Significance of Breast Carcinoma-associated Antigens as a Monitor of Tumor Burden: Characterization by Monoclonal Antibodies

Fernando A. Salinas, Kian H. Wee, and Roberto L. Ceriani

Advanced Therapeutics Department, Cancer Control Agency of British Columbia [F. A. S., K. H. W.]; University of British Columbia [F. A. S.]; Vancouver, British Columbia, Canada; and John Muir Cancer and Aging Research Institute [R. L. C.], Walnut Creek, California 94596

ABSTRACT

Use of monoclonal antibodies (Mc 3 and Mc 8) prepared against human mammary-epithelial antigens of human milk fat globule membranes has enabled characterization of breast carcinoma (BC) associated antigens (BCAA), antibodies, and circulating immune complexes (CIC). For this study, BC patients were grouped on the basis of measurable tumor burden: Group I patients with no evidence of disease at sampling time; Group II patients with tumor burden $\leq 5$ g; and Group III patients with known regional or distal metastases. In an in vitro simulation of tumor burden change, selected BC patients' sera were mixed with Mc 3 and Mc 8 at optimal concentrations. CIC reduction (dissociation) for Groups I and III and increment (formation) for Group II were noted. Unlike Group I sera, Groups II and III sera required 4- to 16-fold dilution of Mc 3 and 4-fold more concentrated Mc 8 to achieve maximal CIC changes. Serum BCAA isolated by use of both Mc 3 and Mc 8 immunobead procedures showed apparent $M$, 33,000 monomer, 66,000 dimer, and 95,000 trimer. When BCAA were added to BC patients' sera, autologous combinations resulted in small (7.7S) CIC for Groups I and III, and medium (9 to 12S) CIC for Group II. Conversely, combinations resulted in mainly small CIC for Group I, and intermediate CIC for Group II and Group III. Evaluation of circulating BCAA concentration by use of a three-step radioligand technique demonstrated significant discrimination between BC patients' sera (mean = 105 ng/ml) and normal control sera ($\leq 20$ ng/ml). BCAA were found to be elevated in 31 of 46 (67%) Group I (mean = 70 ng/ml), 41 of 43 (95%) Group II (mean = 197 ng/ml), and 30 of 46 (65%) Group III (mean = 50 ng/ml) patients' sera, as compared to "background" levels in malignant melanoma and normal controls. Breast carcinoma sera showed moderate BCAA increases (mean = 48 ng/ml) in 20 of 35 (57%) patients. Furthermore, serial sample determination of BCAA in 36 selected BC patients confirmed the above pattern, indicating that this assay can be used with some restriction to monitor tumor burden. Whereas in early breast carcinoma increase in BCAA concentration was concurrent with or antedated clinical objective evidence of tumor burden increase, significant decreased BCAA concentration was observed with tumor burden reduction. Overall, increased BCAA levels were associated with limited tumor burden (Group II) while decreased BCAA levels were observed with no evidence of disease (Group I) and known regional or distal metastatic advanced disease (Group III) during patients' follow-up.

INTRODUCTION

The antigenic heterogeneity occurring on the surface of BC cells is clearly reflected by the immunological reactions observed and reported in BC patients (1–8). Production of monoclonal antibodies to BCAA, breast tumor cell lines, membrane-enriched extracts of BC metastases, and mammary "tissue-specific" antigens have been documented (5, 9–16). Subsequent use of monoclonal antibodies in serotherapy (17), immunodetection, diagnosis (11, 18), prognosis (18, 19), and other clinical applications (2, 20–22) in BC and other cancer types have been reviewed recently (23, 24).

Clinical studies on CIC levels and their relationship to tumor burden have shown that useful clinical correlations of CIC and anti-XOFA levels with the extent of tumor burden were observed in malignant melanoma and other tumor types (25, 26). Similar clinical correlations to both number of tumor cells and antigen concentration have been established (25). The relationship of tumor burden to antigen concentration, size, and composition of CIC in serum of malignant melanoma patients has also been demonstrated (25, 26). In such a patients' group it was shown that the prevalent CIC size is antigen dependent (26). In a preliminary breast carcinoma study the relationship of antigen, antibody, and CIC levels to tumor burden concentration has supported the above findings (1). In addition, Salinas et al. (4) evaluated the prognostic and tumor burden marker role of CIC and noted that CIC changes antedated clinical objective increases or reduction in tumor burden. Conversely, CIC levels were unchanged for patients who remained clinically stable. (4)

