrophoresis for analytical gel was performed at 25 mA per slab for 4 h at room temperature. Sample preparations to be analyzed on SDS-PAGE gel were treated with 2% SDS and 5% 2-mercaptoethanol and heated to 100°C for 10 min prior to electrophoresis on 10 or 12% acrylamide SDS-PAGE resolving gels. SDS-PAGE electrophoresis was performed for a total of 7 h using a Bio-Rad Protein vertical slab gel electrophoresis unit at an initial amperage of 25 mA/gel for 2 h until the sample migrated into the resolving gel and then increased to 40 mA/gel for the final 5 h. The gel was incubated in 12.5% trichloroacetic acid overnight at room temperature prior to staining for 1 h in 0.25% Coomassie Brilliant Blue dissolved in 50% methanol and 10% acetic acid. Gel was destained with 50% methanol containing 10% acetic acid for 4 h in order to visualize the stained bands. Prestained molecular weight standards utilized were myosin (H chain, M, 200,000), phosphorylase (M, 97,400), bovine serum albumin (M, 68,000), ovalbumin (M, 43,000), β-lactoglobulin (M, 18,400), and lysozyme (M, 14,300).

Western Blotting

Analytical or SDS-PAGE separated SER preparations were blotted onto nitrocellulose paper according to the method of Towbin et al. (21). Following blockage of nonspecific sites with 1% gelatin, the nitrocellulose paper was incubated with rabbit anti-normal adult haptoglobin (Sigma) at 1:1000 dilution. Immune complexes were detected by a goat antiserum to rabbit IgG, conjugated with horseradish peroxidase (Flow Laboratories; 1:1000 dilution). When mouse monoclonal antibody was used, the second antibody was a goat antiserum to mouse IgG + IgM, conjugated with horseradish peroxidase (KPL Laboratories, Gaithersburg, MD). Immune complex-bound horseradish peroxidase was detected with chromogenic substrates of either 2,2'-azinobis-3-ethyl benzothiazoline sulfonate (160 μg/ml) or 4-chloro-1-naphthol (160 μg/ml) with 0.003% hydrogen peroxide in 0.1 M citrate buffer, pH 4.0.

Electroelution followed by Amino Acid Sequencing

Affinity column purified SER was denatured under vigorous reducing conditions (100°C, 10 min) and separated on either 10 or 12% SDS-PAGE gel. The M, 17,000–19,000 and 38,000–42,000 components were electroeluted as described by Zalacain et al. (22) using ISCO apparatus. Electroeluted samples were reduced with 2-mercaptoethanol and 4-vinylpyridine (23) and dialyzed exhaustively against 0.1% SDS with 2.5 mM sodium chloride. Each of the SER components was hydrolyzed with constant boiling HCl for 24 h at 110°C in sealed tubes and the amino acid composition of each of these SER components was determined using a Beckman 6300 amino acid analyzer equipped with a ninhydrin detector. The amino acid terminal sequence of each polypeptide was determined by automated Edman degradation using a gas phase sequencer (Applied Biosystem 470A) (24) equipped with an on-line phenyl-thiohydantoin amino acid analysis system. The identity of the N-terminal analysis of each polypeptide was determined by comparison to those sequences contained in the National Biomedical Research Foundation Database (release 7.0).

RESULTS

SDS-PAGE Analysis of SER. The purified SER preparation which shows an apparent electrophoretic homogeneity on 7.5% analytical PAGE gel was separated on 10% SDS-PAGE under vigorous reducing conditions as described in "Materials and Methods." Under these conditions, SER dissociated into M, 38,000–42,000 and 17,000–19,000 moieties as shown in Fig. 1 (Lane a).

Fig. 1 (Lane a) shows that the presence of these two components was consistent with SER preparations obtained from two separate lung cancer patients and seven different ovarian cancer patients. A minor band seen around the M, 68,000 region appeared to be the contaminating serum albumin. Lane b shows the commercial normal adult haptoglobin type 2-2 (Sigma) and Lane c shows the molecular weight standards.

