Immunoelectrophoretic analysis* of mouse fetal antigens obtained by DEAE-sephadex A-25 chromatography

Fraction	Elution (M NaCl) from DEAE-Sephadex A-25	Mobility**	Anti-AFP	Anti-transferrin	Anti-albumin	Anti-fetal mouse serum	Anti-adult mouse serum
1	0	nd***	-	-	-	-	-
2	0	0.33	-	-	-	(1)β	(1)β
3	0.02	0.44	-	(1)β	-	(1)β	(1)β
4	0.05	0.50	-	(1)β	-	(1)β	(1)β
5	0.07	0.73	(1)α	-	-	(1)α	-
6	0.12	nd	-	-	-	-	-
7	0.15	0.78	(1)α	-	-	(1)α	-
8	0.18	0.78 and 1.00	(1)α	-	(1)alb	(2)α,alb	(1)alb
9	0.20	0.80 and 1.00	(1)α	-	(1)alb	(2)α,alb	(1)alb
Albumin	-	1.00	-	-	(1)alb	(1)alb	(1)alb

* The number of precipitin arcs resolved is given in parenthesis; the mobility of the antigen in agarose is appended thereto. ** Electrophoretic mobilities in 7% acrylamide gel, pH 9.0, relative to mouse albumin; when 2 bands were observed, the mobilities of each are given. *** Not detected in the gel.

AFP by immunoelectrophoresis. Ouchterlony analysis of individual day 14–19 fetal mouse extracts with each of these absorbed AFP-specific antisera yielded a single precipitin line of identity in all cases. Staining of the precipitin lines with amidoschwarz[10] also failed to disclose spur formation. Although chromatographically heterogeneous, each AFP species appeared antigenically indistinguishable. Murine AFP is known to exhibit considerable heterogeneity on ion-exchange and electrophoretic support media; AFP produced late in gestation is immunologically similar to AFP made earlier in development[11]. The fetal extracts used here would be expected to contain the 'immature'[4], less sialylated, forms of AFP in addition to the maximally sialylated protein and thus may contribute to the heterogeneity observed. Should post-translational addition of sialyl groups mask potentially unique antigenic determinants, the use of extracts containing just the maximally sialylated protein (day 18 fetal mouse plasma contains only the fully sialylated AFP[3]) as the test antigen may allow only for recognition of those antibody combining sites common to each of the several sialylated variants of AFP. Antigenic differences among fetal mouse AFP's could not be demonstrated, however, in extracts of fetuses as early as day 14 of gestation. This apparent conservation of antigenicity among heterogeneous AFP's contrasts with the well established existence of antigenically distinct variants or 'isoantigens' of carcinoembryonic antigen[12-14].

1 This work was supported in part by Public Health Service Grant CA08748 from the National Cancer Institute. The author expresses his thanks to Dr A. Bendich for critical readings of this manuscript.
2 D.L. Gustine and E.F. Zimmerman, Am. J. Obstetr. Gynec. *114*, 553 (1972).
3 D.L. Gustine and E.F. Zimmerman, Biochem. J. *132*, 541 (1973).
4 J.R. Wilson and E.F. Zimmerman, Dev. Biol. *54*, 187 (1976).
5 E.F. Zimmerman and M.M. Madappally, Biochem. J. *134*, 807 (1973).
6 E.H. Stonehill, Proc. 1st Symp. Serodiagnosis of Cancer, 1973. Armed Forces Radiobiol. Res. Inst. Publ. SP74-1, p. 19, 1974.
7 P. Gold, A. Labitan, H.C.G. Wong, S.O. Freedman, J. Krupey and J. Shuster, Cancer Res. *38*, 6 (1978).
8 C.F. Matson, Analyt. Biochem. *13*, 294 (1965).
9 P.J. Higgins, C. Tong, E. Borenfreund and A. Bendich, Eur. J. Cancer, in press.
10 R. Lardinois and L.A. Page, Devl Biol. *19*, 261 (1969).
11 E.F. Zimmerman, D. Bowen, J.R. Wilson and M.M. Madappally, Biochemistry *15*, 5534 (1976).
12 E.F. Plow and T.S. Edgington, Int. J. Cancer *15*, 748 (1975).
13 R. Vrba, E. Alpert and K.J. Isselbacher, Proc. natl Acad. Sci. USA *72*, 4602 (1975).
14 A.T. Ichiki, K.L. Wenzel, Y.P. Quirin, R.D. Lange and J. Eveleigh, Br. J. Cancer *33*, 273 (1976).

