Pergamon

European Journal of Cancer Vol. 32A, No. 8, pp. 1325–1331, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

PII: S0959-8049(96)00048-2

Original Paper

Humoral Immune Response to Polymorphic Epithelial Mucin (*MUC-1*) in Patients with Benign and Malignant Breast Tumours

S. von Mensdorff-Pouilly,[1] M.M. Gourevitch,[1] P. Kenemans,[1] A.A. Verstraeten,[1] S.V. Litvinov,[2] G.J. van Kamp,[3] S. Meijer,[4] J. Vermorken[5] and J. Hilgers[1]

[1]Departments of Obstetrics and Gynaecology, [3]Clinical Chemistry, [4]Surgery and [5]Medical Oncology, Academic Hospital Vrije Universiteit, De Boelelaan 1117, 1081 HV Amsterdam; and [2]Department of Pathology, University of Leiden, Leiden, The Netherlands

To investigate the clinical significance of an immune response to the MUC-1 encoded polymorphic epithelial mucin (PEM) in breast cancer, circulating immune complexes containing PEM (PEM.CIC) were measured in sera from 96 healthy women, in pretreatment serum samples from 40 patients with benign breast tumours and from 140 patients with breast cancer and in serum samples from 61 breast cancer patients with recurrent or progressive disease. PEM.CIC were measured using a sandwich enzyme-linked immunoassay, and PEM serum levels were measured with CA 15.3 IRMA (Centocor Inc., Malvern, Pennsylvania, U.S.A.). Cut-off levels used for PEM.CIC and CA 15.3 were 120 Optical Density Units (O.D.) $\times 10^3$ and 30 U/ml, respectively. In benign tumours, positivity for PEM.CIC was 37.5% (15/40). 36 of the 140 patients (25.7%) in the breast cancer pretreatment group had elevated PEM.CIC values. In patients with advanced metastatic disease, positivity for PEM.CIC was 18% (11/61). PEM.CIC was elevated in 32% (24/74) of node-negative patients, but only in 20% (12/59) of node-positive patients and absolute values were higher in node-negative patients (Mann–Whitney *U* test, two-tailed $P = 0.0168$). There was an inverse correlation between positivity for PEM.CIC and extent of disease: while 3 of the 6 patients with a carcinoma *in situ* were positive, only 1 of the 15 patients with more than five nodes involved had elevated levels of PEM.CIC. All 7 patients with distant metastases at first diagnosis were PEM.CIC negative. 28 out of 133 patients had a recurrence during the observation period (median 55 months, range 27–84 months). 23 of these 28 patients (82%) were PEM.CIC negative at the moment of first diagnosis. None of the patients with pretreatment elevation of both PEM.CIC and CA 15.3 ($n = 13$) relapsed. Our preliminary clinical results suggest that a humoral immune response to PEM protects against disease progression, and further support the idea of using synthetic peptides or glycopeptides containing the immunogenic core of the mucin as cancer vaccines.

Key words: MUC-1, PEM, auto-antibodies, CA 15.3, breast cancer, prognosis
Eur J Cancer, Vol. 32A, No. 8, pp. 1325–1331, 1996

INTRODUCTION

POLYMORPHIC EPITHELIAL MUCIN (PEM), encoded by the *MUC-1* gene [1, 2], is present at the apical surface of glandular epithelial cells [3]. PEM is a high molecular weight (over 400 kD) transmembrane molecule with a large, highly glycosylated extracellular domain consisting of numerous peptide repeats, varying in number, from 30–90, among the different alleles. These repeats are present as tandemly bound sequences of 20 amino acids enriched by three potential sites for O-linked glycosylation [1, 2, 4–6]. Anti-PEM monoclonal antibodies (MAbs) [7–10] are directed against epitopes on the repeat domain and the majority reacts with a defined cluster of amino acids on the PEM repeat, the PDTRP sequence.

Correspondence to J. Hilgers.
Received 20 Oct. 1995; revised 15 Jan. 1996; accepted 5 Feb. 1996.

This immunodominant region attains its native conformation on synthetic peptides with more than two repeats [11]. The PDTRP region is flanked on both sides by threonines and serines which are O-linked glycosylated in the mature PEM molecule expressed by normal epithelial cells. In cancer cells PEM is overexpressed and deficiently glycosylated [12], the threonines and serines are either not glycosylated or the glycans attached to them are much shorter [13–15]. In consequence, a highly immunogenic molecule with a variable number of exposed immunodominant areas on its peptide core gains access to the circulation in cancer patients.

PEM can be detected in the serum of carcinoma patients by means of various MAb-based assays, such as the CA 15.3 assay [16, 17]. As only very low levels of PEM can be detected in the serum of normal subjects, it is not inconceivable that the enhanced levels present in carcinoma patients lead to a humoral immune response. These PEM-reactive auto-antibodies would bind to the circulating antigen and immune complexes would result.

