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Expression of Epitopes of the Tumour-associated Glycoprotein 72 and Clinicopathological Correlations in Mammary Carcinomas

A. Contegiacomo, M. Alimandi, R. Muraro, C. Pizzi, R. Calderopoli, L. De Marchis, A. Sgambato, G. Pettinato, G. Petrella, M.R. De Filippo and R. Mariani-Costantini

We analysed the immunohistochemical expression pattern of the distinct carbohydrate epitopes of the TAG-72 molecule, defined by the monoclonal antibodies (MAbs) B72.3, CC-49 and CC-83, in 92 breast carcinomas of different histological type, and in other histological components identified in the mammary tissue samples studied. The results were correlated with the clinico-pathological characteristics of the tumours, and with their proliferative activity, assessed by thymidine labelling index (TLI). Expression of the TAG-72 epitopes was detected in all the tumour histotypes analysed, but patterns of immunoreactivity tended to vary in relation to type and level of differentiation. The comparative analysis of the reactivities of the three anti-TAG-72 MAbs revealed differences in their ability to recognise neoplastic lesions. MAb CC-49 reacted with the highest percentage of tumours (82%), and also tended to yield the highest percentages of immunoreactive cancer cells, while B72.3 and CC-83 reacted with lower percentages of tumours (respectively, 55 and 51%), and identified lower percentages of immunoreactive cells. High levels of expression of the three TAG-72 epitopes were detected in areas of *in situ* ductal carcinoma. Comparatively lower levels of immunohistochemical positivity were found in atypical epithelial hyperplasia, normal mammary epithelium and epithelium with cystic disease. TAG-72 epitope expression was correlated with prognostic parameters. The synchronous expression of the three epitopes significantly correlated with large tumour size (> 2 cm), and with high histological grade. No correlations could be demonstrated between TAG-72 phenotypes and nuclear grade, lymph node status and proliferative activity (high versus low).

Key words: breast cancer, antigen, tumour-associated, carbohydrate, cell kinetics
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INTRODUCTION

THE DEVELOPMENT and application of monoclonal antibody (MAb) technology to the study of human tumour antigens has resulted in the immunological detection of an ever increasing variety of carbohydrate antigenic epitopes, some of which have been shown to be associated with the transformed phenotype [1, 2]. Among these, the core carbohydrate epitopes of mucins tend to demonstrate a pan-carcinoma distribution and can be utilised for a variety of clinical applications [1, 3, 4]. However, one of the major limitations to the clinical utility of this class of tumour-associated antigens (TAAs) is the high degree of antigenic heterogeneity that has been found among different types of carcinoma, among distinct carcinomas of the same type, and even within single neoplastic lesions [1, 5–7]. The nature and the biological implications of such a high degree of antigenic variability are still debated [1, 2].

The tumour-associated glycoprotein 72 (TAG-72), a pan-carcinoma marker, is expressed in a variety of human epithelial tumours, most notably mammary, gastrointestinal, ovarian and non-small cell lung cancer [1, 8, 9]. The expression of TAG-72 was initially evaluated using MAB B72.3, generated against the membrane fraction of a metastatic breast carcinoma [5, 8], and reacting with sialosyl-Tn, a core region structure of *O*-linked carbohydrates [10]. A series of MAbs detecting different carbohydrate epitopes of the TAG-72 molecule was subsequently generated [11]. Among these MAbs, two, respectively designated CC-49 and CC-83, were shown to identify mucin-carried epitopes expressed in higher percentages of carcinomas than the B72.3 epitope [7]. Several studies suggest that the expression of core sialosylated carbohydrate epitopes of mucins may play an important role in determining the biological behaviour of carcinoma cells [1, 12, 13]. In addition, the inter- and intra-tumour heterogeneity of mucin-related epitopes may be associated with differences in the type and level of differentiation of the tumour [1].

Correspondence to A. Contegiacomo.
A. Contegiacomo, M. Alimandi and L. De Marchis are at the Dipartimento di Medicina Sperimentale, Università degli Studi "La Sapienza", Viale Regina Elena 324, 00161 Roma; R. Muraro and R. Mariani-Costantini are at the Istituto di Patologia Umana e Medicina Sociale, Università "G. D'Annunzio", Chieti; C. Pizzi, R. Calderopoli and A. Sgambato are at the Cattedra di Oncologia Medica; G. Petrella and M.R. De Filippo are at the Cattedra di Oncologia Chirurgica; and G. Pettinato is at the Istituto di Anatomia ed Istologia Patologica, Università "Federico II", Napoli, Italy.
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In this work, the three distinct TAG-72 carbohydrate epitopes, defined by MAbs B72.3, CC-49 and CC-83, [8, 10, 11, 14], were evaluated in a series of biopsy specimens of primary breast cancers, which were also studied for proliferative activity, considered to be one of the most important biological indicators of aggressiveness in primary human breast cancer *in vivo* [15–17].

