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Effects of Neoplastic Disease and Local X-Irradiation on Polymorphonuclear Leukocyte Functions

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PMN: Polymorphonuclear leukocytes

ABSTRACT

The aim of this study was to study polymorphonuclear leukocyte (PMN) functions in patients with solid tumor prior to or following X-irradiation. We observed that PMNs from patients with head and neck cancer exhibited decreased chemotaxis, while phagocytosis and intracellular killing of *Candida albicans* were normal. In contrast, PMNs from other solid cancer exhibited decreased intracellular killing of *C. albicans*, while chemotaxis and phagocytosis functions were normal. Local body X-irradiation significantly decreased PMN chemotaxis and intracellular killing of *Candida albicans*, but had no effect on phagocytosis. The effect on PMN function was dose-dependent and observed in 46 out of 65 patients (71%) that received a dose above 1000 Rad. Patients whose PMNs exhibited normal chemotaxis after receiving a dose above 1000 Rad, had comparable numbers of granulocytes and lymphocytes than those showing significant depression. No correlation was found between chemotaxis and circulating leukocyte number. Also, no correlation was found between the effects of X-irradiation on PMN functions and circulating lymphocyte number. In summary, patients with solid tumors show selective defects in PMN functions.

Introduction

Degradation of antigens by polymorphonuclear (PMN) leukocytes and monocytes/macrophages is essential for protecting the host against microbial and other foreign invaders [1, 2]. PMN and macrophages are also important players in modulating tumor development and metastasis formation [3, 4]. Accumulation of circulating PMNs and monocytes at sites infected by foreign antigens is essential for the first line of defense as well as for the induction of later adaptive immune responses [5, 6]. The local accumulation of these cells is dependent on proper chemotaxis. Impaired chemotaxis may lead to increased susceptibility to infections. Defective monocyte chemotaxis has been demonstrated in patients with a variety of solid tumor [7-9]. There are also some reports suggesting altered chemotaxis or other PMN functions in selected cancer patients [10-14].

In the present study, we have evaluated the effects of solid cancer and local X-irradiation on three essential PMN functions (chemotaxis, phagocytosis and intracellular killing of *Candida albicans*). The data clearly demonstrate that neoplastic diseases have selective effects on circulating PMN. Especially, local body X-irradiation at a dose above 1000 Rad significantly reduced chemotaxis and intracellular killing capacity. The latter effects were dose- and site-dependent and most pronounced in patients with high functional capacity prior to X-irradiation.

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Materials and methods

Patients

One hundred twenty-five patients with a variety of solid tumors were studied (Table 1). The patients mean age was 56.4 (range 16-84), 68 were males and 57 females. Fifty-four of 125 patients had surgery, 22 of them with less than 6 months and 29 patients with more than 6 months from the day of surgery to the day evaluating their PMN functions. Fifty-five had epidermoid carcinoma of the head and neck region. Eight patients with either stage III or IV cancer were studied at the time of their initial diagnosis, while another 25 were examined at the time of their first recurrence. In the latter group, 8/25 patients had been treated with surgery, 8 with X-irradiation and the other 9 with both procedures, and at least 6 months had passed since the last treatment. The breakdown according to tumor site for these 33 patients is presented in Table 4. The 22 patients examined while receiving X-ray therapy included 10 patients who were being treated with curative intent and 12 patients who were treated for palliation.

Table 1: Origin of primary tumor in the patient population investigated. The table represents the number of patients in each category. Patients receiving X-irradiation were categorized into two groups, those receiving less or higher doses than 1000 Rad. The untreated cancer patient group includes patients evaluated at the time of their initial presentation or during the first recurrence when the relapse occurred more than 6 months after last therapy.

Origin of Tumor	Untreated	<1000 Rad	>1000 Rad
Lung	7	3	14
Head and Neck	33	5	17
Breast	4	1	11
Prostate	-	-	4
Melanoma	1	-	2
Cervix	2	2	3
Lymphoma	-	-	4
Uterus	-	1	3
Bladder	1	-	-
Thyroid	-	-	2
Rectum	-	-	2
Brain	-	-	1
Thymoma	-	-	1
Stomach	-	-	1
Total	**48**	**12**	**65**

Sixteen patients with breast cancer with a mean age of 58.4 (36-74) were evaluated. All had metastatic visceral and/or bone disease. Only 4/16 were evaluated prior to starting X-irradiation or chemotherapy. Twenty-four patients with lung cancer with a mean age of 54 (40-72) were tested. All had unresectable disease with extrathoracic metastasis. Eleven were evaluated following initial diagnosis, while the others were evaluated while receiving palliative radiation therapy. Twenty of 24 gave a history of heavy tobacco smoking (>15 cigarettes/day for over 15 years). One hundred-four healthy subjects (65 males and 39 females), whose mean age was 54 (range 24-70) without any regular medication, served as controls. Only 10 of the 104 subjects gave a history of alcoholism and 30 were heavy smokers (>15 cigarettes/day).

