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Letter to the Editor

Epitopes with Diagnostic and Prognostic Significance Co-expressed on a Human Breast Carcinoma-associated Antigen*

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THE MONOCLONAL antibodies NCRC–11 and 115D8 define epitopes which are of prognostic and diagnostic significance in breast cancer respectively [1, 2].

NCRC–11 (murine IgM), which was prepared against dissociated breast carcinoma cells, is expressed variably in breast cancer, and in an immunocytochemical study of 126 women with primary disease there was a highly significant relationship between the proportion of cells staining with NCRC–11 on paraffin sections of tumours and patient survival (minimum follow-up time, 5 yr or to death) [1]. Staining related weakly to histological grade but not to oestrogen receptor status or pathological lymph node stage, and the relation of staining to survival was independent of other known prognostic indicators.

The monoclonal antibody 115D8 (murine IgG2b) was raised against human milk fat globule (HMFG) membranes [3] and reacts with the glycoprotein antigen, MAM–6 [2]. A sandwich immunoassay has been developed for the detection of MAM–6 in the circulation. The level of MAM–6 in the serum of benign tumour patients was similar to that of healthy persons whereas increased levels were found in 18, 43, 51 and 85% of sera of stage I, II, III and IV breast cancer patients respectively; also, MAM–6 levels correlated with tumour load [2].

The present study was designed to evaluate the relationship between these two antibodies and the epitopes they define in breast cancer tissue. It is known that the target antigens for these antibodies are both large, complex glycoproteins (molecular weight > 400 kD), although NCRC–11 antigen was isolated from breast carcinoma tissue [4] and MAM–6 from human skim milk [2]. A detailed description of the methodologies employed in this investigation has been reported elsewhere [4].

When NCRC–11 and 115D8 were tested against immunoadsorbent purified NCRC–11 antigen, both antibodies reacted positively. As shown in Table 1 using a solid-phase radioisotopic antiglobulin assay with NCRC–11 antigen adsorbed to the wells of Terasaki Microtest Plates (Falcon 3034 Tissue Culture Plates, Becton Dickinson, CA, U.S.A.), the binding of NCRC–11 and 115D8 antibodies was clearly demonstrable with hybridoma ascitic fluids diluted to 10^{-5}.

A sandwich immunoassay was employed to determine whether NCRC–11-defined and 115D8-defined epitopes were co-expressed upon the same molecules in the NCRC–11 antigen preparation. In this test diluted ascitic fluids, and normal mouse serum as control, were adsorbed to the wells of Microtest Plates and, after washing the plates, NCRC–11 antigen or buffer was added. After incubation and washing ^{125}I-labelled NCRC–11 antibody was added (10^5 cpm/well) and, after further incubation and washing, the radioactivity bound to each well was determined. As shown in Table 2, ^{125}I-labelled NCRC–11 antibody bound to wells coated with NCRC–11 and 115D8 antibodies, indicating that epitopes defined by these antibodies are co-expressed upon the same molecules.

In order to determine whether NCRC–11 and 115D8 epitopes are represented by the same determinants or whether they are topographically closely associated on the NCRC–11 breast carcinoma

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Table 1. *Reactivity of monoclonal antibodies NCRC* 11 *and* 115*D*8 *with purified NCRC*-11 *antigen*

Ascitic fluid/ serum dilution	Mean cpm ± S.D. (−background) bound using antibodies:		
	NCRC–11	115D8	NMS*
10^{-3}	6572 ± 140	11226 ± 482	162 ± 51
10^{-4}	6184 ± 144	12231 ± 282	17 ± 33
10^{-5}	1610 ± 88	8715 ± 166	25 ± 16
10^{-6}	179 ± 27	1229 ± 66	3 ± 9

*NMS, normal mouse serum.

antigen, the antibodies were assayed for their capacity to inhibit the binding of ^{125}I-labelled NCRC–11 antibody to NCRC–11 antigen adsorbed to the wells of Microtest Plates. Unlabelled NCRC–11 antibody effectively inhibited the binding of ^{125}I-labelled NCRC–11 antibody to antigen at a dilution of ascitic fluid of 10^{-3} (Table 3). Similar findings were obtained with the anti-HMFG antibody LICR-LON-M8 [5], which is consistent with previous findings [4]. However, 115D8 antibody failed to inhibit the binding of [^{125}I]NCRC–11 antibody to antigen at any of the dilutions of hybridoma ascitic fluid tested (Table 3), even though the antibody at dilutions of 10^{-3} and 10^{-4} is at saturation (Table 1).

The findings indicate that the antibodies NCRC–11 and 115D8 both react with the breast-carcinoma-derived NCRC–11 antigen (Table 1) and that the epitopes they define are co-expressed upon the same molecules (Table 2) but that they are not topographically closely associated (Table 3). The results further illustrate the complexity of

Table 2. *Analysis of epitopes on NCRC*-11 *antigen using a sandwich immunoassay*

Microtest Plate wells coated with ascitic fluids (1/500):	Binding of ^{125}I-labelled NCRC–11 antibody (mean cpm ± S.D.) to ascitic fluid coated wells treated with:	
	Buffer	NCRC–11 antigen
NCRC–11	51 ± 37	7198 ± 760
115D8	150 ± 190	5945 ± 725
NMS*	61 ± 57	143 ± 120

*NMS, normal mouse serum.

Table 3. *Competitive inhibition of binding of* ^{125}I-*labelled NCRC*–11 *antibody to NCRC*–11 *antigen*

Dilution of inhibiting ascitic fluid	Binding of ^{125}I-labelled NCRC–11 antibody (mean cpm ± S.D.) in admixture with:		
	NCRC–11	115D8	LICR-LON-M8
10^{-3}	515 ± 69 (10%)*	5765 ± 702 (106%)	948 ± 81 (20%)
10^{-4}	3079 ± 196 (57%)	6043 ± 455 (111%)	2292 ± 122 (48%)
10^{-5}	4874 ± 405 (90%)	5627 ± 297 (103%)	3940 ± 196 (82%)
Medium alone	5393 ± 92 (100%)	5439 ± 243 (100%)	4786 ± 430 (100%)

*Figures in parentheses represent the percentage binding for ^{125}I-labelled NCRC–11 antibody to NCRC–11 antigen with the antibody in admixture with diluted ascitic fluids, as compared with binding of ^{125}I-labelled antibody diluted in medium alone.

epitope expression upon the NCRC–11 antigen which previously has been shown to express epitopes for other anti-HMFG antibodies [4] such as HMFG–1 and 2 [6] and LICR-LON-M8 [5]. The latter antibodies have not as yet been found to define particularly reliable markers for diagnostic and/or prognostic studies, although they are practically useful in the identification of epithelial and differentiation markers.

It is clear that the NCRC–11 antigen is an important marker in breast cancer and its properties and epitopes may be exploited for diagnosis and prognosis. Parallel studies are now required with the NCRC–11 and 115D8 antibodies to evaluate the additional information that may be obtained by using the two antibodies in conjunction.

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