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PROGNOSTIC RELEVANCE OF RADIATION INDUCED IMMUNE SUPPRESSION IN BREAST CARCINOMA

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Impaired lymphocyte reactivity has been found in patients with malignant disease (DUCOS et coll. 1970, WHITTAKER & CLARK 1971, BROOKS et coll. 1972, KUMAR & TAYLOR 1973, WATKINS 1973). The degree of this impairment was found to be correlated to the extent of the disease (CATALONA et coll. 1973, LEHANE & LANE 1974, KNIGHT & DAVIDSON 1975). This finding raised the question whether the immunodeficiency is primary, i.e. necessary for the development and growth of a malignant tumour or secondary, i.e. consequential to the presence of the tumour. The finding that an immune suppression was present at the time of diagnosis of metastatic breast malignancy, while being absent in the same patients at the time of initial diagnosis (GLAS et coll. 1976), indicates that the second alternative is more likely in most instances.

Recently, no association between prognosis and some immunologic variables at the time of diagnosis of breast carcinoma was found (BARAL et coll. 1977 a), i.e. the presence of certain autoantibodies, lymphocytes counts and lymphocyte stimulability

Submitted for publication 14 February 1979.

by PHA and PPD did not correlate either to prognosis or to the clinical condition of the patients at the time of diagnosis as defined by tumour size, axillary node involvement and grade of malignancy.

It was considered of interest to extend this investigation in order to assess whether any difference in the extent of radiation induced immune suppression existed between patients with mammary carcinoma who developed a recurrent disease and those who remain clinically free of disease several years following treatment. At the same time it was also analysed whether any correlation existed between certain clinical features of the disease and the extent of radiation induced immune suppression.

Materials and Methods

The material consisted of 100 women with operable carcinoma of the breast without evidence of distant metastases. The patients were included in a randomized trial aiming at establishing the value of pre- and postoperative radiation therapy. The patients entered the trial during June 1971 through June 1974. After an initial clinical assessment they were randomly allocated into 3 treatment groups: pre- or postoperative radiation therapy or modified radical mastectomy only. A detailed account of the patients included in this trial, the clinical condition of the patients and the randomization procedures have been described previously (WALLGREN et coll. 1978). Of the 100 irradiated patients, 56 received preoperative and 44 postoperative irradiation.

Radiation therapy. Preoperative irradiation was given with a ^{60}Co source and directed to the breast, the internal mammary node region, axilla and the supraclavicular fossa. A total target dose of 45 Gy (4 500 rad) was delivered during approximately 6 weeks. Postoperative irradiation was given using high energy electrons to the chest wall and the regional lymph nodes as described; the dose and treatment period being similar. Details of these techniques have been given previously (WALLGREN et coll.).

Blood sampling was performed at the time of diagnosis and within one month after completion of the radiation therapy.

Clinical follow-up. After completion of the primary treatment the patients were examined at 3-month intervals during the first 2 years and thereafter every 4 to 6 months. Appropriate radiographic and laboratory examinations were performed when recurrence was possible. The median follow-up time (i.e. time elapsing between entry into the trial and the development of a recurrence or the end of the investigation in October 1978) for all patients was 68 months ranging from 3 to 88. The shortest follow-up for disease-free patients was 55 months. Of the 26 patients

who developed distant metastases by the end of October 1978, 6 did so within one year of diagnosis.

Malignancy grading of tumours. The microscopic slides were reviewed by one of the authors (C. S.). Only cases not given preoperative irradiation (44 patients) were considered as eligible for this review. Malignancy grading was carried out as recommended by the International Reference Centre for Breast Tumours (WHO; SCARFF & TORLONI 1968).

Immunology. Venous blood was defibrinated by agitation in beakers containing glass pearls. Lymphoid cells were obtained after gelatine sedimentation of erythrocytes according to the method of COULSON & CHALMERS (1964, 1966).