Detection, isolation, and characterization of differentiation HME-Ags in BC patients' sera have been previously reported (2, 9, 25). Peterson et al. (7), Ceriani et al. (8, 12), and Taylor-Papadimitriou et al. (10) have characterized HME-Ags and showed their heterogeneity by the occurrence of $M$, 46,000, 70,000, and 400,000 moieties. Whether the largest component represented antigen-antibody complexes is yet to be established. A solid phase assay that quantitated and analyzed HME-Ags aimed at identifying cell membrane components involved in cell adhesivity, invasiveness, and metastatic capability (12) has been developed. Availability of monoclonal antibodies allowed detailed analysis of the antigenic heterogeneity in malignant, premalignant, and normal mammary epithelial cells (1, 2, 5, 18, 21). It also facilitated demonstration of differential expression of several BCAA and associated reactants reported to occur in BC and BBD, even within a tumor mass (5, 10, 12, 18, 20).

Recently, several radioimmunoassays have been developed (11, 16, 27, 28) to detect BCAA as defined by monoclonal antibodies, predominantly in sera from advanced disease BC patients. Use of a similar assay but using polyclonal antibodies to human milk fat globule membranes has been reported by Ceriani et al. (9). Preliminary reports on BCAA have demonstrated antigenic heterogeneity as reflected by detection of several antigenic moieties (1). These BCAA have been shown to occur in a pattern that related to the extent of tumor burden and immune complexes' concentration in BC (1, 2). In the present study we have extended these observations to other patients at all levels of tumor burden expression. Results are presented to demonstrate relative use advantages of the BCAA assay to monitor the course of disease and also to illustrate how

Received 11/7/85; revised 9/26/86; accepted 10/21/86.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by grants from National Cancer Institute, Canada and CA 39932 and 39933 from the National Cancer Institute, NIH, Department of Health, Education, and Welfare.

2 To whom requests for reprints should be addressed, at Cancer Control Agency of British Columbia, B. C., 600 West 10th Avenue, Vancouver, B. C., V5Z 4E6, Canada.

3 The abbreviations used are: BC, breast carcinoma; BCAA, breast carcinoma-associated antigens; CIC, circulating immune complexes; HME-Ag, human mammary epithelial antigen; BBD, benign breast disease; XOFA, xenogeneic oncofetal antigen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline (0.13 M NaCl-2.7 mM NaHPO$_4$-7H$_2$O-1.6 mM KH$_2$PO$_4$, pH 7.2).


907

Downloaded from cancerres.aacrjournals.org on October 18, 2017. © 1987 American Association for Cancer Research.
BCAA levels antedated clinical objective evidence of tumor burden changes.

In summary, the purpose of the present study was (a) to evaluate by use of an in vitro model the reactivity of monoclonal antibodies prepared against HME-Ags with BC-associated immune reactants, (b) to isolate BCAA and examine by means of an in vitro model that simulates increasing tumor burden the changes of CIC and associated immune reactants upon addition of BCAA to BC patients’ sera, and (c) to quantitate BCAA of selected BC patients’ sera in order to assess their relationship to in vivo tumor burden.

MATERIALS AND METHODS

Monoclonal Antibodies
Monoclonal antibodies, IgG2a (hereafter called Mc 3 and Mc 8) were prepared against HME-Ag of human milk fat globule membranes as per details described elsewhere (12, 29). Mc 8 biotinylation with biotin-p-nitrophenyl ester (control No. 34828; US Biochemical Corp., Cleveland, OH) was performed as described by Kendall et al. (30).

Preparation of Monoclonal Antibody-coated Immunobeads

This procedure was performed according to the manufacturer’s specifications. Briefly, cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Ltd., Uppsala, Sweden) were swollen, washed, and incubated with either Mc 3 or Mc 8, then washed three times to remove noncovalently bound protein.