All human materials were obtained in accordance with the local regulations of California at the time of performing this study (1978-1982).

PMN Isolation

Peripheral blood leukocytes were isolated by dextran (6%) sedimentation followed by hypotonic lysis of contaminating red blood cells. The cells were washed and resuspended in Hanks' balanced salt solution (HBSS) containing 10% heat-inactivated fetal calf serum (FCS) to a density of 5 x 10^6 PMN/ml. When studying the effect of X-irradiation on PMN functions, PMN were isolated 24 hours after treatment.

Chemotaxis

PMN chemotaxis was measured by a modification of the Boyden technique [15]. The chemoattractant was prepared by incubating fresh AB serum with lipopolysaccharide (from *Shigella flexneri*, 1.5 μg/ml, Sigma) at 37°C for 90 min to activate complement and then heated to 56°C for 30 min to cease complement activation and to inactivate inhibitors. Following centrifugation at 2500 rpm for 10 min the activated serum was aliquoted in tubes and stored at -20°C. The activated serum was diluted to 6-10% with HBSS and placed in the lower compartment. 0.2 ml PMN suspension (5 x 10^6/ml) in HBSS/FCS was added to the upper compartment, separated from the lower one by a 5μ-pore size filter (Nucleopore polycarbonate filter, Wallaby, San Rafael, California). The chambers were incubated at 37°C for 60 min. The filters were removed, fixed in methanol and stained with Giemsa. The number of migrated PMN were determined by counting 20 high-power fields (HPF) using a microgrid. Results are expressed as the number of cells per HPF. To ascertain assay variability, 4 healthy subjects were examined on 3-5 different occasions and their mean ± SD migration calculated. As shown in Table 2, the SD was less than 20% in all 4 subjects and even less than 5% in 2 of them. The data clearly demonstrate a low intra-individual variance which supports the reliability of the changes observed in patients studied serially.

Table 2: Serial measurements of PMN chemotaxis in 4 healthy donors. PMN were isolated from 4 healthy donors at different days and their chemotaxis monitored. The numbers present the average number of migrated PMN per high-power field after counting 20 high-power fields. Net Migration = (Migration in the presence of chemoattractant)-(Random migration).

Subject	Mean Net Migration	Net Migration (Individual values)
1	143.3 ± 19.9	120, 135, 109, 149, 157
2	111.3 ± 3.17	111, 108, 114
3	129.8 ± 4.31	128, 125, 120
4	139.4 ± 16.50	148, 157, 147, 156, 119
Average	**131.43±18.24**	

To determine the number of cells detached from the filters, circular cover slips were placed at the bottom of the lower compartment. At the end of the incubation, the slides were fixed, stained with Giemsa and the number of cells counted. In different sets of experiments, two filters were inserted between the two compartments, an upper filter with a pore size

of 5μm and a lower one with a pore size of 0.4μm. The number of cells migrating into the two filters was determined as described above and compared to the number of cells migrated when just one filter was used.

Phagocytosis and Intracellular Killing of *Candida albicans*

A modification of the methods described by Lehrer and Cline and Territo and Cline was applied [16, 17]. *Candida albicans* grown in Sabouraud's dextrose broth for 3-4 days were washed twice in HBSS and resuspended in HBSS to a concentration of 5 x 10^6 yeasts/ml. To measure phagocytosis, 0.4 ml of a PMN suspension (5 x 10^6/ml) was incubated in polystyrene plastic tubes (12 x 75 mm) with 0.8 ml of a *Candida albicans* suspension (5 x 10^6 yeasts/ml) (ratio 1:2) in 0.4 ml HBSS/FCS and 0.4 ml AB serum for 30 min at 37°C on a rocker platform. At the end of incubation, cytocentrifuge smears were prepared and stained with Giemsa. Two hundred PMN were counted each time, and the mean number of phagocytosed *Candida albicans* per 100 PMN and the average number of *Candida albicans* per PMN were determined.