The lymphocyte stimulation tests have been described previously (GLAS & WASSERMAN 1974). In brief, 2×10^6 lymphoid cells were cultured in glass tubes containing 1.0 ml Eagle's Minimal Essential Medium supplemented with Earle's salts (MEM), 10 per cent heat inactivated human AB serum and antibiotics. The stimulants used were: Phytohaemagglutinin (PHA-M, Bacto Phytohaemagglutinin-M, Difco Lab., Detroit, Michigan, USA) and PPD-tuberculine (PPD, RT22, Statens Seruminstitut, Copenhagen, Denmark). The cells were cultured with PHA at a final concentration of 0.6 mg/ml and with PPD at a final concentration of 1.0 μg/ml. Control cultures, which were set up in parallel, received no stimulant. After 4 days of incubation at 37°C in a humidified 5 per cent CO_2 air atmosphere, each tube received 14 kBq (0.4 μCi) of ^{14}C-thymidine (The Radiochemical Centre, Amersham, England, specific activity 1.998 GBq (54 mCi)/mmol). Twenty-four hours later the cells were harvested and incorporated radiation activity was determined and expressed as counts per minute (cpm). The activity of the control cultures was subtracted from the values obtained in the corresponding test cultures. Mean values of triplicate cultures were calculated on an arithmetic basis.

Data processing and statistical methods. The variables used in this analysis, their median values and standard deviations or distributions are listed in Table 1. Variables 1 to 4 are in the following termed clinical, 5 to 6 follow-up and those showing the relative changes of lymphocyte counts and their reactivities to PHA and PPD are termed immunologic variables. The relative change, expressed as per cent, is defined as the difference between the values obtained following and before radiation therapy. In order to examine whether the immunologic variables were associated with any of the clinical, each of the latter was divided into 2 subgroups (Table 2). The hypothesis that the distribution of the values of the immunologic variables is identical in the 2 subgroups of each clinical variable has been tested using the distribution-free Wilcoxon rank sum test (HOLLANDER & WOLFE 1973). As a measure of the central point in the distribution of the variables the median value was chosen due to its stability with respect to extreme values.

Table 1

List of the variables used with medians and standard deviations (SD) or distributions

No.	Variable	Median	SD	Percentage distribution
1	Age	54	8.73	
2	Tumour size (mm)	35	13.55	
3	Grade of malignancy (I, II or III)			(4, 32, 64)
4	Tumour involvement of axillary nodes (yes/no)*			(39/61)
5	Distant metastases (yes/no)			(26/74)
6	Local recurrence (yes/no)			(9/91)
7	Change of PHA-reactivity after irradiation (%)**	− 3	126	
8	Change of PPD-reactivity after irradiation (%)**	−63	262	
9	Change of lymphocyte counts after irradiation (%)**	−53	91	

* Axillary nodes were considered as involved only when confirmed by microscopy.

** Absolute pre-treatment stimulations with PHA and PPD (cpm) were (46 958, 21 534) and (13 822, 20 881), respectively, and pre-treatment lymphocyte counts/μl were (1 909, 576; Median, SD).

Results

The description of the variables is given in Table 1. The radiation induced changes of the blood lymphocyte counts and the lymphocyte responses to PHA and PPD are not significantly influenced by either the age of the patient or the local extension of the disease (Table 2). Neither was any correlation found between the grade of malignancy to either of the immunologic variables (data not presented). Moreover, Table 2 demonstrates that post-treatment values of the immunologic variables were essentially the same in those patients who developed recurrent disease and in those who remained clinically disease-free during a follow-up period of 4.5 to 7 years.

Discussion

Radiation therapy of malignant tumours may result in a marked reduction of circulating lymphocytes and their reactivities to mitogens and antigens (GOSWITZ et coll. 1963, MILLARD 1965, THOMAS et coll. 1971, BARAL et coll. 1977 b, GLAS & WASSERMAN, BLOMGREN et coll. 1976, 1977). Since it has been claimed that radiation induced immunosuppression may accelerate growth of tumour cells outside the irradiated volume in breast carcinoma (MEYER 1970, STJERNSWÄRD 1974) it was investigated whether those patients who developed recurrent disease became more

Table 2

Relative changes of lymphocyte counts and stimulations by PHA and PPD, expressed as per cent, following radiation therapy in various subgroups of patients with mammary carcinoma. The absolute pre-treatment lymphocyte counts, PHA and PPD responses did not differ between the subgroups of patients (data not given)