Immunological profile of breast cancer patients in early or advanced disease[1]

E. Menconi, A. Barzi, M. Grecó[2], M.C. Caprino, L. De Vecchis and F. Muggia[3]

Institute of Pharmacology, University of Perugia, Via del Giochetto, I-06100 Perugia (Italy), 15 January 1979

Summary. Immune reactivity of patients with early or advanced breast cancer, compared with healthy controls, has been measured using in vivo and in vitro tests. The results of our study show that impairment of cellular responsiveness occurred in women with advanced disease.

The data accumulated in the past few years suggest that impairment of immunologic reactivity occurs in breast cancer patients[4-6]. In the present report we describe our studies on the immune reactivity of breast cancer patients with early or advanced disease as compared with healthy controls.

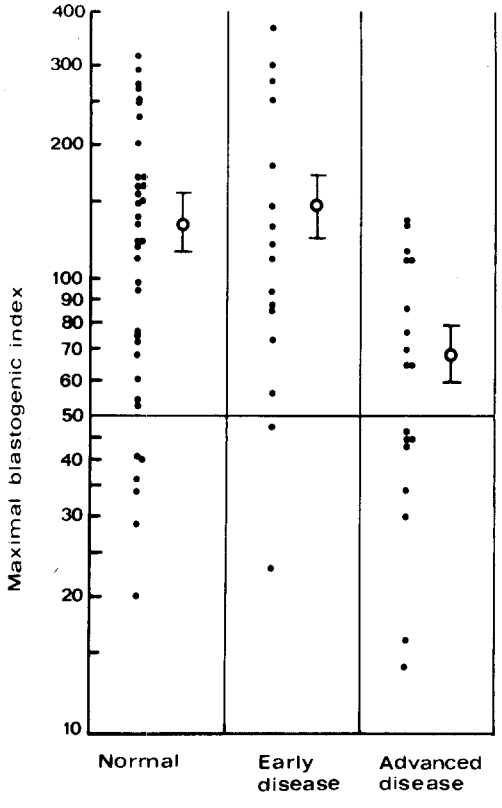
The studies were performed on 34 women with histologically diagnosed breast cancer: 16 with early disease (average age 50, range 28–65) and 18 with advanced disease (average age 50, range 36–65). The 16 patients with early cancer included 10 at the 2nd stage of TNM classification (U.I.C.C.) and 6 at the 3rd stage. All immunological tests were performed prior to any treatment; all patients had a performance status (PS) higher than 40, according to the Karnofsky scale. Healthy controls were 36 volunteers of both sexes (average age 27, range 23–45). Immunological

Blastogenic response to PHA and percentages of E-rosettes in healthy controls or in patients bearing early or advanced breast cancer

Group	No. of observations	Mean age (range)	No PHA (cpm, mean ± SE)	PHA 7.5 µg/ml (cpm, mean ± SE)	PHA 15 µg/ml (cpm, mean ± SE)	PHA 60 µg/ml (cpm, mean ± SE)	% E-rosettes (mean ± SE (range))
Normal BL	36	27 (23–45)	352 ± 51	26,383 ± 1750	29,054 ± 1781	24,023 ± 1731	48.50 ± 2.43 (29–68%)
Early BL	16	50 (28–65)	157 ± 29	17,319 ± 3260	18,245 ± 3077	15,284 ± 2976	37.28 ± 3.16 (20–65%)
Advanced BL	18	50 (36–65)	253 ± 51	9750 ± 1834	11,620 ± 1451	10,741 ± 1366	28.10 ± 2.80 (7–52%)

BL: blood lymphocytes, separated by Ficoll-Hypaque from heparinized blood samples.
For PHA stimulation test, lymphocytes were cultivated in flat bottom microtiter plates, according to the technique already described[8]. In brief 2×10^5 lymphocytes in 0.1 ml of culture medium[9] were incubated either without or with PHA[10] in a humidified atmosphere at 37 °C with 5% CO_2 for 72 h. Blastogenic response was measured in terms of incorporation of ^{125}I deoxyuridine[11] which was added to the cultures (0.4 µCi/ml) along with 5-fluoro-2′-deoxyuridine[12] (0.04 µg/ml), after 54 h and then incubated for 18 h. The results are expressed as arithmetic mean ± SE of cpm showing the isotope incorporation by cultures in quadruplicate.
E-rosettes were determined by a modification of the method of Nemoto et al.[6]. In brief, 0.1 ml of lymphocytes suspension (2×10^6 cells/ml in RPMI + 20% FCS) was mixed in a test tube with 0.1 ml of 0.5% suspension of sheep red blood cells (SRBC) in the same medium. The mixture was centrifuged at 200 × g for 5 min and then incubated at 4 °C for 4 h. E-rosettes (lymphocytes surrounded by 3 or more SRBC) were read after gentle resuspension of the pellet and counting at least 200 lymphocytes in a hemocytometer. Each test was done in duplicate. The results represent the mean percentages of the 2 simultaneous readings for each subject. The mean of percentages and SE were calculated following angular transformation of the percentages[13].