Evidence exists to support the idea of a humoral immune response to PEM. Circulating antibodies against a peptide epitope on this molecule have been detected in ulcerative colitis [18]. Rughetti and associates [19] have demonstrated the existence of a B-cell immune response to PEM. Human antibodies produced by immortalised B-cells obtained from tumour draining negative lymph nodes of an ovarian cancer patient were shown to react with the PEM protein core and with PEM-expressing tumour cells [20]. Kotera and colleagues [21] detected anti-mucin antibodies of the IgM isotype in sera from breast, colon and pancreas cancer patients and Croce and colleagues [22] identified MUC-1 immune complexes by Western blotting tests in sera from breast cancer patients.

Our group reported the presence of PEM-containing immune complexes (PEM.CIC) in the serum of adenocarcinoma patients [23]. The present study investigates the incidence of PEM.CIC in benign breast tumour and breast cancer patients, as well as its possible clinical significance for the diagnosis, prognosis and treatment of breast cancer.

PATIENTS AND METHODS

Controls, patients and serum samples

A total of 337 serum samples were obtained from 40 patients with benign breast tumours (median age 46.5 years, range 22–83 years) and 140 breast cancer patients (median age 57.5 years, range 31–88 years) before primary treatment, and from 61 breast cancer patients (median age 57 years, range 33–79 years) with recurrent or progressive disease, treated in the Academic Hospital Vrije Universiteit, Amsterdam, between 1987 and 1992. Patients with a past or concomitant history of malignancy were excluded from the study. Sera obtained from 96 apparently healthy women (median age 45 years, range 39–72 years) were used as controls. Serum samples were collected, aliquoted and stored at −70°C until analysed.

The benign breast tumour group consisted of 10 patients with fibroadenomas and 30 with fibroadenosis.

Breast cancer patients sampled in the course of disease included 18 patients with metastases at multiple sites and 18 patients with bone, 13 with locoregional, 7 with lung, 3 with brain and 2 with liver metastases at the moment of sampling.

Table 1 describes the characteristics of the node-negative and node-positive breast cancer pretreatment group (6 carcinoma *in situ*, 32 Stage I, 79 Stage II and 16 Stage III patients). 7 patients had distant metastatic disease at diagnosis. 5 of these Stage IV patients died during the observation period, 4 of them in the course of the first follow-up year, 1 of these 4 at a very early stage in the course of treatment from a cardiotoxic reaction to doxorubicin.

Disease-free survival (DFS) was defined as the time elapsed between primary surgery and the first confirmed metastasis or recurrent growth. The duration of follow-up ranged from 3 to 84 months (median 48 months). The median duration of follow-up at the time of current analysis for disease-free survivors is 55 months with a range of 27 to 84 months (Table 1).

Survival was defined as the time elapsed between primary treatment and death from breast cancer. Follow-up duration ranged from 0.3 to 84 months. The median follow-up period of survivors was 54 months (range 27–84 months).

Assay description

The assay for the detection of circulating PEM-immune complexes (PEM.CIC) has been developed in our laboratory and has been described previously [23]. The PEM.CIC assay is an enzyme-linked immunoassay using the anti-PEM MAb 139H2, directed to the peptide core of the molecule [24], as catcher and a horseradish peroxidase-conjugated anti-human immunoglobulin antibody as tracer. An upper level of normal of 120 Optical Density Units (O.D.) $\times 10^3$, including 97.5% of the healthy population (Table 2) was used.

CA 15.3 IRMA is a commercial heterologous double determinant radioimmunoassay for the quantification of PEM in serum (Centocor, Malvern, Pennsylvania, U.S.A.), that uses the monoclonal antibody 115D8 [25], raised against human milk fat globules (HMFG) as catcher, and the MAb DF3 [8, 26], raised against a membrane enriched fraction of a human breast carcinoma, labelled with 125-I as tracer. A cut-off level of 30 U/ml was used.

All measurements were performed in duplicate and without knowledge of the corresponding clinical data.

Statistical methods

Statistical analysis was performed using the SPSS Advanced Statistics 6.1 software package (SPSS Inc., Chicago, Illinois, U.S.A.). The diagnostic value (sensitivity, specificity, positive predictive value, negative predictive value and accuracy) of the test results was calculated according to Büttner [27]. CA 15.3 levels and PEM.CIC assay results in the different groups were analysed using the Mann–Whitney *U*-Wilcoxon Rank Sum *W* Test. The impact of the different variables in the prediction of events was analysed by logistic regression. Multivariate analysis included as potential predictors the following features: stage, nodal involvement, menopausal status, CA 15.3 and PEM.CIC. Disease-free survival and survival were computed using the Kaplan–Meier technique, and univariate comparisons between subgroups were made using a two-tailed log-rank test.