Isolation of BCAA Using Immunobeads

This technique was performed essentially as previously described (9). Briefly, BC patients’ serum (0.2 ml) was incubated with equal volumes of Mc 3- or Mc 8-coated Sepharose 4B immunobeads and PBS. Immunobeads carrying BCAA recovered from the serum were labeled with 125I (31) and washed with PBS to remove unreacted 125I. Bound 125I-labeled BCAA were released from the monoclonal antibodies by incubating the immunobeads for 20 min at 20°C with 0.3 ml NaSCN or 0.3 ml 1 M CH3COOH, and dialyzed overnight. A parallel antigen isolation procedure was performed using selected BBD sera.

Characterization of BCAA by SDS-PAGE

Tube Gel Procedure. Duplicate samples of 125I-labeled BCAA (15 µl) were analyzed on 7.5% (w/v) polyacrylamide gels (Bio-Rad, Inc., Cleveland, OH) was performed as described by Kendall et al. (30).

Preparation of Monoclonal Antibody-coated Immunobeads

This procedure was performed according to the manufacturer’s specifications. Briefly, cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Ltd., Uppsala, Sweden) were swollen, washed, and incubated with either Mc 3 or Mc 8, then washed three times to remove noncovalently bound protein.

Isolation of BCAA Using Immunobeads

This technique was performed essentially as previously described (9). Briefly, BC patients’ serum (0.2 ml) was incubated with equal volumes of Mc 3- or Mc 8-coated Sepharose 4B immunobeads and PBS. Immunobeads carrying BCAA recovered from the serum were labeled with 125I (31) and washed with PBS to remove unreacted 125I. Bound 125I-labeled BCAA were released from the monoclonal antibodies by incubating the immunobeads for 20 min at 20°C with 0.3 ml NaSCN or 0.3 ml 1 M CH3COOH, and dialyzed overnight. A parallel antigen isolation procedure was performed using selected BBD sera.

Characterization of BCAA by SDS-PAGE

Slab-gel Immunoblot Procedure. Slab-gel SDS-PAGE was performed essentially as reported (33). Briefly, treated BCAAs samples (10 µg protein) were applied to a 4% w/v stacking gel over a 10% w/v slab gel. After electrophoresis, transfer of BCAA from the gel to nitrocellulose paper was achieved by use of Bio-Rad Trans-Blot cell according to the manufacturer’s specifications. Thereafter, samples were reacted with 1 ml serially diluted Mc 3 or Mc 8, washed, and followed by peroxidase-conjugated goat anti-mouse IgG (Lot No. 23637, Cooper Biomedical, Inc., Malvern, PA) enzyme-linked immunodetection. Resulting bands were measured by a densitometer, Model 620 (Bio-Rad).

In Vitro Model of Tumor Burden Change

The interaction of Mc 3, Mc 8, or isolated BCAA with selected BC patients’ sera was studied by use of an in vitro model, a method used to generate and dissociate immune complexes (25, 34). By using this model, CIC size changes were examined by titration of either Mc 3 or Mc 8 with selected Groups I, II, and III BC patients’ sera followed by CIC determination. Conversely, this model was used to simulate defined increases in tumor burden by addition of BCAA to autologous (self) or allogenic (non-self) patients’ sera. Briefly, this consisted of admixing (17 h at 4°C) 50 µl 1:8 PBS-diluted patients’ sera with isolated 125I-labeled BCAA (1 µg). In preliminary experiments this concentration of BCAA was determined to render maximal immune complex size changes (1, 34). The reaction mixture was analyzed for CIC size changes by SDS-PAGE procedure.

Three-step Radioligand Assay

This immunoassay was performed essentially as previously described (9) with minor noted modifications. Briefly, instead of specific antigen, Raji-cell-extracted BCAA (25) was used as standard antigen, at 1 to 100 ng in 50 µl normal control sera. Test sera (50 µl, undiluted) from cancer patients and controls were similarly prepared and incubated with packed immunobeads (100 µl). Beads were washed with radioligand serum assay buffer (0.5% w/v human serum albumin in Hanks’ balanced salt solution, pH 7.2), and then reacted with (50 µl) biotin-conjugated Mc 8. The beads were washed again, incubated with 3 µg of 125I-labeled avidin (control No. 28227; US Biochemical), specific activity 35 µCi/µg, and after further washing their radioactivity was determined. The least detectable BCAA concentration was 10 ng/ml serum with a combined intra- and interassay 9% coefficient of variation. The cut-off value (220 ng/ml serum) used for classification of results as either normal or elevated has been selected on the basis of preliminary results, as analyzed by the Statland et al. (35) technique to minimize the costs of misclassifications.

