Cancer Letters, 27 (1985) 339–345
Elsevier Scientific Publishers Ireland Ltd.

HIGH AFFINITY ROSETTE FORMING CELLS IN CARCINOMA OF THE ORAL CAVITY, UTERINE CERVIX AND BREAST

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(Received 24 January 1985)
(Revised version received 28 March 1985)
(Accepted 2 May 1985)

SUMMARY

Total rosette forming cells (TRFC) and high affinity rosette forming cells (HARFC) were enumerated in 534 patients with carcinoma of oral cavity, uterine cervix or breast, and the results were compared with adult controls. The HARFC levels were found to be significantly decreased in cancer patients, and this decrease was more pronounced in advanced clinical stages. The HARFC levels returned to normal in patients who had a clinical cure but remained low in patients having residual disease. Enumeration of HARFC may therefore be useful in assessing the cell mediated immunity in cancer patients.

INTRODUCTION

It has been well established that the human thymus derived lymphocytes (T cells) can form rosettes with sheep erythrocytes (SRBC) [6,11,16]. A close association between cellular immunity and malignancy is reported [4,5] and hence much attention was given to the rosette forming cells (RFC) [8,13]. Two types of RFC were identified and differentiated [15]. The TRFC included all T cells which form rosettes with SRBC at low temperature on prolonged incubation with excess SRBC, whereas the HARFC form rosettes at elevated temperatures (29°C) with fewer number of SRBC [1,15]. The HARFC were reported to be decreased in patients with carcinoma of breast, lung, uterine cervix and in hematological neoplasms [1,7,15]. But similar reports on head and neck tumours are scanty. Moreover, no attempts were made to correlate the decrease in HARFC with the

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0304-3835/85/$03.30
Published and Printed in Ireland

clinical stage of the disease. The aim of this study was to explore the value of HARFC in assessing the cell mediated immunity in patients with selected malignant tumours and in predicting their prognosis.

MATERIALS AND METHODS

The study was conducted on 534 cancer patients of which 196 had carcinoma of the oral cavity, 172 had carcinoma of the uterine cervix and the remaining 166 had carcinoma of the breast. Thirty two women with mild to moderate cervical dysplasia and 18 women with mammary dysplasia were included in the study. Sixty normal healthy adult males and 40 normal adult females from the staff and students of the Medical College services formed the control group. None of them had any diseases or immunological deficiencies. The patients were in the age group of 30—60 years and the normals were in the age group of 25--55 years. None of the patients had any form of treatment prior to the study. Ninety patients were available for follow up study, out of which 67 had no residual disease and 23 were on chemotherapy after surgery or radiotherapy. The follow up tests were done 6 months after the therapy. Ten millilitres of venous blood was collected from all subjects using 100 units/ml of preservative-free heparin. The lymphocytes were separated using Lymphoprep (Nyegaard & Co., Norway) and the TRFC and HARFC were enumerated by the method described by Weese et al. [15]. Duplicates of 300 cells were counted in all cases and the results were expressed as percentage of total lymphocytes. Statistical analysis was done using Student's *t*-test.

RESULTS

The age and sex distribution of patients and control subjects are given in Table 1. The number of patients in stage I was less because of the delay in detection and report to the hospital. The mean values of the TRFC and HARFC are given in Table 2. It can be seen that the difference was signifi-

TABLE 1

AGE AND SEX DISTRIBUTION OF SUBJECTS

	Control		Oral cavity		Benign uterine lesions	Benign breast lesions	Cervical cancer	Breast cancer
	Males	Females	Males	Females				
No. of subjects	60	40	123	73	32	18	172	166
Age (mean ± S.D.)	35.4 ± 6.4	36.8 ± 7.5	47.4 ± 11.2	49.6 ± 9.3	40.7 ± 8.6	43.5 ± 6.9	44.2 ± 10.9	46.8 ± 12.1

TABLE 2

ENUMERATION OF TRFC AND HARFC IN CANCER PATIENTS, BENIGN CONDITIONS AND IN CONTROLS

Subjects	% of TRFC (mean ± S.D.)	% of HARFC (mean ± S.D.)
Normal controls (n = 100)	71.42 ± 8.49	50.67 ± 4.78
Benign lesions of breast (n = 18)	71.62 ± 8.91	51.15 ± 5.36
Benign cervical lesions (n = 32)	68.94 ± 10.12	48.74 ± 4.87
Cancer of oral cavity (n = 196)	68.54 ± 7.85	41.35 ± 6.13**
Cancer of uterine cervix (n = 172)	69.04 ± 6.72	38.75 ± 5.82**
Cancer breast (n = 166)	67.46 ± 8.92*	35.08 ± 5.14**

*$P < 0.05$
**$P < 0.001$.