evaluation included leukocyte and lymphocyte counts, skin tests with tuberculin purified protein derivative (PPD), counts of E-rosette forming cells (E-RFC), and in vitro lymphocyte stimulation with phytohemagglutinin (PHA).
Skin tests with PPD[7] were carried out only in breast cancer patients; in the group with early disease, 80% had positive responses (induration of 5–5 mm or more in 2 perpendicular diameters after 48 h was considered positive); in the group with advanced disease, only 30.7% had positive responses. The statistical analysis by contingency table (2 × 2) showed a significant difference between these 2 groups ($p < 0.02$). The Student t-test showed no significant difference in leukocyte and lymphocyte counts between controls and cancer patients, nor between the varying stages within the cancer groups ($p > 0.05$ in all cases). The arithmetic mean ± SE and the range of E-RFC are described in the table. A significant difference (according to Student's t-test) was found between healthy controls and breast cancer patients, and, among this group, women with advanced disease had lower percentages of E-rosettes than those with early disease ($p < 0.05$). The results of the tests on blastogenic responses to PHA are also illustrated in the table. There was significant difference ($p < 0.05$) in responses between healthy controls and patients bearing early cancer with all the PHA concentrations used; however, the mean age difference of 23 years between these 2 groups seems to rule out any possible significance regarding this finding[4,5]. The responses of patients with early disease were significantly higher than those of women with advanced disease, as evidenced at the PHA concentrations of 7.5 and 15 µg/ml ($p < 0.05$ and $p = 0.05$ respectively). However, no constant relationship was found between the depression of E-RFC and PHA responses in the same patient.
Maximal blastogenic indexes are reported in the figure; low reactivity (i.e. index lower than 50) was observed in 16.6% of the healthy controls, in 12.5% of early, and in 44.4% of advanced breast cancer patients. The percentage of low-reactive patients was significantly higher in women bearing advanced breast cancer.
This study confirms that patients with breast cancer show an impairment of cell-mediated immunity, related to the clinical stage of the disease. This was demonstrated by reduction of delayed hypersensitivity reaction to PPD, decreased percentages of E-RFC and impairment of blastogenic lymphocyte response to PHA.



Blastogenic response to PHA (for details see legend of the table) expressed as individual maximal blastogenic index, i.e. the ratio between the cpm at the highest reactivity recorded (regardless of the PHA concentration at which it occurred) and those of the unstimulated control cultures, for each individual. Open circles represent the mean; vertical bars represent SE. Cut-off value (determined by exploratory analysis) is 50, below which the proliferative response is considered depressed. The statistical analysis by contingency table (2 × 2) shows significant difference between early and advanced cancer groups ($p < 0.05$).

1 This work was supported in part by the CNR, Italy-USA Contract No. 7801062.65, and in part by the Region of Umbria, Italy. Acknowledgment. We wish to thank Mr Mario Andrielli for his expert technical assistance.
2 M.G., Institute of Hygiene, University of Perugia, Italy.
3 F.M., Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md., USA.

4 P.M. Bolton, C. Teasdale, A.M. Mander, S.L. James, J.M. Davidson, R.H. Whitehead, R.G. Newcombe and L.E. Hughes, Cancer Immunol. Immunother. *1*, 251 (1976).
5 W.J. Catalona, W.F. Sample and P.B. Chretien, Cancer *31*, 65 (1973).
6 T. Nemoto, T. Han, J. Minowada, V. Angkur, A. Chamberlain and T.L. Dao, J. natl Cancer Inst. *53*, 641 (1974).
7 PPD, Sclavo, Siena, Italy, 10 U in 0.1 ml intradermal.
8 E. Menconi, M. Neri, V. Sorci and L. Frati, J. Immunol. Meth. *22*, 191 (1978).
9 RPMI - 1640, GIBCO, at pH 7.2, containing 100 U/ml penicillin, 100 µg/ml streptomycin and supplemented with 10% fetal calf serum (FCS, GIBCO, heat-inactivated at 56 °C for 30 min).
10 PHA-P, Difco, added to the stimulated cultures in 0.1 ml at final concentrations of 7.5, 15, and 60 µg/ml.
11 Sp. act. 38 Ci/mM, Sorin Biomedica, Saluggia, Italy.
12 Supplied by the National Cancer Institute, NIH, Bethesda, Md., USA.
13 R.A. Fisher and F. Yates, in: Statistical Tables, p. 74. Oliver and Boyd, Edinburgh 1963.