RESULTS

PEM.CIC and CA 15.3 results for healthy controls, benign breast tumour and breast cancer patients are shown in Table 2. PEM.CIC was elevated in 37.5% of patients with benign breast tumours. In breast cancer, the number of patients with values above the cut-off level was higher in the pretreatment group (25.7%) than in the group of patients with advanced metastatic disease after primary treatment (18%).

Table 1. Clinical characteristics of the breast cancer patients in the pretreatment group

	Node − ve $n = 74$	Node + ve $n = 59$	Total $n = 133$
Age in years (mean) (S.D.)	60 (12)	57 (13)	58 (13)
Premenopausal	20	27	47
Postmenopausal	54	32	86
Primary treatment			
Breast-conserving surgery	35	20	55
Modified radical mastectomy	39	39	78
Irradiation	41	57	98
Adjuvant systemic therapy			
chemotherapy	1	23	24
tamoxifen	9	31	40
Follow-up time in months, median (range)	51 (27–84)	56 (28–83)	55 (27–84)
Number of recurrences	15	13	28
Site of recurrence			
Local	3	3	6
Lung	2	2	4
Pleura	1	1	2
Bone	2	1	3
Liver	—	2	2
Brain	—	1	1
Multiple sites	7	3	10
Deaths from breast cancer	9	11	20

Table 2. PEM.CIC and CA 15.3 serum levels in healthy controls and in benign and malignant breast tumour patients

	Healthy controls	Benign breast tumours	Breast cancer pretreatment	Breast cancer high tumour load
n	96	40	140	61
PEM.CIC >120*	2	15	36	11
%	2.1	37.5	25.7	18
Mean	17.8	96	90	65
Median	13.5	99.5	64	57
S.D.	37.7	67.2	93	82.3
Two-tailed *P*†		<0.0001	<0.0001	<0.0001
CA 15.3 >30 U/ml	12	2	54	41
%	12.5	4.2	38.6	67.2
Mean	18.5	18	40	263
Median	18	17.5	25.5	43
S.D.	8.2	6.8	92	471

*PEM.CIC is expressed in O.D. × 10^3. †Mann–Whitney *U* test: breast tumour patients/healthy controls.

Diagnostic value

Evaluation of the diagnostic value of PEM.CIC and CA 15.3, individually and in combination, is shown in Table 3. A concomitant elevation of PEM.CIC and CA 15.3 had a high specificity (99%) for breast cancer, but sensitivity was very low (9%).

Relation to extent of disease

PEM.CIC was positive in 24/74 (32.4%) of breast cancer patients with no nodal involvement, but only in 12/59 (20.3%) of patients with nodal involvement. All 7 patients with distant metastases at diagnosis had PEM.CIC values under the cut-off level. PEM.CIC absolute values were significantly higher in node-negative patients in comparison with node-positive ($P = 0.0168$) and Stage IV patients ($P = 0.0259$; Table 4). CA 15.3 values are shown in Table 4. CA 15.3 was elevated in 6/7 Stage IV patients.

Whilst 3 of the 6 pTisN0M0 patients had elevated pretreatment PEM.CIC levels, positivity for PEM.CIC in pretreatment serum decreased steadily with increasing stage of disease and was only 6.7% in the 15 patients with more than five positive nodes. Comparison of absolute PEM.CIC values among extremes showed significant differences (Table 5).

Disease-free survival (DFS)

Logistic regression analysis showed stage to be the only independent variable ($P < 0.0001$) for prediction of recurrence in the study population. DFS probability was higher for pT1 in comparison to pT2 node-negative patients ($P = 0.0381$) and for Stage I in relation to Stage II and Stage III breast cancer patients. These latter differences were not significant ($P = 0.0897$ and $P = 0.1003$, respectively). No difference in DFS was found between node-negative and node-positive patients during the observation period ($P = 0.8793$).

Table 3. Diagnostic value of CA 15.3 and PEM.CIC in breast cancer

Healthy controls	$n = 96$
Benign breast tumours	$n = 40$
Breast cancer pretreatment	$n = 140$

%	CA 15.3*	PEM.CIC†	CA 15.3 and PEM.CIC	CA 15.3 and/or PEM.CIC
Sensitivity‡	39	26	9	55
Specificity§	90	88	99	79
Positive predictive value‖	79	68	88	73
Negative predictive value¶	59	53	51	63
Accuracy**	64	56	53	67

Cut-off: *CA 15.3 = 30 U/ml. †PEM.CIC = 120 O.D. × 10^3. ‡Percentage of patients with breast cancer with elevated test results. §Percentage of subjects with no breast cancer with non-elevated test results. ‖Probability that a positive test result will truly be associated with breast cancer. ¶Probability that a negative test result will truly be associated with absence of breast cancer. **Proportion of the total test results that are correctly positive or negative.