Patient Population

In a retrospective study, 135 BC patients with histopathological confirmation of diagnosis were assigned to groups on the basis of objective evaluable tumor burden, as previously described (1, 25, 36). Briefly, Group I included 46 patients with no evidence of residual tumor at 4 to 6 weeks after surgical excision of all known carcinoma; Group II comprised 43 patients with limited recurrent disease confined to the chest wall with tumor mass estimated at ≥5 g; and Group III consisted of 46 patients known to have advanced regional or distal metastatic disease, with estimated tumor mass at >5 g. Serial-sample BCAA determinations were performed on 36 BC patients’ sera selected on the basis of known objective clinical episodes of tumor burden changes.

All patients were participants in the breast carcinoma program of the Cancer Control Agency of British Columbia. They were evaluated at 1 to 3 monthly intervals for objective evidence of tumor burden reduction, stabilization, or increase according to criteria reported elsewhere (36, 37) in the absence of BCAA results. Briefly, objective increase in tumor burden was defined as at least 25% increase in the product of diameters of measurable lesions, the development of new lesions or the appearance of cytologically confirmed malignant effusion. Objective decrease in tumor burden consisted of a reduction in the product of diameters of measurable lesions, the development of new lesions or the appearance of cytologically confirmed malignant effusion. Stabilization was scored only when there has been a clear change in growth pattern.

In view of reported multiple marker evaluation advantages, and in order to examine how other markers relate to circulating BCAA, patients were monitored for CIC by fetal liver cell or Raji cell radioligand assay (31, 34), and anti-XOFA by isotopic antiglobulin test (25, 26, 32). Thirty-five BBD patients having biopsy-proven diagnosis of fibrocytic disease or fibroadenoma were similarly evaluated. Seventy serum controls (35 males, 35 females) from healthy nonhospitalized volunteers with no known medical illness were selected for comparison from the serum bank maintained at the Cancer Control Agency of British Columbia. Also included from the same source were pretreated malignant melanoma, sarcoma, colon, and ovarian carcinoma serum samples.

Statistical Analysis

The selection of sample size per tumor burden group reflects minimal requirement to achieve statistical significance at a value of P ≤ 0.05 and with a power of 95%, assuming a discrepancy limit between groups of ±20%. This projection exceeds discrepancies observed in preliminary results.

The results from 40 to 60 patients’ sera/tumor burden group have been analyzed and compared (correlation coefficient r for data in a pair...
assay and their significance) to assess statistical differences among themselves and from those results of other unrelated malignancies or normal control sera. Such assessment has been performed by use of an independent pair difference rank analysis, Mann-Whitney test, or by $\chi^2$ analysis (38).

RESULTS

Isolation of BCAA and BBD Antigen by Immunoaffinity Beads. Isolation and characterization of BCAA from selected BC patients’ sera were achieved by in situ radioiodination of BCAA-coated immunobeads followed by antigen release and subsequent SDS-PAGE analysis. Characterized BCAA showed heterogeneity as depicted in Fig. 1. A parallel isolation procedure but using selected BBD sera showed less heterogeneity.

Reactivity of BCAA with Me 3 and Me 8. Isolated BCAA from 9 selected BC patients’ sera (3 from each tumor burden group) were reacted with both Me 3 and Me 8 by use of SDS-PAGE immunoblot procedure. The results showed a single-band reactivity at $M_r$ 33,000 for all BCAA tested. Regardless of tumor burden patients’ sera used for BCAA extraction, a 10-fold stronger band density was observed with Me 8 (Fig. 2) than with Me 3. Parallel analysis performed with antigens isolated from 9 selected BBD patients’ sera demonstrated minimal reactivity with Me 3 or Me 8 (data not shown).