Amino Acid Composition and N-Terminal Amino Acid Sequence. Table 1 shows that the amino acid composition of the two components of SER obtained by electroelution of SDS-PAGE gels run under reducing conditions correlate best with the α₂ and β subunits of normal adult haptoglobin (type 2-2),
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Table 1 Amino acid composition of SER

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Table 2 N-terminal sequence of M, 17,000-19,000 and 38,000-42,000 peptides

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Table 2 N-terminal sequence of M, 17,000-19,000 and 38,000-42,000 peptides

Fig. 1. SDS-PAGE of purified SER. Purified SER (15 μg, lane a), normal adult haptoglobin type 2-1 (15 ng, Lane b), and 10 μg each of prestained molecular weight standards (Lane c) were analyzed on 10% SDS-PAGE gel. The molecular weight standards utilized were myosin (H chain, M, 200,000), phosphorylase b (M, 97,400), bovine serum albumin (M, 68,000), ovalbumin (M, 43,000), α-chymotrypsinogen (M, 25,700), β-lactoglobulin (M, 18,400), and lysozyme (M, 14,300).

given the limitation of the analysis performed (i.e., cysteine, methionine, and tryptophan have not been analyzed and the high content of glycine was not included in the analysis due to the glycine contained in the SDS-PAGE buffer).

Table 2 shows that the N-terminal amino acid sequences of the two peptides (M, 17,000–19,000 and 38,000–42,000) of SER coincide with the sequences of α2 and β chains of normal adult haptoglobin translated from the cDNA sequences by Maeda (25). In order to confirm the identity of SER with normal adult haptoglobin, commercially prepared normal adult haptoglobin and graded concentrations of SER were subjected to Western blotting analysis, using a goat antiserum to normal adult haptoglobin as detecting antibody.

Fig. 2 shows that the SER pattern as examined on SDS-PAGE under vigorous reducing conditions coincides with that of normal adult haptoglobin. Presence of contaminating AGP can be demonstrated with anti-AGP antiserum by Western blotting of the duplicate gel run simultaneously (data not shown). However, the AGP content in SER accounted for 3–10% of the total protein whereas haptoglobin content occupied greater than 95% of the total protein in SER preparations (data not presented). The chemical identity of the minor band near a molecular weight of 14,300 is not known. However, judging from the cross-reactivity of this band with the polyclonal anti-haptoglobin serum, this may represent the α1 chain of the normal adult haptoglobin, type 2-1.

Immunosuppressive Activity of SER. The immunosuppressive potency of SER was examined in PHA and anti-T3-induced mitogenesis assays.

Fig. 3 shows that SER exhibited similar immunosuppressive potency in both of these assays and that it was more effective when added during the early phase of lymphocyte activation.

Fig. 4 shows that SER is a much more effective immunosuppressant than that of normal adult haptoglobin or AGP (at least 100 to 1000-fold). Neonatal haptoglobin isolated from cord blood in a similar manner to SER also exhibited a similar immunosuppressive potency as SER obtained from malignant effusions. Previous work of Raam et al. (26) showed that greater than 50% of the haptoglobin found in cancer patients shared immunological cross-reactivity with that of macromolecular haptoglobin isolated from neonatal cord serum (27).

Immunochernical Identity of SER. In order to further define the immunochemo-moieties of SER, purified SER and crude ascites fluids obtained from metastatic cancer patients were compared with normal adult haptoglobin and rod haptoglobin by radial immunodiffusion and immunoelctrophoresis as described in “Materials and Methods.” In Fig. 5, the left panel radio immunodiffusion shows that the antiserum to normal adult haptoglobin reacted with all 3 forms of haptoglobin contained in the purified SER, haptoglobin isolated from malignant ascites fluids, as well as with crude ascites fluids of four different ovarian cancer patients. In contrast, an antiserum to cord blood reacted both with the SER and with the crude ascites fluids obtained from ovarian cancer patients, but it failed to
Fig. 2. Western blotting of SER with a polyclonal antiserum to haptoglobin. SDS-PAGE-separated protein resolved on 12% acrylamide gel were blotted onto nitrocellulose according to the method of Towbin et al. (21). Following blockage of nonspecific sites with 1% gelatin, the nitrocellulose was incubated with a rabbit antiserum to normal adult haptoglobin (Hp). Immune complexes were detected by incubation with a goat anti-rabbit IgG conjugated with horseradish peroxidase. The substrate utilized was 2,2'-azinobis-3-ethyl-benzothiazoline sulfonate (160 μg/ml) and 0.003% hydrogen peroxide in 0.1 M sodium citrate buffer (pH 4.0). Lane a, the same molecular weight (MW) standards as were used in Fig. 1; Lane b, normal adult haptoglobin (100 ng); Lanes c-e, SER at 100, 500, and 1000 ng, respectively.