Table 4. Pretreatment CA 15.3 and PEM.CIC serum levels in breast cancer patients (n = 140)

	Node –ve $n = 74$	Node + ve $n = 59$	Metastatic disease $n = 7$
CA 15.3 > 30 U/ml	24 (32%)	24 (41%)	6 (86%)
Mean ± S.D.	27.2 ± 11.8	28.9 ± 14.9	227.4 ± 385
Median	25	24	114
PEM.CIC > 120*	24 (32%)	12 (20%)	0 (0%)
Mean ± S.D.	105.3 ± 94.1	76.3 ± 92.4	38 ± 33.6
Median	87	48	37

Mann–Whitney *U* test on PEM.CIC levels, two-tailed *P* nodal – ve/nodal + ve 0.0168, nodal – ve/metastatic disease 0.0259, nodal + ve/metastatic disease n.s.
*PEM.CIC is expressed in O.D. × 10^3.

Table 5. PEM.CIC pretreatment serum levels in breast cancer patients in relation to extent of disease at first diagnosis

		Tumour size	PEM.CIC > 120*	%	Mann-Whitney *U* test two-tailed *P*
node – ve $n = 74$	a	pTis	3/6	50	a/h 0.0194
	b	pT1	10/32	31	b/h 0.0062
	c	pT2	11/33	33	c/h 0.0158
	d	pT3	0/1	—	n.s.
	e	pT4	0/2	—	n.s.
	no. of + ve nodes				
node + ve $n = 59$	f	1 to 3	8/33	24	n.s.
	g	4 to 5	3/11	27	g/h 0.0355
	h	>5	1/15	6.7	

*PEM.CIC is expressed in O.D. × 10^3.

Postmenopausal patients had a lower probability of recurrence than premenopausal patients ($P = 0.0569$). No relation was found between DFS and CA 15.3 values above or below cut-off level.

Mean DFS was 74 months (95% confidence interval 67–81 months) for node-negative and node-positive patients with PEM.CIC above cut-off level ($n = 36$) and 69 months (95% confidence interval 63–74 months) for those with PEM.CIC below cut-off level ($n = 97$), but these differences were not significant ($P = 0.2166$). 23 of the 28 patients with recurrences were PEM.CIC negative (82%), 12/15 in the nodal negative group and 11/13 in the nodal positive group (Table 6). None of the 13 patients with simultaneous elevation of CA 15.3 and PEM.CIC relapsed during the observation period. All tumour stages were present in this group of 13 patients (1 carcinoma *in situ*, 4 Stage I, 6 Stage II and 2 Stage III). Difference in DFS approached significance when comparing the group of patients with simultaneous elevation of CA 15.3 and PEM.CIC, to patients with low pretreatment levels of both PEM.CIC and CA 15.3 ($P = 0.0586$) and to patients with

Table 6. CA 15.3 and PEM.CIC pretreatment serum levels in breast cancer patients in relation to number of recurrences and death from breast cancer

	Node – ve		Node + ve		Total	
	rec.	death	rec.	death	rec.	death
PEM.CIC neg.	12/50	8	11/47	9	23/97	17
CA 15.3 neg./PEM.CIC neg.	10/34	7	5/28	4	15/62	11
CA 15.3 pos./PEM.CIC neg.	2/16	1	6/19	5	8/35	6
PEM/CIC pos.	3/24	1	2/12	2	5/36	3
CA 15.3 neg./PEM.CIC pos.	3/16	1	2/7	2	5/23	3
CA 15.3 pos./PEM.CIC pos.	0/8	0	0/5	0	0/13	0

Cut-off: CA 15.3 = 30 U/ml. PEM.CIC = 120 O.D. x 10^3.

elevated CA 15.3 levels and low PEM.CIC ($P = 0.0758$). DFS in this group of 13 patients in relation to all other 120 patients is shown in Figure 1.

Survival

Survival analysis in relation to stage showed, as expected, a significantly poor outcome for Stage IV patients ($P < 0.0001$) in comparison to each of the other stages. Patients with Stage I had a significantly better survival than those with Stage II ($P = 0.0414$), and probability of survival was significantly higher for pT1 node-negative patients in comparison to pT2 node-negative patients ($P = 0.0403$). There was no significant difference in survival between node-negative and node-positive patients during the observation period. Menopausal status was not significantly related to survival and no relationship was found between CA 15.3 values and survival.