Reactivity of Me 3, Me 8, and BCAA with BC and BBD patients’ sera. By use of an in vitro model that simulates tumor burden changes, the reactivity of Me 3, Me 8, or isolated $^{125}$I-labeled BCAA with 2 sets of BC patients’ sera was determined. When serially diluted (50 $\mu$l of 1:32 to 1:2048) Me 3 or Me 8 were mixed with each of selected Groups I, II, and III BC patients’ sera, changes in patients’ serum CIC levels were observed. Maximal CIC changes were achieved with Me 3 and Me 8 at 1:128 dilution for Group I serum, 1:2048 and 1:32 dilution for Group II, and 1:512 and 1:32 for Group III sera. At optimal Me 3 and Me 8 dilutions, CIC levels were reduced by 32 and 28% for Group I, reduced by 64 and 65% for Group III, but increased by 89 and 41% for Group II patients (Fig. 3). However, in a parallel experiment no CIC change was observed by use of an “indifferent” monoclonal anti-p 97a melanoma-associated antigen (Hybritech, Inc., La Jolla, CA).

Upon addition of $^{125}$I-labeled BCAA to BC patients’ sera, size analysis of major CIC moieties by SDS-PAGE demonstrated 1- to 8-fold dissociation of CIC, as compared to unreacted BC serum samples. Autologous combinations resulted in mainly small (7.7S) CIC for Groups I and III BC and intermediate size (9-12S) for Group II patients’ sera. However, BCAA in allogenic combinations resulted in small CIC for Group I and intermediate size for Groups II and III. A parallel control dissociation was performed with 2 sets of BCAA isolated from BC Groups I, II, and III patients but reacted with 2 selected sera samples from BBD patients. The results showed lack of reactivity as demonstrated by unchanged CIC size moieties in all combinations tested (Table 1).

Detection of BCAA in Tumor Burden-Grouped BC Patients. One hundred and thirty-five BC, 20 each of sarcoma and malignant melanoma, 10 each of colon and ovarian carcinoma,
TUMOR MARKER ROLE OF BCAA

35 BBD patients, and 70 normal control sera were evaluated for circulating BCAA concentration. A reference standard curve by use of Raji-cell isolated BCAA as illustrated in Fig. 4 was used. The results showed significantly elevated levels (mean = 105 ng/ml; \( P \leq 0.001 \); Mann-Whitney test) of BCAA in 102 of 135 (76%) BC patients as compared to "background" levels (≤20 ng/ml) in 20 each of sarcoma and malignant melanoma patients and 70 normal control sera. A slight increase in BCAA levels was observed in ovarian and colon carcinoma and BBD samples. In 41 of 43 (95%) Group II patients, elevated BCAA concentration (mean = 197 ng/ml) was 3-fold higher than that observed in 31 of 46 (67%) Group I patients, and 4-fold higher than that of 30 of 46 (65%) Group III patients tested (Fig. 5). There was a relationship between tumor burden and their respective mean free-BCAA levels. Group II showed higher BCAA values than Groups I and III patients. In addition, 4 patients with increased levels of BCAA who were originally diagnosed as BBD were thereafter histologically proven to have malignant breast disease.

Predictive Value of BCAA. Twenty-six BC patients' sera were retrospectively evaluated for circulating free BCAA during intervals at which there were changes in clinical course as reflected by either objective evidence of tumor burden increase or reduction, as compared to 26 patients whose tumor burden and BCAA ratio (mean = 1.03) remained unchanged (Table 2A). The BCAA ratio for those 26 patients whose tumor burden changed depended on initial and final tumor burden. For 6 patients with early episodes of increasing tumor burden (Groups I to II), there was a concurrent decreased BCAA ratio (initial/final), mean = 0.25 (Table 1 to 6) (Table 2B). For 8 patients

Table 2 Serum BCAA in BC patients at timed intervals

<table>
<thead>
<tr>
<th>Episode</th>
<th>Group tumor burden change</th>
<th>Time interval between serum samples (mo)</th>
<th>BCAA ng/ml</th>
<th>BCAA ratio, initial/final</th>
</tr>
</thead>
</table>

A. Stable

<table>
<thead>
<tr>
<th>Episode</th>
<th>Group tumor burden change</th>
<th>Time interval between serum samples (mo)</th>
<th>BCAA ng/ml</th>
<th>BCAA ratio, initial/final</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
</table>

- Stable

- Progression

- Regression

* Tumor burden group as described in "Materials and Methods."

* No statistical differences between initial and final BCAA levels as calculated by Mann-Whitney test.