The objectives of our investigation were (a) to define and compare the immunohistochemical expression and distribution of the three mucin epitopes in different histotypes of invasive carcinoma, and in other histological components identified in the biopsy samples studied (normal epithelium, epithelium with benign or atypical proliferative changes, intraductal carcinoma; and (b) to correlate the TAG-72 antigenic phenotypes with clinico-pathological characteristics of the tumours (size, histological type, lymph node status, histological and nuclear grade), and with cell kinetics, as evaluated by thymidine labelling index (TLI).

MATERIALS AND METHODS

The study was performed on 92 primary breast carcinomas obtained from the Department of Surgery, 2nd Medical School, University of Naples, Italy. The tumours were histologically classified according to the criteria of the World Health Organization [18]. Invasive ductal carcinomas were graded following the system of Bloom and Richardson [19], and the nuclear grade was also scored [20]. Data concerning tumour size were available for 85 of the 92 lesions, lymph node status was determined in 91 cases. The majority of the 92 tumours (86 cases, i.e. 93%) were invasive ductal carcinomas, the remaining 6 cases (7%) were classified as invasive lobular carcinomas. The invasive ductal carcinomas were histologically subclassified as follows: 63 not otherwise specified (NOS), five tubular, five cribriform, three papillary, three medullary, six mixed mucinous, one secretory. The ductal tumours, with the exception of the three medullary carcinomas, were graded as G1 in 12 cases, G2 in 35 cases and G3 in 36 cases. Mixed mucinous tumours were graded on the basis of their associated infiltrating ductal carcinoma components. Nuclear grade was assessed in 77 ductal carcinomas. The sections often included other histopathological components. Intraductal carcinoma was found in 37 of the 86 cases of invasive ductal carcinoma, atypical epithelial hyperplasia in 4 cases (three of ductal type, one of lobular type). Epithelium with benign proliferative changes (fibrocystic disease, including sclerosing adenosis and/or intraductal papillomatosis), was associated with invasive ductal carcinoma in 15 cases. *In situ* neoplasia of lobular type was detected in 2 of the 6 cases of invasive lobular carcinoma.

A small portion of the tumour was placed in TC 199 tissue culture medium (Gibco, U.K.) immediately after surgery, and processed for the determination of the proliferative activity by the TLI technique [21]. The remaining part was fixed in 10% buffered formalin and embedded in paraffin. Sections, cut at 5 μm, were used for pathological and immunohistochemical studies.

The immunohistochemical analysis was performed using a panel of MAbs directed against the tumour-associated glycoprotein TAG-72, which were selected for their specificity to distinct epitopes and for their well-established reactivity with breast carcinomas [10, 11, 14]. Immunohistochemistry was performed using a streptavidin–biotin system for mouse primary antibodies (Zymed, San Francisco, California, U.S.A.) and diaminobenzidine tetrahydrochloride (DAB) as reporter chromogen. Endogenous peroxidase activities were blocked by immersion in 0.3% (vol/vol) hydrogen peroxide in absolute methanol for 10 min. In order to minimise non-specific binding, the sections were incubated for 15 min at room temperature with normal rabbit serum, diluted 1/10 in RPMI 1640 tissue culture medium. Serial tissue sections were incubated for 30 min at room temperature in a humidified chamber with 200 μl/slide of primary MAb (ascitic fluids, containing MAbs B72.3, CC83 and CC49, diluted at 1:1000, 1:1000 and 1:1500, respectively, in RPMI 1640 containing 5% fetal calf serum). The dilutions were selected by end-point titration, and allowed maximal immunoreactivity with carcinoma cells without background staining of tumour stroma. Negative control sections were immunostained under the same conditions, substituting murine non-specific MAbs for the primary antibodies. The immunoreactivity was analysed on the basis of the percentage of immunostained cells. The number of DAB-labelled cells was approximated by combining the observation of the overall reactivity of the tumour at low magnification with the count of reactive cells in five different microscopic fields at ×400.

Statistical analyses for the correlations of TAG-72 epitope expression with clinicopathological characteristics and proliferative activity were performed by the χ^2 Fisher's test as appropriate. *P* values were computed after combining the cases with no immunostaining versus those with immunostaining.

Table 1. *Reactivity of MAbs B72.3, CC83 and CC49 with the various histotypes of invasive primary carcinomas*

Histotype	No. positive/total B72.3	CC83	CC49
NOS	39/63	32/63	55/63
Tubular	0/5	2/5	3/5
Cribriform	2/5	2/5	3/5
Papillary	1/3	0/3	1/3
Medullary	1/3	1/3	2/3
Mixed mucinous	5/6*	6/6*	6/6*
Secretory	0/1	0/1	0/1
Total ductal (%)	48/86 (56)	43/86 (50)	70/86 (81)
Lobular	3/6	4/6	5/6
Total carcinomas (%)	51/92 (55)	47/92 (51)	75/92 (82)

*Diffuse positivity of extracellular mucin associated with focal reactivity of neoplastic cells in mucoid components. NOS, not otherwise specified.