cant only in cancer of the breast ($P < 0.05$). But HARFC values showed a drastic reduction in all types of cancers studied and the reduction was highly significant ($P < 0.001$). Benign lesions of the breast and uterine cervix showed no change either in TRFC or HARFC. The changes in TRFC and HARFC values were maximum in carcinoma of the breast and minimum in carcinoma of the oral cavity. Table 3 shows the TRFC and HARFC values in different stages of patients in different stages. When compared with normal controls the TRFC values in patients in stages III and IV were significant ($P < 0.05$) in all 3 types of cancer, but the changes were not significant in stages I and II. In all patients the HARFC values were significant when compared to normal controls and in stages III and IV the changes were highly significant. Table 4 represents the percentage of patients whose TRFC values are higher than 47 (the value arbitrarily fixed as the mean lower limit [15]). In our normal control group 92% were above the limit and in the non-malignant patients 86% were above this level, but out of 534 cancer patients studied only 8% were seen to be above this limit. Table 5 shows the effect of treatment on the HARFC values. The patients who had a clinical cure had normal HARFC values and 73% of them had HARFC more than 47 whereas in patients on chemotherapy the HARFC values remained low and only 3 patients had HARFC more than 47.

DISCUSSION

The failure in immune surveillance has been reported as an important mechanism in the development of cancer [2] and in tumour progression [12]. Djeu et al. [3] originally reported a decrease in the RFC at 29°C (which was later named as HARFC) in cancer patients. We employed this method with minor modifications suggested by others [15]. Many workers had tried to correlate the RFC with the clinical staging of cancer with

TABLE 3

ENUMERATION OF TRFC AND HARFC ACCORDING TO THE STAGES AND TYPES OF DISEASE

Stage of disease	RFC	Carcinoma oral cavity		Carcinoma uterine cervix		Carcinoma breast	
		No. of patients	RFC	No. of patients	RFC	No. of patients	RFC
I	Total	26	70.41 ± 6.36	12	69.85 ± 7.12	8	68.76 ± 5.98
	high affinity		40.16 ± 5.42*		43.45 ± 3.67*		41.62 ± 4.05*
II	Total	82	68.56 ± 6.73	76	68.66 ± 5.68	61	66.38 ± 6.22
	High affinity		44.32 ± 3.74*		40.78 ± 3.98*		37.13 ± 3.95*
III	Total	51	67.16 ± 4.98*	64	66.08 ± 5.18*	57	65.78 ± 5.47*
	High affinity		40.58 ± 4.26**		35.92 ± 4.72**		34.15 ± 4.52**
IV	Total	37	66.85 ± 6.05*	20	65.15 ± 4.80**	40	64.04 ± 4.05*
	High affinity		36.42 ± 3.80**		30.45 ± 3.05**		30.25 ± 4.75**

P values were compared with values of normal healthy controls: TRFC = 71.42 ± 8.79; HARFC = 50.67 ± 4.78.

*$P < 0.05$.

**$P < 0.001$.

TABLE 4

PATIENTS SHOWING HARFC MORE THAN 47

The value 47 is observed to be the mean lower limit in normal persons.

Type of patients	HARFC >47/total cases studied (%)[a]	HARFC >47 in cancer cases distributed in different clinical stages (%)			
		I	II	III	IV
Normal healthy controls	92/100 (92)				
Benign lesions	43/50 (86)				
Carcinoma of oral cavity	25/196 (12.75)	7/126 (26.92)	13/82 (15.85)	4/51 (7.84)	1/37 (2.70)
Carcinoma of uterine cervix	8/172 (4.65)	3/12 (25.00)	2/76 (10.52)	3/64 (4.78)	0/20
Carcinoma of breast	11/166 (6.63)	3/8 (37.50)	6/61 (9.83)	2/57 (3.50)	0/40

contradictory results. Normal levels of TRFC in cancer patients were reported by Nemoto et al. [9] and Stjernsward et al. [14]. These are in accordance with the present findings. However, a significant reduction in TRFC in cancer patients was reported by others [10,17]. Such difference in findings may be due to variation in the methods employed. We kept all the variables such as pH, temperature, time of incubation, age of SRBC, etc. to the minimum. In our study HARFC were found to be highly useful in distinguishing the immunological status of the normal and cancer patients as reported earlier by others [3,15]. A change in HARFC in patients with moderate and severe dysplasia of the cervix was reported by Bashford and

TABLE 5

EFFECT OF TREATMENT ON HARFC

Subjects	HARFC (mean ± S.D.)	Subjects with HARFC >47 (%)
Normal healthy controls (n = 100)	50.37 ± 4.78	92/100 (92)
Patients after surgery and/or radiotherapy and having no clinical disease (n = 67)	48.47 ± 5.68	49/67 (73.13)
Patients on chemotherapy with residual tumour (n = 23)	37.08 ± 6.14*	3/23 (13.04)*

*$P < 0.001$.

Gough [1], which could not be observed in our patients. The effect of treatment on the RFC seems to be more important. The patients who had no residual lesions had normal HARFC levels whereas the patients on chemotherapy had a very low percentage of HARFC. However, there was no change in their TRFC levels.

Monoclonal antibodies are available to characterise the different subsets of human T cells and the use of these antibodies could help to understand better the role of cell mediated immunity in the development of human cancer. Further studies are in progress. From the present study it is reasonable to assume that HARFC is useful in assessing and monitoring cell mediated immunity in cancer and will be of help in predicting the prognosis.

ACKNOWLEDGEMENT

Financial support from the Indian Council of Medical Research is gratefully acknowledged.

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