* Statistical differences between initial and final BCAA levels for group changes I to II, \( P \leq 0.004 \); II to III, \( P \leq 0.002 \); II to I, \( P \leq 0.001 \); and III to II, \( P \leq 0.02 \).
with late episodes (Table 2B, episodes 7–14) of increasing tumor burden (Groups II to III) there were concurrent increased BCAA ratios (mean = 9.2). There were 8 episodes of early objective tumor burden reduction in 8 patients, with concurrent significant increase of BCAA ratio (mean = 8.3) in all. These patients experienced tumor burden regression to Group I and decreased BCAA levels 2 to 6 months later (Table 2B, episodes 15–22). Of those 4 patients (Table 2B, episodes 23–26) presenting tumor burden reduction from Groups III to II there was a decreased BCAA ratio (mean = 0.3).

Exceptional results from this BCAA evaluation were noted and listed below. Despite unchanged tumor burden, an exceptional Group III patient showed a significant (>4-fold) reduction of BCAA. Also, an exceptional Group II patient (CIC = 26 μg/ml) who presented background BCAA level underwent disease progression to Group III status (CIC = 7 μg/ml) 9 months later, and 5 Group I patients with BCAA ratio of approximately 1 showed clinical objective tumor burden increase within 4 months (data on exceptions not shown). In addition, a 4-fold reduction in serum BCAA was observed 3 days after mastectomy in 3 BC patients tested before and after surgery.

Serial-sample BCAA determinations were performed on 10 selected BC patients over a 21- to 66-month (median = 29) period. During this follow-up patients were also monitored for serum CIC, anti-XOFA, and for tumor burden changes resulting from disease progression or therapeutic interventions. Overall BCAA levels showed a tendency to fluctuate with increased values for tumor burden Group II and lower levels for both Groups I and III patients. Using the most stringent criterion for evaluation of tumor size change (50%) as described for tumor regression, an assessment of how BCAA changes related to tumor burden was undertaken. The results showed that in 7 of 10 patients BCAA changes antedated 8 times (2 regressions and 6 progressions) clinical objective evidence of tumor burden changes by 8 to 24 (mean = 18) weeks. Concurrent changes in BCAA levels and tumor burden were observed in 2 patients, while a 2-month delay was noted in the remaining patient. In all follow-ups, an irregular inverse relationship between BCAA and CIC levels was noted. Also, a correlation between BCAA and anti-XOFA levels (r = 0.67; P ≤ 0.05, by Spearman’s test) was observed in one patient. A profile representative of one of these patients, including ongoing clinical and therapeutic interventions, is illustrated in Fig. 6.

DISCUSSION

Monoclonal antibodies prepared against HME-Ag of human milk fat globule membrane were used in an earlier described in vitro model of tumor burden change (2, 25, 34). The present evaluation with both Mc 3 and Mc 8 (Fig. 3) suggested that Group III patients’ CIC were at or close to antigen:antibody molecular equivalence, Group II patients’ CIC were in antigen excess, while Group I patients’ CIC were in relative antibody excess.

Previous results from criss-cross combinations of melanoma-associated antigens and sera from malignant melanoma patients harboring different tumor burden demonstrated that the prevalent CIC size was antigen dependent (25, 34). In a complementary report on BC, predominant large size (25S) CIC for Group I patients, intermediate to large size (16 to 19S) for Group II, and large (17.5 to 25S) for Group III unreacted BC patients’ sera has been described. In this study, by addition of optimal concentration of BCAA to selected BC and BBD patients’ sera, we assessed how tumor burden changes relate to BCAA concentration, and size and composition of resulting immune complexes. In brief, CIC formation and dissociation illustrated a kinetic balance of CIC size changes as they relate to antigen and antibody concentrations. These results are in keeping with those of a comparable analysis on malignant melanoma (25, 34) in that autologous reactivity resulted in CIC size reduction. However, unlike earlier observations (2, 25, 26, 31, 34), current allogenic combinations demonstrated CIC size reduction.