For TLI determination, tumour samples were minced into eight to 12 fragments of about 1 mm^3 after removal of adipose or necrotic tissue, placed in complete TC199 medium (20% fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin), containing 6 μmCi/ml [^{3}H]thymidine (specific activity 25 Ci/mmol; Amersham, Buckinghamshire, U.K.) and incubated under slow agitation at 37°C for 1 h. The fragments were then fixed in Bouin's solution and processed for paraffin embedding. Serial sections, cut at 4 μm and mounted on gelatin-coated slides, were autoradiographed using K5 emulsion (Ilford, U.K.). The autoradiographs were developed after 3 days of exposure at 4°C, fixed, stained with haematoxylin and eosin, and examined under oil immersion at a magnification of ×1000. Cells were considered thymidine-labelled when more than 20 grains were counted on the nucleus. The TLI values were determined by counting the thymidine-labelled neoplastic cells on a total of 3×10^3 to 10×10^3 tumour cells, from different fragments of the same tumour. A TLI value of 2.8% was used as the cut-off point to define tumours with low and high cell kinetics (respectively, < 2.8 and ≥ 2.8%). This value corresponded to the median value of the series studied.

RESULTS

Expression of the three TAG-72 epitopes appeared to be frequent in all the breast cancer histotypes studied (i.e. NOS, tubular, mixed mucinous, cribriform, papillary, medullary and secretory varieties, Table 1). The only case of secretory type ductal carcinoma tested was negative for the expression of the three epitopes. TAG-72 immunostaining was predominantly localised at the level of the apical cell membranes and secretion products in well differentiated ductal carcinomas, whereas it shifted to the entire cell membrane, cytoplasm and intracytoplasmic lumina in ductal carcinomas with solid or alveolar growth patterns, and in lobular carcinomas. All six mixed mucinous carcinomas studied were positive for TAG-72. In the mucinous components of these tumours, immunostaining co-localised with the extracellular mucinous secretion rather than with the neoplastic cells, that were only focally positive (Figure 1). Strong TAG-72 expression was observed at the level of the cytoplasm, the cell membranes and luminal secretions in areas of intraductal carcinoma, while areas of *in situ* lobular carcinoma, detected in two cases, were negative (Table 2). In some cases, the TAG-72 epitopes were also expressed in areas of atypical terminal duct hyperplasia, and of benign proliferative disease, and in normal ductal and lobular epithelium (Table 2).

Expression of the epitopes defined by MAbs B72.3, CC-83 and CC-49 was, respectively, detected in 51 (55%), 47 (51%) and 75 (82%) of the 92 carcinomas studied (Table 1). The immunoreactivities of the three anti-TAG-72 MAbs revealed inter- and intra-tumour heterogeneity in the percentages of positive cells (Figure 2). When tumours were scored as positive on the basis of immunostaining for any of the three MAbs, TAG-72 was detected in 75/92 cases (82%); however, the synchronous expression of the B72.3, CC83 and CC49 epitopes was found in only 46/92 tumours (50%). Immunoreactivity for CC-49 was detected in all the cases positive for B72.3 and/or CC-83. Moreover, 21 cases negative for CC-83 and 24 cases negative for B72.3 were immunostained by CC-49. CC-49 also yielded the strongest immunostaining and the highest percentages of immunoreactive tumour cells in those cases that demonstrated synchronous expression of the three epitopes (Figure 3). The three anti-TAG-72 MAbs reacted at different levels, and with morphologically distinct patterns, with the extracellular mucin