Subgroups compared	PHA			PPD			Lymphocyte counts		
	Median value	No. of cases	p-value*	Median value	No. of cases	p-value*	Median value	No. of cases	p-value*
Age									
< 50 years	14.6	24	0.36	−52.4	23	0.11	−58.1	21	0.57
⩾ 50 years	−10.3	70		−68.4	74		−52.5	67	
Tumour size									
< 50 mm	− 0.3	74	0.38	−59.9	78	0.89	−53.1	69	0.82
⩾ 50 mm	1.1	20		−72.7	19		−51.5	19	
Involvement of axillary nodes									
yes	16.4	17	0.67	−70.9	16	0.27	−54.9	15	0.90
no	−19.3	25		−78.4	26		−50.4	25	
Distant metastases									
yes	−16.5	26	0.16	−69.8	24	0.71	−51.8	22	0.47
no	11.3	68		−63.2	73		−52.9	66	
Local recurrence									
yes	−13.1	9	0.25	−77.3	7	0.28	−49.3	9	0.74
no	3.4	85		−60.4	90		−53.1	79	

* Probability of falsely rejecting the hypothesis of distributions being 'equal' between the two subgroups of patients.

immunosuppressed than those who did not. It was also examined whether some clinical features (Table 1) correlated to the extent of this impairment. The results may be summarized as follows: (1) The degree of radiation induced lymphopenia and impairment of PHA and PPD reactivities of the cells were not associated with the subsequent course of the disease and (2) the radiation induced immune suppression was essentially the same in patients of different ages and the size of the primary tumour, its spread to axillary nodes and malignancy grade.

These results are in accordance with the clinical data obtained in the randomized Stockholm Breast Cancer trial, in which no increased frequency of metastatic disease was observed in the irradiated patients as compared to those who had been treated by surgery alone (WALLGREN et coll. 1978). In addition, it may be mentioned that further analysis of the prognostic importance of blood lymphocyte counts and their reactivities to PHA and PPD before and at various times following radiation therapy

did not reveal any major differences between patients who developed a recurrent disease and those who did not (BLOMGREN et coll., to be published).

In conclusion, the results of this investigation do not give any support for the view that the immuno-suppression which follows radiation therapy for mammary carcinoma promotes growth of occult distant deposits of malignant cells.

Acknowledgements

This investigation was supported by grants from King Gustaf Vth Jubilee Foundation.

SUMMARY

The extent of radiation induced immune suppression was analysed in 100 patients with carcinoma of the breast. The relative changes of lymphocyte counts and stimulations by PHA and PPD were similar in patients who differed with regard to age, size of the tumour and its malignancy grade or axillary node condition. Moreover, no difference in the degree of radiation induced immune suppression existed between patients who developed recurrent disease and those who remained free of disease during a follow-up period of 4 1/2 to 7 years.

ZUSAMMENFASSUNG

Der Grad der strahleninduzierten Immunsuppression wurde bei 100 Patienten mit Brustkarzinom analysiert. Die relativen Veränderungen der Lymphozytenwerte und der Stimulation durch PHA und PPD waren ähnlich bei Patienten, die hinsichtlich ihres Alters, Grösse des Tumors und Malignitätsgrads oder hinsichtlich des Axilla-Lymphknoten-Stadiums unterschiedlich waren. Weiterhin bestand kein Unterschied im Grad der strahleninduzierten Immunsuppression zwischen Patienten, die eine rezidivierende Erkrankung entwickelten und solchen, die frei von Erkrankung während einer Beobachtungsperiode von 4 1/2 bis 7 Jahren waren.

RÉSUMÉ

L'étendue de l'immuno-suppression induite par les radiations a été étudiée chez 100 malades atteintes de carcinome du sein. Les modifications relatives du nombre des lymphocytes et des stimulations par PHA et PPD ont été semblables chez les malades qui présentaient des différences concernant l'âge, le volume de la tumeur et son grade de malignité ainsi que l'état des ganglions lymphatiques axillaires. De plus, il n'y a pas eu de différence dans le degré de l'immunosuppression induite par les radiations entre les malades qui ont présenté une récidive et celles qui sont restées guéries pendant une période d'observation de 4 ans 1/2 à 7 ans.

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