All 7 patients with metastatic disease at diagnosis were PEM.CIC negative, and 5 of them died during the observation period. Probability of survival in the total population in relation to PEM.CIC was, though not significant, higher for PEM.CIC positive patients ($P = 0.0865$). In the group of patients with elevated CA 15.3, survival analysis showed a significant difference for the group of patients with concomitant elevation of PEM.CIC (Figure 2).

DISCUSSION

A successful immune surveillance on the part of the host could be one of the reasons for the variable extent of tumour dissemination found at diagnosis among patients. Tumour–host interactions could also explain differences in disease outcome among patients with, to all intents, the same lesions at diagnosis [28]. In the present study, we found evidence for a natural humoral immune response to PEM in breast cancer patients that was inversely related to extent of disease at diagnosis. It is not possible to rule out that the decrease of circulating antibody in patients with a high tumour load is due to antibody trapping within the tumour tissue. However, the presence of high serum levels of immune complexes in patients with early stage disease is probably indicative of a restraining influence of this immune reaction on tumour growth and dissemination.

In breast cancer, natural immunity against PEM may have a restrictive effect on disease progression through one or more mechanisms. Studies show that MUC-1 expression reduces intercellular adhesion and participates in epithelial sheet differentiation and lumen formation during organogenesis [29]. MUC-1 overexpression strongly decreases cell–cell and cell–

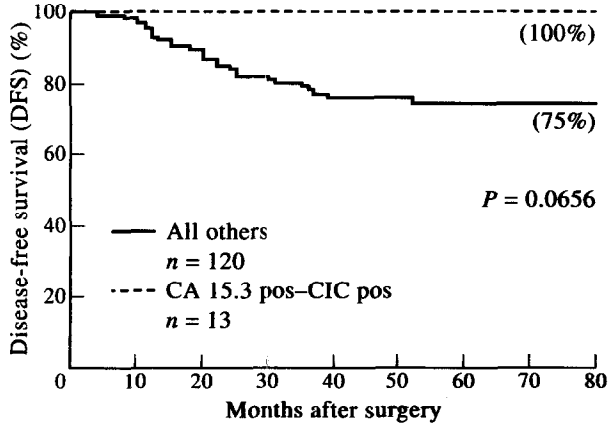

Figure 1. Disease-free survival (DFS) related to pretreatment CA 15.3 and PEM.CIC serum levels in node – ve and node + ve breast cancer patients ($n = 133$). No recurrences were observed in the group of patients with concomitant elevation of CA 15.3 and PEM.CIC.

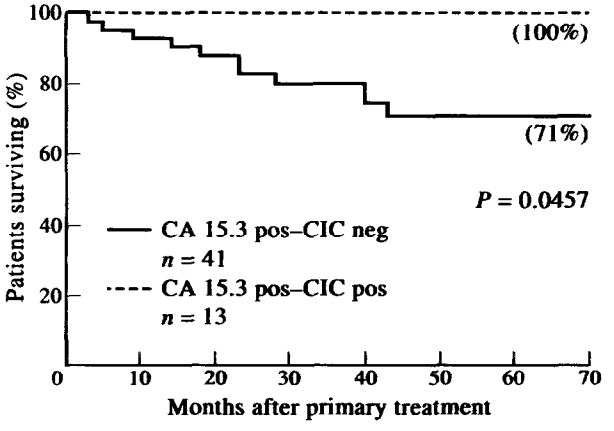

Figure 2. Survival in breast cancer patients with elevated pretreatment CA 15.3 levels in relation to pretreatment PEM.CIC serum levels.

matrix interactions [30]. The extracellular component of the PEM molecule extends high above all other cell surface molecules and may reach a length of several hundred nanometers. As is the case with MAbs to the PEM repeat domain [31], immune complexes fixed on the cell surface could lead to a capping of PEM, thus uncovering adhesion molecules masked by it. As a consequence, cell adhesion would be restored, limiting cancer invasion. MUC-1 transfected cells are less susceptible to lysis by cytotoxic effector cells [32], a redistribution of PEM could also be instrumental in unmasking cell surface antigens involved in immune recognition processes. Other possible mechanisms involved in tumour destruction could be antibody-dependent cellular cytotoxicity against cancer cells or complement-dependent cytolysis of these cells.

Furthermore, several recent studies seem to indicate that humoral immunity reflects the presence of cellular immunity. Induction of an immune network cascade in cancer patients, with development of anti-anti-idiotypic antibodies and cellular immunity after MAb therapy with HMFG1 [33, 34], with OC 125 [35] and with 17-1A and its anti-idiotype [36, 37], led to a prolonged DFS. Adjuvant therapy with 17-1A extended life and prolonged remission in Dukes' stage C colorectal cancer patients [38]. Immune complexes could be taken up and presented by dendritic cells and thus initiate T cell responses [39, 40]. Cytotoxic T cells from patients with breast, pancreas and ovarian carcinomas recognise PEM core peptides and mediate lysis of tumour targets *in vitro* [41–44]. Immunisation of mice with a vaccinia virus construct carrying the cDNA for the MUC-1 antigen produced a moderate MUC-1 specific immune response and protected 30% of the treated mice against growth of murine tumours expressing MUC-1 [45].