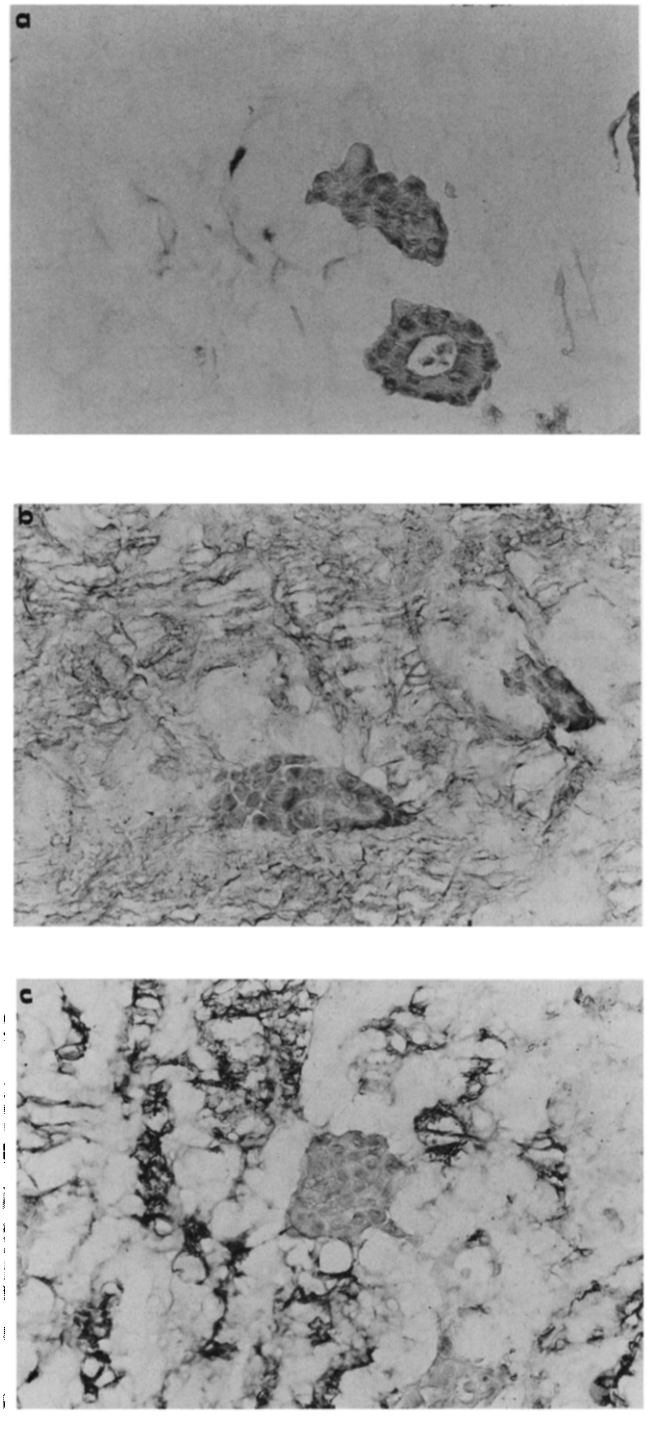

Figure 1. Patterns of immunoreactivity with the anti-TAG-72 MAbs observed in the mucoid component of a mixed mucinous carcinoma: (a) B72.3, focal positivity of mucin; (b) CC83, relatively diffuse positivity of mucin; (c) CC49, strong, foamy and granular positivity of mucin. Immunoperoxidase with haematoxylin counterstaining, original magnification ×400.

of mixed mucinous carcinomas: B72.3 tended to demonstrate focal reactivity, CC-83 yielded relatively diffuse mucin immunostaining, while CC-49, again the most reactive of the three MAbs, demonstrated a densely granular, foamy pattern of immunopositivity (Figure 1, panels a–c).

Table 2 and Figure 4 compare the immunoreactivities and the percentages of positive neoplastic cells obtained using each of the three MAbs in the infiltrating ductal and lobular carcinomas,

Table 2. Reactivity of MAbs B72.3,CC83 and CC49 with various histological components identified in the primary breast cancers

Histological component	No. positive/total B72.3	CC83	CC49
In situ ductal	22/37	20/35*	26/32*
In situ lobular	0/2	0/2	0/2
Atypical hyperplasia	2/3*	2/4	2/4
Benign proliferative disease	2/15	3/15	3/12*
Normal ductal epithelium	3/30	1/24*	2/30
Normal lobular epithelium	1/2*	1/2*	2/4

*The decrease in the number of samples studied is related to loss of the component in serial sections.

and in the other histological components identified in the biopsy samples studied. Altogether, 42 (56%) of the 72 invasive ductal and lobular tumours that were positive for CC-49 had more than 20% immunostained cells, whereas only 22 of the 51 B72.3-positive invasive tumours (43%), and 15 of the 47 CC-83-positive invasive tumours (32%) scored above 20% immunostained cells (Figure 3). CC-49 was also the most reactive of the three antibodies when evaluated against *in situ* ductal carcinoma. The intraductal carcinoma components containing > 20% immunostained cells, respectively, were 9/22 positive for B72.3 (41%), 8/20 positive for CC-83 (40%) and 13/26 positive for CC-49 (50%).

Figure 5 correlates the expression of the TAG-72 epitopes with histopathological grade in invasive ductal carcinomas. The immunostaining obtained with each of the three MAbs tended to increase with the increase in histological grade. MAbs B72.3, CC-83 and CC-49, respectively, immunostained 5/12 (42%), 5/12 (42%) and 7/12 (58%) G1 cases, 18/35 (51%), 18/35 (51%) and 27/35 (77%) G2 cases, and 26/36 (72%), 26/36 (72%) and 34/36 (94%) G3 cases. The percentages of immunostained cells also tended to increase with the increase in tumour grade (data not shown). Again, it should be noted that MAb CC-49 yielded the highest levels of immunoreactivity with each class of histological grade.