We have developed a modified 3-step radioligand immunodetection technique for quantitation of circulating free BCAA in patients’ sera. An overall assessment of this assay demonstrated that despite BCAA not correlating linearly with tumor burden, BCAA represents a useful marker to monitor tumor burden. This is more in keeping with the MAM-6 antigen assay (27) than the diagnostic and/or prognostic value reported for other monoclonally detected antigens (16, 18, 19, 21, 39). The use of Raji cell-extracted BCAA proved to be a satisfactory alternative to specific HME-Ag. Although observed results appear qualitatively unaffected, Raji cell-extracted BCAA may limit, to a certain extent, assurance that one is measuring the same antigen. Results of this study have allowed us to estimate antigen to antibody proportions occurring in sera of BC patients with differing tumor burden, confirming higher free BCAA concentration for Group II than for Groups I and III (Fig. 5).

Induction of a preterm delivery may account for the unusual increased initial BCAA level noted in a stable Group III patient. The only Group II patient with low BCAA progressed to Group III 9 months later. This change has been interpreted as an exception of otherwise clinically unsuspected tumor burden change. Conversely, Group II patients with initial elevated free BCAA concentration who 3.5 months later experienced tumor burden regression to Group I shifted concurrently to background BCAA level.

The strong association of increased BCAA level to Group II tumor burden and decreased BCAA level to Groups I and III was further confirmed by a preliminary study on serial-sample BCAA determinations. The increased expression of circulating BCAA in the sera of 65 to 95% of BC and 57% of BBD patients is in keeping with figures reported for monoclonal antibodies binding to BC histological sections (3, 13, 14, 21, 30) and their reactivity to tumor and normal tissues and cell lines (3, 5, 10–12, 28). Detection of increased levels of BCAA in BBD and adenocarcinomas of non-breast origin reflects the expression of heterogeneity in antigenic epitopes and their noted different glycosylation degrees known to be recognized by these monoclonals (9–11, 13, 14, 16). In keeping with reported results, the degree of cross-reactivity observed appears mostly restricted to adenocarcinomas (secretory epithelial cell differentiation) in both clinical and experimental conditions (11, 13, 16, 28, 40). Our experience with BCAA has been similar to reported observations on the expression of ductal carcinoma antigen in BC patients (15). Despite the apparent nonidentity of our BCAA to ductal carcinoma antigen, it is difficult to disregard the analogy of antigenic expression detected in BC patients with differing extents of disease. Such results provide supporting evidence for similar observations reported on BC patients (15), and with a reported suggestion of a temporal relationship between CIC and malignant cell proliferation (41).

The novelty of our approach consisted of dealing, to some extent, with the effect of CIC and anti-XOFA on free circulating BCAA levels and their relationship to tumor burden. Such findings, if further substantiated, may have direct implications
Fig. 6. Representative profile of BC patient for BCAA (Δ), fetal liver cell-radioimmunoassay-determined CIC (●), and anti-XOFA (○), including ongoing clinical and therapeutic interventions. Amino, aminoglutethimide; mets, metastases; AR, adsorption ratio.

for the understanding of clearance, pathogenetic deposition, and ultimate fate of CIC (25, 34), as related to CIC size and composition and also affecting the planning and identification of problems implicit in the use of nonspecific immunotherapeutic and immunorestorative modalities (23, 34). The observed interactions of Mc 3 and Mc 8 with BC sera provide supporting evidence for suggesting alternative views to confront clinical limitations in the use of monoclonal antibodies. Such limitations have been encountered in serotherapy (24, 25), in radiodetection or targeting of micrometastases (42), as carriers of chemotherapeutic agents or toxins (23, 29, 43), and as agents for detection of adjunct markers for the clinical monitoring of BC patients.

ACKNOWLEDGMENTS

We thank Kek-Lim Lee for his technical assistance; Dr. P. Rebeck and St. Vincent's Hospital, Vancouver, for supplying BBD serum samples; Andy Coldman, Division of Epidemiology, Biometry, and Occupational Oncology, Cancer Control Agency of B.C. for assistance in statistical analysis; Terry Nuyten, Bio-Rad Laboratories, Ltd. for assistance in densitometer determinations; and Linda Wood and Joan Bethune for their secretarial expertise.

REFERENCES


Significance of Breast Carcinoma-associated Antigens as a Monitor of Tumor Burden: Characterization by Monoclonal Antibodies

Fernando A. Salinas, Kian H. Wee and Roberto L. Ceriani


Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/47/3/907](http://cancerres.aacrjournals.org/content/47/3/907)

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

To request permission to re-use all or part of this article, contact the AACR Publications Department at [permissions@aacr.org](mailto:permissions@aacr.org).