Enhancement by caffeine of sister-chromatid exchange frequency induced by antineoplastic agents in human lymphocytes

D.C. Mourelatos[1]

Cytogenetics Laboratory, University of Dundee, Ninewells Hospital and Medical School, Dundee (Scotland), 27 September 1978

Summary. The SCE frequency induced by Thiotepa and the effect of this antineoplastic drug in combination with caffeine have been studied in cultures of human peripheral blood. Caffeine was found to enhance SCE and breakage frequencies induced by Thiotepa in human lymphocytes.

Caffeine has been reported to be without effect on DNA repair mechanisms in human cells[2-4] but we have already reported that this substance enhances the effect of several SCE-inducing chemicals in cultured human lymphocytes[5]. Caffeine which is known to increase the frequency of chromosome aberrations induced by UV and many chemical substances in rodent and plant cells, apparently reduces the frequency of SCEs produced by UV, MMC, 4-NQO and Triaziquone in rodent cells[6,7] but in human lymphocytes following treatment with MMC, 8-Methoxypsoralen plus UV-light[8], 4-NQO or Trenimon the effect of post-treatment with caffeine is to enhance SCE formation[5]. Caffeine does not potentiate chromosome damage induced in human lung embryonic (LU 106) cells by Thiotepa and MMC[9] although caffeine post-treatment does potentiate the chromosome aberration frequency induced by MMC in a normal human fibroblast strain and in 2 *Xeroderma pigmentosum* strains (XP4LO and XP7TA) but at different threshold values[10]. I now report the effect of caffeine on SCE rate and breakage frequency in human lymphocytes following treatment with Thiotepa, a trifunctional cross-linking agent. Results are also presented concerning the time factor in caffeine potentiation of the MMC-induced SCE frequency in these cells.

Material and methods. SCEs were demonstrated in human lymphocytes from normal subjects by growing PHA stimulated cultures of whole blood in the presence of 4 µg/ml BUDR for 72 h. Thiotepa was given with BUDR at 18 h after initiation of the culture with PHA. Caffeine at 100 µg/ml (5×10^{-4} M) was added at 18 h of culture life. The effect of adding caffeine at different times after treatment with MMC was investigated by introducing the caffeine (5×10^{-4} M) immediately after treatment, 18 h post-treatment and 48 h post-treatment. In these experiments MMC was added at the time of initiation of the culture with PHA and the cultures harvested, as usual, at 72 h of culture life.

Results and discussion. The results are shown in table 1 and indicate that when caffeine is added to the cultures exposed to Thiotepa at doses sufficiently great to induce SCEs the effect of the caffeine is synergistic, the SCE level achieved being consistently much greater than that expected by the simple addition of the effect by the Thiotepa and caffeine. A synergistic effect can also be seen for chromosome aberrations (table 2). Caffeine produces a relative reduction in the number of cells that reach 2nd and later divisions. Potentiation of MMC-induced SCE level was found when caffeine was present for any of the 3 periods tested but the extent of synergism varied being highest when caffeine was introduced at 18 h (54 h post-treatment) after initiation of the culture (table 3).

The difference between these results on human lymphocytes and the findings on rodent material[6,7] might be accounted for by the different cell types used. In cultured rodent cells in which there is little excision repair caffeine interferes with post-replication repair[11] which may be responsible for the drop found in SCE frequency. Kato[6] concluded that SCEs were the result of errors in a repair process that includes a caffeine sensitive step. Vogel and Bauknecht[7] refrained from drawing conclusions about their rodent data because they were distorted by the treatment (agent plus caffeine) influencing the cell cycle and reducing the mitotic index, which they consider may distort any influence of caffeine on the incidence of SCEs mediated by post-replication repair. In the present experiments using human lymphocytes, although the rate at which the cells are cycling appears to be reduced by Thiotepa and caffeine (table 2), the treatment results in an enhancement, instead of suppression, of SCE frequency. The finding that caffeine

Table 1. Comparison of the effect of Thiotepa on SCE rates in the presence and absence of caffeine

Thiotepa concentration (ng/ml)	Without caffeine		100 µg/ml caffeine	
	Mean SCE/cell	No. of cells	Mean SCE/cell	No. of cells
Control	7.3	53	8.7	75
3	7.7	60	10.3	60
30	14.8	53	18.1	60
60	22.6	39	35.7	60
150	35.7	60	57.7	60
300	59.2	55	69.2	22