Table 3 correlates anti-TAG-72 immunoreactivity with the clinicopathological characteristics of the tumours (size, lymph node status, histological and nuclear grade), and with cell kinetics. For this purpose, we evaluated the cases that were positive for the synchronous expression of the three TAG-72 epitopes (i.e. +,+,+) versus the cases that demonstrated any other combination of immunoreactivity with at least one negative. The expression of the three TAG-72 epitopes was statistically correlated with large tumour size (T2/T3, i.e. greater than 2.0 cm in diameter, $P = 0.002$), and with high histological grade (G2/G3, $P = 0.02$). We could not find correlations between positivity for the three epitopes and nuclear grade and lymph node status. The TLI values, that could be evaluated in 88 of the 92 tumours studied, ranged from 0.01 to 12.2%. No relationships between TLI (low versus high) and TAG-72 positivity could be detected. There were also no correlations between TLI (low versus high) and tumour size, histotype and histological grade (data not shown).

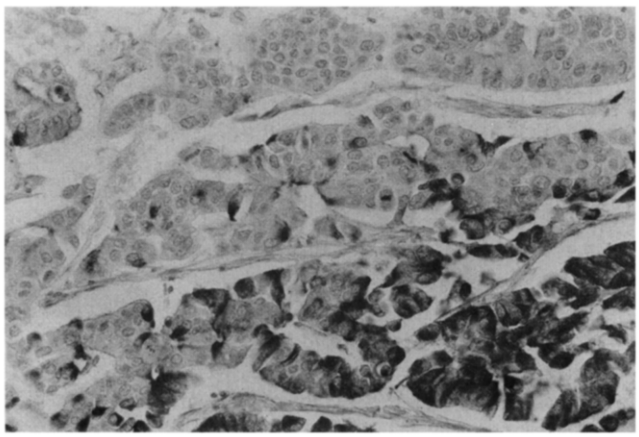

Figure 2. Invasive ductal carcinoma (NOS), demonstrating marked intratumoral heterogeneity in the expression of the TAG-72 epitope, defined by MAb CC49. Immunoperoxidase with haematoxylin counterstaining, original magnification ×400.

DISCUSSION

Altered glycosylation patterns of cell surface glycoconjugates, that result in the expression of novel carbohydrate epitopes, are associated with neoplastic transformation and may serve as antigenic targets for diagnosis, monitoring and therapy of cancer [1–4]. The tumour-associated glycoprotein, TAG-72, one of the most extensively studied TAAs, exposes unmasked core carbohydrate *O*-linked sequences, defined by a series of MAbs that are currently used in the clinico-pathological study of carcinomas [1, 3, 10, 11, 14, 22, 23]. Several immunohistochemical studies, based on MAb B72.3, demonstrated that the TAG-72 molecule is differentially expressed in neoplasms compared with normal tissues [5–7, 9]. More recently, newly generated anti-TAG-72 MAbs defined distinct epitopes of the molecule [24, 25]. In several instances, tumours that appeared TAG-72-positive for the expression of one specific epitope were negative for other anti-TAG-72 MAbs. These findings suggested that heterogeneity in the glycosylation pattern of TAG-72 could result in the differential exposure of distinct epitopes among and within tumours [6, 25].

In the present study, we analysed the expression of three distinct carbohydrate epitopes of the TAG-72 molecule in 92 mammary carcinomas of different histological type, and correlated the results with clinico-pathological characteristics of the tumours, and with their proliferative activity. The expression of each of the three TAG-72 antigenic determinants was evaluated in the different histological components identified in the tumour samples (invasive carcinoma, *in situ* carcinoma, atypical hyperplasia, cystic disease and normal mammary epithelium). Immunopositivity for TAG-72 was not exclusively associated with the neoplastic phenotype, as immunostaining was also