Fig. 3. Inhibition kinetics of SER on lymphocyte mitogeneses induced with PHA (1 μg) or anti-T3 antibody (5 ng) per 1 x 10^6 lymphocytes as described in "Materials and Methods." Purified SER (2 μg/ml) was added to the culture at the points indicated in the figure and the DNA synthesis was measured at the end of 72-h culture. Normal human serum IgG (1 mg/ml) served as a control protein.

Fig. 4. Comparative immunosuppressive properties of purified SER, normal adult haptoglobin, α1-acid glycoprotein, and neonatal cord blood haptoglobin. Various concentrations of SER, normal adult haptoglobin, α1-acid glycoprotein, and neonatal cord blood haptoglobin were tested on lymphocyte mitogenesis induced with PHA as described in the "Materials and Methods."
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Fig. 5. A, Radial immunodiffusion of normal haptoglobin (Hp) type 2-2, purified SER (1.38 mg/ml), and crude ascites fluids from ovarian cancer patients using a rabbit anti-normal adult haptoglobin antiserum (Well a) and a rabbit anti-neonatal cord blood antiserum (Well b). The samples and antisera were used undiluted (15 µl/well) and allowed to form precipitin lines in 1% agarose gel for 3 days at room temperature. Center Well a, anti-normal adult haptoglobin. Center Well b, anti-cord blood. Peripheral Wells: 1, normal adult haptoglobin (type 2-2); 2, purified SER (1.38 mg/ml); 3, ascites from ovarian cancer patient A; 4, ascites from ovarian cancer patient B; 5, ascites from ovarian cancer patient C; 6, ascites from ovarian cancer patient D. B, Immunelectrophoresis of whole cord blood, SER, and normal adult haptoglobin (type 2-2) versus anti-cord blood haptoglobin and anti-normal adult haptoglobin. Well 1, bromphenol blue tracking dye; Well 2, whole cord blood; Well 3, purified SER (1.38 mg/ml); Well 4, normal adult haptoglobin (type 2-2). Trough 1, blank; Trough 2, anti-cord blood serum; Trough 3, anti-normal adult haptoglobin.

Fig. 6. Western blotting (right) and analytical PAGE (left) of SER. Purified SER (10 µg) (Lanes a,a.), 15 µg of haptoglobin purified from the same fluid as the SER using the hemoglobin-Sepharose media (lanes b,b.), 15 µg of normal adult haptoglobin (type 2-1) (lanes c,c.), and 15 µg of normal adult haptoglobin (type 1-1) (lanes d,d.) were electrophoresed in duplicates on a 4% acrylamide gel as described by Raam et al. (20). One gel was stained with Coomassie brilliant blue and the other gel was blotted onto nitrocellulose according to the procedure of Towbin et al. (21). A monoclonal antibody to SER was obtained by fusion of splenocytes immunized with SER and the murine myeloma SP2/0 as described by Gefter et al. (15). Immune complexes were detected using a goat antiserum to mouse IgG + IgM, conjugated with horseradish peroxidase enzyme in phosphate buffered saline containing 0.05% Tween 20. Addition of substrate solution containing 12.5 ml of 0.1 M sodium phosphate buffer containing 1 mM citrate (pH 4.0), 1 µl of 30% hydrogen peroxide, and 100 µl of 4-chloro-1-naphthol stock solution (0.163 g/1.1 ml of methanol) allowed detection of components.

with the native form of macromolecular haptoglobin-like component in SER and fails to react with normal adult haptoglobin. Therefore, it is clear that SER bears an additional epitope(s) that is absent in normal adult haptoglobin. Interestingly enough, this monoclonal antibody failed to react with SER when the SER was separated on SDS-PAGE under denaturing conditions. Therefore, it is possible that SER is a macromolecular form of haptoglobin and that the specificity of the monoclonal antibody to SER may be directed to the multimeric form of haptoglobin such as that contained in SER preparations.

DISCUSSION

Serum haptoglobin is an acute phase reactant produced by the liver in response to a cytokine, presumably interleukin-1 or hepatocyte-stimulating hormone (29). Studies of Apffel and Peter suggested that the acute phase reactants function to self-limit the autoimmune aggression of the host during an intense immune response to infection, trauma, or tissue injury (30). Elevated levels of haptoglobin have been reported in the serum of women with inflammatory, benign, or neoplastic lesions of the ovary (31). Elevated levels of haptoglobin in serum have been reported to correlate with the extent of smoking in heavy smokers (32) as well as with the tumor load in patients with metastasized tumors (33). Grange et al. (34) reported that the elevated levels of haptoglobin in the serum of patients with tuberculosis appears to inversely correlate with their lymphocyte function. Baseler and Burrel (35) isolated haptoglobin from acute phase rabbit serum and showed that purified haptoglobin derived from these animals significantly inhibited the mitogenic response of murine splenic lymphocytes to PHA, concanavalin A, and lipopolysaccharide.
In contrast to this acute phase haptoglobin, the effect of the tumor-associated SER-haptoglobin appears to be limited to the T-dependent immune responses in vivo and in vitro as the phagocyte functions of macrophages, natural killer cells, or immunoglobulin synthetic functions of B-lymphocytes were minimally affected by the purified SER (data not shown).