While stage and tumour size in node-negative patients were determinant variables for disease outcome, the lack of impact of nodal involvement in outcome of disease in this study population may be due to its limited size and the relatively short follow-up time for some of the patients in each group, showing the effect of aggressive tumours, metastasising early, on DFS evaluation. This effect is later lost in prolonged follow-ups. As nodal status is one of the strongest prognostic indicators in breast cancer, this study will be repeated in a larger breast cancer population. Nevertheless, PEM.CIC positive patients had a better, albeit not significant, outcome than PEM.CIC negative patients in all subgroups considered. The fact that the 5 patients positive for PEM.CIC with a recurrence during the observation period were CA 15.3 negative and that none of the patients with elevation of both PEM.CIC and CA 15.3 relapsed, suggests that sustained elevated levels of CA 15.3 would be necessary to provoke a prolonged specific immune response. This natural immune response does not happen in all patients: 6 of the 23 patients that relapsed and 6 of 7 patients with distant metastatic disease at diagnosis all had elevated levels of CA 15.3 but were PEM.CIC negative. Survival in the group of patients with elevated CA 15.3 was significantly higher for those patients with elevated levels of PEM.CIC. The molecular characteristics of the circulating PEM glycoforms, with variable exposure of the immunodominant peptide epitopes according to the presence of shorter or longer glycans, associated to the genetic polymorphism in the number of repeats, could be a decisive factor in provoking a prolonged immune response.

Altered PEM glycoforms could access the circulation during pregnancy and lactation and also in benign breast diseases and unleash an immune response. Circulating free antibodies to PEM are present not only in sera from breast cancer patients but also in healthy women and benign breast tumour patients (Gourevitch and associates, unpublished observations). Agrawal and associates [46] demonstrated the presence of T cells specifically proliferating in response to PEM in multiparous, but not in nulliparous women. Furthermore, the formation of immune complexes could impair CA 15.3 measurements [23], and the presence of PEM.CIC could be considered as indirect evidence of circulating mucin, even in patients with normal CA 15.3 levels.

Thus, a natural humoral immune response to PEM seems to protect against disease progression, while lack of immune reaction, or immune tolerance developed in the course of disease, could be an additional risk factor more frequently associated with an unfavourable outcome. Our preliminary clinical results, in addition to the growing evidence of an immune response to tumours, further support the idea of the use of mucins or, better still, synthetic peptides or glycopeptide derivatives containing the immunogenic core of the molecule as a vaccine for cancer therapy. Furthermore, the PEM.CIC assay provides a tool for vaccine therapy monitoring. Characterisation of the PEM epitopes involved in this immune response may define antibody subclasses with higher diagnostic and prognostic value and provide information on the best material for vaccine therapy.

1. Gendler S, Lancaster C, Taylor-Papadimitriou J, *et al.* Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 1990, **265**, 15286–15293.
2. Ligtenberg MJL, Vos B, Gennissen A, Hilkens J. Episialin, a carcinoma-associated mucin, is generated by a polymorphic gene encoding splice variants with alternative amino termini. *J Biol Chem* 1990, **265**, 5573–5578.
3. Zotter Z, Hageman PC, Lossnitzer A, Mooi WJ, Hilgers J. Tissue and tumor distribution of human polymorphic epithelial mucin. *Cancer Rev* 1988, **11–12**, 55–101.
4. Price MR, Hudezc F, O'Sullivan C, Baldwin RW, Edwards PM, Tendler SJB. Immunological and structural features of the protein core of human polymorphic epithelial mucin. *Mol Immunol* 1990, **27**, 795–802.
5. Nishimori I, Perini F, Mountjoy KP. *N*-Acetylgalatosamine glycosylation of MUC1 tandem repeat peptides by pancreatic tumor cell extracts. *Cancer Res* 1994, **54**, 3738–3744.
6. Stadie TRE, Chai W, Lawson AM, Byfield PGH, Hanisch FG. Studies on the order and site specificity of GalNAc transfer to MUC1 tandem repeats by UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferase from milk or mammary carcinoma cells. *Eur J Biochem* 1995, **229**, 140–147.
7. Taylor-Papadimitriou J, Peterson J, Arklie J, Burchell J, Ceriani RL, Bodmer WF. Monoclonal antibodies to epithelium specific components of the human milk fat globule membrane: production and reaction with cells in culture. *Int J Cancer* 1981, **28**, 17–21.
8. Kufe D, Inghirami G, Abe M, Hayes D, Just-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma* 1984, **3**, 223–231.
9. Hilkens J, Buijs F, Hilgers J, *et al.* Monoclonal antibodies prepared against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. *Int J Cancer* 1984, **34**, 197–206.
10. Taylor-Papadimitriou J. Report on the First International Workshop on Carcinoma-Associated Mucin. *Int J Cancer* 1991, **49**, 1–5.
11. Fontenot DJ, Tjandra N, Bu D, Ho C, Montelarco RC, Finn OJ. Biophysical characterization of one-, two-, and three-tandem repeats of human mucine (muc-1) protein core. *Cancer Res* 1993, **53**, 5386–5394.
12. Burchell J, Taylor-Papadimitriou J. Effect of modification of