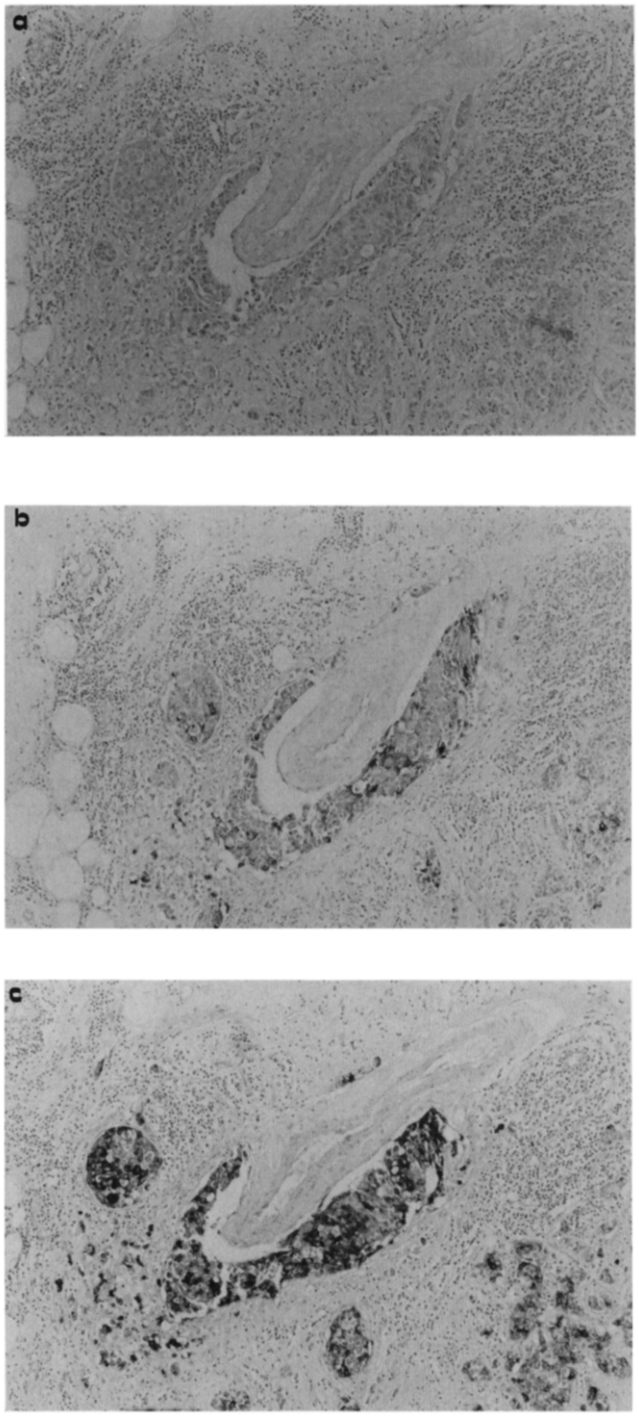

Figure 3. Differential expression of the three TAG-72 epitopes in a mammary carcinoma with perivascular lymphatic node invasion: (a) B72.3, negative; (b) CC49, strong and diffuse positivity of tumour cells; (c) CC83, heterogeneous positivity of tumour cells. Immunoperoxidase with haemotoxylin counterstaining, original magnification ×250.

detected in benign cystic disease and in morphologically normal ductal and lobular epithelium. This further indicates that the exposure of core antigenic structures of mucins may occur independently from malignant transformation [1].

Differences in TAG-72 expression between normal and neoplastic mammary epithelia were considerable in terms of intensity, distribution and pattern of localisation of the immunoreactivity. As observed for other tumour cell membrane glycoproteins [26], the pattern of TAG-72 expression tended to vary according to the level of histomorphological differentiation of the tumours, shifting from predominantly apical distribution in well differentiated neoplasms to diffuse cytoplasmic and plasma membrane localisation in less differentiated tumours. Mucoid areas in mixed mucinous tumours were remarkable for their expression of TAG-72. In these areas, the immunoreactive epitopes were detected in association with the extracellular mucin, while focal or no antigenic reactivity was observed in the cytoplasm or on the plasma membrane. This characteristic might have important implications, since it implies that mucinous carcinomas preferentially release TAG-72 into the extracellular environment. However, therapeutic strategies aimed at eliciting anti-tumour immune responses, based on the exposure of TAG-72 epitopes, could be negatively affected by the abundance of extracellular TAG-72.

The pattern of TAG-72 epitope expression observed in areas of intraductal carcinoma was similar to that of invasive ductal tumours, as opposed to atypical hyperplasia, normal epithelium and cystic disease. This would suggest that quantitative changes in the oligosaccharide composition of TAG-72 take place in the pre-invasive phase of ductal tumours. In contrast, the *in situ* component of lobular carcinomas failed to demonstrate immunopositivity for any of the three TAG-72 epitopes. Further studies on a larger series of pre-invasive ductal and lobular lesions will be necessary to determine whether expression of TAG-72 epitopes may be employed as an additional criterion to differentiate the two histomorphological types of *in situ* mammary cancer [6, 18, 20].

The comparative immunohistochemical analysis of the reactivities of the three anti-TAG-72 MAbs revealed differences in their ability to recognise neoplastic lesions. MAb CC-49 reacted with the highest percentage of tumours (81%), and also tended to yield the highest percentages of immunoreactive tumour cells. B72.3 and CC-83 gave lower percentages of positive tumours, (respectively, 55 and 51%), and tended to yield lower percentages of immunoreactive tumour cells. All the tumours that were immunostained by B72.3 and/or CC-83 were also recognised by CC-49. The results did not vary considerably when the intraductal components were evaluated for their immunostaining with each MAb. Together, these observations confirm that distinct epitopes of TAG-72 may not be simultaneously expressed in mammary tumours, and that the inter- and intratumoral heterogeneity of TAG-72 expression can be significantly reduced by targeting the CC-49 epitope [7, 25]. The complementation of MAb CC-49 with B72.3 and/or CC-83 failed to result in advantages in terms of immunodetected lesions. This was in contrast with results obtained for colorectal carcinomas, where the generally larger tumour size probably emphasised the regional heterogeneity of epitope expression [25].