The instability and scarcity of the immunosuppressive substance in cancer have hampered its biochemical identification for the past three decades. In fact, the conventional purification procedure utilizing ion-exchange chromatography under acidic conditions might have denatured the native biological activity of haptoglobin as the acidification of whole serum is reported to denature 80% of the haptoglobin in 30 min (36). Furthermore, the antigenic sites of the normal adult haptoglobin were identical to those of the denatured SER as examined by SDS-PAGE followed by Western blotting using a polyclonal anti-serum to normal adult haptoglobin (Fig. 2). Therefore, it is possible that our purification procedure utilizing sequential affinity chromatographies may have preserved the biological reactivity of haptoglobin better than the conventional purification method. However, this argument can be discounted by our observation that we have not been able to obtain any measurable quantities of the SER type of potent immunosuppressive factor from normal human plasma, using the same purification procedures applied to SER. Therefore, the immunosuppressive property of SER-haptoglobin may be attributable to both the way in which it was purified and the new antigenic site(s) expressed on the SER-haptoglobin moiety. Further support for the presence of a unique antigenic determinant in SER-haptoglobin comes from the results obtained with a monoclonal antibody specific to SER-haptoglobin as shown in Fig. 6. Since the monoclonal antibody to SER reacts only with the native, macromolecular form of SER, analogous to neonatal haptoglobin, this tumor-associated SER-haptoglobin may be immunologically analogous to a neonatal or fetal form of haptoglobin. Furthermore, it suggests that overproduction of this tumor-associated SER-haptoglobin may be the culprit of immune dysfunction in metastatic cancer patients. Unfortunately, the quantity of neonatal blood necessary to obtain a sufficient quantity of fetal haptoglobin was prohibitively large (at least 1 liter). Therefore, we could not make a direct immunochromatographic comparison of the neonatal haptoglobin with the SER in this study. However, it is clear that SER has larger molecular mass than any of the normal adult type haptoglobin. In addition, it appears to bear a unique epitope in this tumor-associated haptoglobin that is not present in normal adult haptoglobin. In this respect, SER appears to be a unique molecule: on one hand it manifests an immunosuppressive property as an acute phase reactant, and on the other hand it appears as an oncofetal antigen during tumor development.

At the molecular level, fetal haptoglobin is believed to be different from the normal adult type in its electrophoretic mobility, molecular weight, and ability to bind hemoglobin (20, 27, 37). However, the chemical or genetic basis of antigenic differences between the fetal or tumor-associated SER-haptoglobin and the normal adult haptoglobin is not known. In spite of direct and indirect evidences suggesting that there are at least two separate haptoglobin genes, one for fetal and the other for adult life (38), only one gene transcript has been detected in fetal or adult liver (39). Recent studies by Raugei et al. (40) also presented evidence that there are at least two copies of haptoglobin gene per haptoglobin genome. However, the genetic product of the second gene, i.e., the haptoglobin-related gene product, has never been identified. Our SER-haptoglobin is clearly not the product of the haptoglobin-related gene product since the N-terminal sequence of the haptoglobin-related gene, if translated, would have different amino acid sequence in the α chain from that of normal adult haptoglobin. Earlier studies on the genomic sequence of haptoglobin predicted a hot spot for a recombination in the fetal gene (41). Whether the SER-haptoglobin represents a product of an unmodified primary transcript of the adult gene appearing during the tumor development, a product of a mutated haptoglobin gene, or a product of a normal adult gene that became modified under the influence of tumor has yet to be determined. It is also possible, although unlikely, that the gene product of the haptoglobin-related gene occupied less than 5% of the SER-haptoglobin preparation and that we might have missed this gene product during amino acid sequencing of SER-haptoglobin. Availability of a monoclonal antibody to the SER-haptoglobin should enable us to further differentiate the antigenic sites of this tumor-associated macromolecular SER-haptoglobin from the normal adult haptoglobin and should help to assess the pathophysiological role of SER in vivo.

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