carbohydrate side chains on the reactivity of antibodies with core-protein epitopes of the MUC1 gene product. *Epith Cell Biol* 1993, **2**, 155–162.
13. Burchell J, Gendler S, Taylor-Papadimitriou J, *et al.* Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. *Cancer Res* 1987, **47**, 5476–5482.
14. Devine PI, Warren JA, Ward BG, McKenzie IFC, Layton GT. Glycosylation and the exposure of tumor-associated epitopes on mucins. *J Tumor Marker Oncol* 1990, **5**, 11–26.
15. Litvinov S, Hilkens J. The epithelial sialomucin, episialin, is sialylated during recycling. *J Biol Chem* 1993, **268**, 21364–21371.
16. Hayes DF, Zurawski VR, Kufe DW. Comparison of circulating CA 15.3 and carcinoembryonic antigen levels in patients with breast cancer. *J Clin Oncol* 1986, **4**, 1542–1550.
17. Bon GG, Kenemans P, van Kamp GJ, Yedema CA, Hilgers J. Review on the clinical value of polymorphic epithelial mucin tumor markers for the management of carcinoma patients. *J Nucl Med Allied Sci* 1990, **34**, 151–162.
18. Hinoda Y, Nakagawa N, Nakamura H, *et al.* Detection of a circulating antibody against a peptide epitope on a mucin core protein, MUC1, in ulcerative colitis. *Immunol Letters* 1993, **35**, 163–168.
19. Rughetti A, Turchi V, Ghetti CA, *et al.* Human B-cell immune responses to the polymorphic epithelial mucin. *Cancer Res* 1993, **53**, 2457–2459.
20. Nuti M, Rughetti A, Turchi V, Apolloni-Ghetti C, Scambia G, Frati L. Human B cell immune response to selected epitopes of the polymorphic epithelial mucin (PEM) in cancer patients. *In vivo* 1993, **7**, 645–648.
21. Kotera Y, Fontenot DJ, Pecher G, Metzgar RS, Finn OJ. Humoral immunity against a tandem repeat epitope of human mucin MUC-1 in sera from breast, pancreatic, and colon cancer patients. *Cancer Res* 1994, **54**, 2856–2860.
22. Croce MV, Price MR, Segal-Eiras A. Expression of monoclonal-antibody-defined antigens in fractions isolated from human breast carcinomas and patients' serum. *Cancer Immunol Immunother* 1995, **40**, 132–137.
23. Gourevitch MM, von Mensdorff-Pouilly S, Litvinov SV, *et al.* Polymorphic epithelial mucin (MUC-1)-containing circulating immune complexes in carcinoma patients. *Br J Cancer* 1995, **72**, 934–938.
24. Hilkens J, Buijs F, Ligtenberg M. Complexity of MAM-6, an epithelial sialo-mucin associated with carcinomas. *Cancer Res* 1989, **49**, 786–793.
25. Hilkens J, Kroezen V, Bonfrer JMG, De Jong-Bakker M, Bruning PF. MAM-6, a new serum marker for breast cancer monitoring. *Cancer Res* 1986, **46**, 2582–2587.
26. Hayes DF, Sekine H, Ohno T, Abe M, Keefe K, Kufe DW. Use of a murine monoclonal antibody for detection of circulating plasma DF3 antigen levels in breast cancer patients. *J Clin Invest* 1985, **75**, 1671–1678.
27. Büttner J. Die Beurteilung des diagnostischen Wertes klinisch-chemischer Untersuchungen. *J Clin Chem Clin Biochem* 1977, **15**, 1–12.
28. Devitt JE. Breast cancer: Have we missed the forest because of the tree? *Lancet* 1994, **344**, 734–735.
29. Braga VMM, Pemberton LF, Duhig T, Gendler SJ. Spatial and temporal expression of an epithelial mucin, Muc-1, during mouse development. *Development* 1992, **115**, 427–437.
30. Hilkens J, Ligtenberg JL, Vos HL, Litvinov S. Cell membrane-associated mucins and their adhesion-modulating property. *Trends Biochem Sci* 1992, **17**, 359–363.
31. Wesseling J, Van der Valk SW, Vos HL, Sonnenberg A, Hilkens J. Episialin (MUC1) overexpression inhibits integrin-mediated cell adhesion to extracellular matrix components. *J Cell Biol* 1995, **129**, 255–265.