Based on *in vivo* and *in vitro* studies, it has been proposed that the expression of sialosylated core carbohydrate epitopes of mucins may correlate with poor prognosis and advanced disease, at least in colorectal carcinomas [1, 12, 13, 22, 27–30]. The potential clinical utility of the TAG-72 molecule as a tumour marker has been extensively explored both *in vitro* and *in vivo* [1, 3, 4, 23]. However, few studies attempted to correlate TAG-72 expression with other clinicopathological parameters of breast carcinomas [17]. We obtained statistically significant correlations between a pattern of TAG-72 epitope expression, i.e. the synchronous positivity for B72.3, CC83 and CC49, and both large tumour size ($P = 0.002$) and high histological grade ($P = 0.02$). This finding was consistent with the concept that

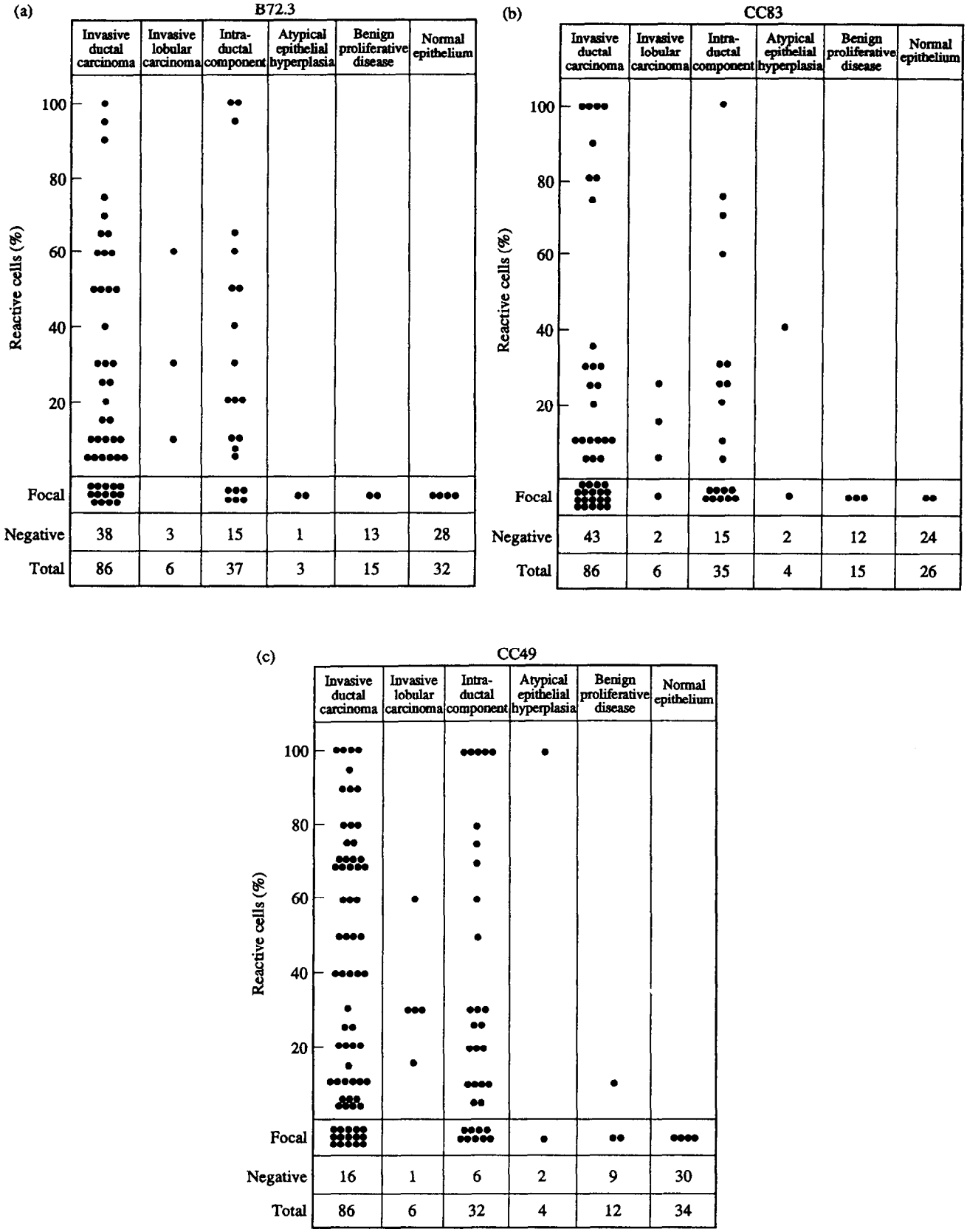

Figure 4. Reactivity of the anti-TAG-72 MAbs B72.3, CC83 and CC49 with the invasive ductal and lobular carcinomas and with the other histological components identified in the biopsy samples studies.

Table 3. Correlation between immunoreactivity for the TAG-72 epitopes B72.3, CC83 and CC49 and pathological characteristics in 92 breast cancers

Characteristics	TAG-72 immunoreactivity a	b	Total	*P*
Tumour size				
≤ 2 cm	13	28	41	
> 2 cm	30	14	44	0.002
Lymph node status				
N−	29	32	61	
N+	17	13	30	0.6
Histological grade				
G1	2	10	12	
G2G3	37	34	71	0.02
Nuclear grade				
G1G2	10	15	25	
G3	28	24	52	0.4
Cell kinetics				
Low	34	34	68	
High	10	10	20	0.8

a: synchronous expression of the B72.3, CC83 and CC49 epitopes(+++); b: negative for the expression of one or more of the three epitopes.

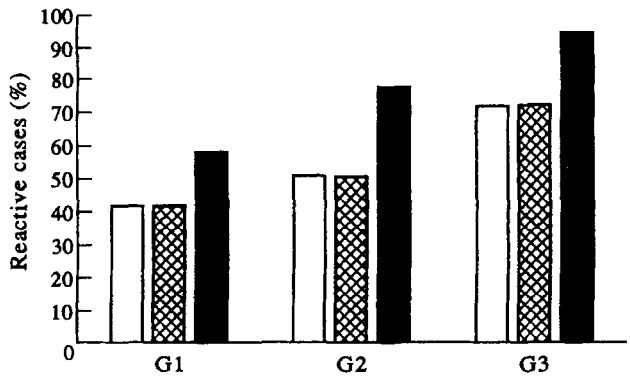

Figure 5. Reactivity of the anti-TAG-72 MAbs B72.3 □, CC83 ▨, and CC49 ■ according to histopathological grading in the invasive ductal carcinomas.

alterations in TAG-72 glycosylation pattern tend to increase in parallel with the level of tumour progression, and suggest that these changes could contribute selective growth advantages to mammary carcinoma cells [1].

Cell kinetics are considered one of the strongest prognostic indicators for breast cancer [15, 16]. In agreement with previous studies, no relationships between TLI (low versus high) and tumour size, histotype and/or histopathological grading could be demonstrated [31, 32]. In this study, no correlation between a pattern of TAG-72 epitope expression and proliferative activity (low TLI versus high TLI) could be shown.

In conclusion, the pattern of expression of TAG-72 epitopes may represent a biological parameter that could contribute to the evaluation of the level of tumour progression and degree of differentiation of breast carcinomas.

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Increased Bone Marrow Toxicity of Doxorubicin Bound to Nanoparticles

S. Gibaud, J. P. Andreux, C. Weingarten, M. Renard and P. Couvreur

The *in vivo* myelosuppressive effects of free and polyalkylcyanoacrylate-bound doxorubicin were compared in a mouse model. After intravenous administration of 11 mg/kg body weight of doxorubicin either free or bound to polyisobutyl (doxo-PIBCA) or polyisohexylcyanoacrylate (doxo-PIHCA) nanoparticles, we studied the total and differential counts of blood, bone marrow and spleen cells; the number of granulocyte progenitors (CFU-GM) was determined by culture. Doxorubicin concentrations were measured with an HPLC method in the bone marrow and the spleen. Doxo-PIHCA nanoparticles showed the highest and longest myelosuppressive effects which correlated well with a high concentration of the drug in the bone marrow and the spleen. Moreover, it was found that PIHCA nanoparticles induced the release of colony stimulating factors, which might account for the observed increase of toxic effects of doxorubicin on bone marrow progenitors. These data also indicate that a more precise evaluation of the myelosuppressive effects of targeted formulations of anticancer drugs is needed, which may be attained by studies on bone marrow progenitors.

Key words: doxorubicin, drug carriers, cyanoacrylates, bone marrow, stem cells
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INTRODUCTION

A MAJOR CONCERN in cancer chemotherapy has been to develop drugs with greater tumour specificity: more effective against the tumour and less toxic towards normal tissues. This may be attained by using new drugs or by targeting known ones. Recently, the potential usefulness of polyalkylcyanoacrylate nanoparticles as anticancer drug carriers has been well demonstrated. These biodegradable colloidal systems have several advantages, especially modifying tissue distribution of bound drugs [1,2]. Indeed, a higher accumulation of drugs in the liver, spleen and lungs has been reported after intravenous administration of anticancer compounds loaded on to these