32. Van de Wiel-van Kemenade E, Ligtenberg MJL, De Boer AJ, *et al.* Episialin (MUC1) inhibits cytotoxic lymphocyte–target cell interaction. *J Immunol* 1993, **151**, 767–776.
33. Courtenay-Luck NS, Epenetos AA, Sivalopenko GB, Larche M, Barkans JR, Ritter MA. Development of anti-idiotypic antibodies against tumour antigens and autoantigens in ovarian cancer patients treated intraperitoneally with mouse monoclonal antibodies. *Lancet* 1988, **ii**, 894–897.
34. Kosmas C, Epenetos AA, Courtenay-Luck NS. Activation of cellular immunity after intracavitary monoclonal antibody therapy of ovarian cancer. *Cancer* 1994, **73**, 3000–3010.
35. Wagner U, Chronides A, Mallmann P, *et al.* Induktion einer tumorspezifischen zellulären Immunität durch Idiotypen-Vakzination für das Ovarialkarzinom mit dem MAB OC 125. *Tumordiagn u Ther* 1993, **14**, 125–131.
36. Fagerberg J, Hjelm AL, Ragnhammar P, Frödin JE, Wigzell H, Mellstedt H. Tumor regression in monoclonal antibody-treated patients correlates with the presence of anti-idiotype-reactive T lymphocytes. *Cancer Res* 1995, **55**, 1824–1827.
37. Fagerberg J, Steinitz M, Wigzell H, Askelöf P, Mellstedt H. Human anti-idiotypic antibodies induced a humoral and cellular immune response against a colorectal carcinoma-associated antigen in patients. *Proc Natl Acad Sci USA* 1995, **92**, 4773–4777.
38. Riethmüller G, Schneider-Gädicke E, Schlimok G, *et al.* Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes'C colorectal carcinoma. *Lancet* 1994, **343**, 1177–1183.
39. Melief CJM. Dendritic cells as specialized antigen presenting cells. *Res Immunol* 1989, **140**, 902–926.
40. Caux Ch, Liu YJ, Banchereau J. Recent advances in the study of dendritic cells and folicular dendritic cells. *Immunol Today* 1995, **16**, 2–4.
41. Barnd DL, Lan MS, Metzgar RS, Finn OJ. Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic T cells. *Proc Natl Acad Sci USA* 1989, **86**, 7159–7163.
42. Jerome KR, Barnd DL, Bendt KM, *et al.* Cytotoxic T-lymphocytes derived from patients with breast adenocarcinoma recognize an epitope present on the protein core of a mucin molecule preferentially expressed by malignant cells. *Cancer Res* 1991, **51**, 2908–2916.
43. Jerome KR, Domenech N, Finn OJ. Tumor-specific cytotoxic T cell clones from patients with breast and pancreatic adenocarcinoma recognize EBV-immortalized B cells transfected with polymorphic epithelial mucin complementary DNA. *J Immunol* 1993, **151**, 1654–1662.
44. Ionnanides CG, Fisk B, Jerome KR, Irimura T, Wharton JT, Finn OJ. Cytotoxic T cells from ovarian malignant tumors can recognize polymorphic epithelial mucin core peptides. *J Immunol* 1993, **151**, 3693–3703.
45. Acres RB, Hareveuni M, Balloul J-M, Kieny M-P. Vaccinia virus MUC1 immunization of mice: immune response and protection against the growth of murine tumors bearing the MUC1 antigen. *J Immunother* 1993, **14**, 136–143.
46. Agrawal B, Reddish MA, Krantz MJ, Longenecker BM. Does pregnancy immunize against breast cancer? *Cancer Res* 1995, **55**, 2257–2261.

Acknowledgements—This work was supported by a grant from the Biocare Foundation (grant no. 94–23), a European Communities concerted Action (contract no. BMH1-CT94-1462) and grants from Centocor Inc. (Malvern, Pennsylvania, U.S.A.) and Abbott (Abbott Park, Illinois, U.S.A.). Monoclonal antibody (MAb) 139H2 was a gift from Centocor Inc. (Malvern, Pennsylvania, U.S.A.). We thank Astrid Kok for expert technical assistance with the CA 15.3 determinations.