To measure intracellular killing of *Candida albicans*, 0.4 ml of a PMN suspension (5 x 10^6/ml) was incubated with 0.4 ml of a *Candida albicans* suspension (5 x 10^6 yeasts/ml) (ratio 1:1) in 0.8 ml HBSS/FCS and 0.4 ml AB serum for 2 hrs at 37°C on a rocker platform. At the end of incubation, cytocentrifuge preparations were made and stained with Giemsa. Live yeast stained uniformly with a dark blue color, whereas dead yeast was partially or completely decolorized. The percentage of intracellular killing of *Candida albicans* was calculated by determining the viability of 200 intracellular yeast particles.

Statistics

Comparisons of the various patients with healthy subjects were done using the paired Student's t test. Each patient was paired with his simultaneous day control. Comparisons between patient populations were made using the two sample Student's t test.

Results and Discussion

PMN Functions in Patients with Malignant Solid Cancer

Three different PMN functions were evaluated in 48 patients with a variety of solid tumors, the majority (33 patients) having squamous carcinoma of the head and neck region. Despite the fact that these patients comprised a highly heterogeneous group, their white blood cell count, percent and absolute number of granulocytes, lymphocytes and monocytes were comparable to that of a control population examined during the same period of time (Table 3). Patients with squamous carcinoma of the head and neck showed significantly reduced PMN chemotaxis, whereas phagocytosis and intracellular killing of *Candida albicans* were comparable with healthy subjects (Table 4). In contrast, patients with other solid tumors showed normal PMN chemotaxis and phagocytosis, while intracellular killing of *Candida albicans* was decreases (Table 4). Of note, the mean chemotaxis of 8 head and neck patients examined at the time of their initial diagnosis was lower than the whole group (Table 4). The decrease in PMN chemotaxis seen in patients with head and neck cancer was not dependent on the particular site of tumor origin (Table 5). All showed depressed chemotaxis. Breakdown of this group on the basis of age, sex or stage of disease did not influence the results obtained (data not shown). The reduced PMN chemotaxis might contribute to the impaired delayed hypersensitivity reactions observed in head and neck patients [18].

Effect of Local Body X-Irradiation on PMN Chemotaxis

The effect of local body X-irradiation on PMN function was examined in 77 patients. Twelve patients were evaluated following a dose less than 1000 Rad and 65 patients after receiving a dose above 1000 Rad (in most cases $\geq$2000 Rad). Significant differences in the absolute lymphocyte numbers were seen between these treatment groups (Table 3). Patients who received a dose above 1000 Rad showed on the following day a dramatic drop in lymphocyte number and a slight rise in PMN number with no significant alterations in total white blood cell (WBC) count (Table 3). Outstanding, was the pronounced rise in total WBC and PMN numbers in patients who received a dose less than 1000 Rad (Table 3).

Local X-irradation at a dose above 1000 Rad significantly decreased patients PMN chemotaxis in most kind of solid cancer, except for those suffering from head and neck cancer (Table 6). The reason for this difference is not known, but the former patients received X-irradiation to the chest, abdomen or cranial vault, while the irradiation area of head and neck cancer patients was more restricted. Two patients with head and neck cancer that got irradiation to the cranial vault showed marked decrease in chemotaxis, lending support to the notion that the irradiation area might be a factor influencing PMN chemotaxis. Also, the PMN of head and neck patients before treatment showed reduced chemotaxis (Tables 4-5) which might be a reason for no further reduction following X-irradiation. For each disease category investigated, there were some patients whose PMN showed normal chemotaxis even after receiving a dose above 1000 Rad, with the exception of patients with breast cancer. In the latter group, all the 11 patients examined exhibited chemotactic migration that was below the mean of controls. Altogether, decreased chemotaxis was observed in 71% (46/65) X-irradiated (>1000 Rad) patients. The other patients had values in the normal range.

Table 3: White blood cell (WBC) counts in untreated and X-irradiated patients with a variety of solid tumors. Patients receiving X-irradiation were categorized into two groups, those receiving less or higher doses than 1000 Rad. Blood samples were taken 24 hrs after treatment. The numbers present average number (K/l) ± standard deviation.

Subjects	Number	WBC	PMN	Lymphocytes	Monocytes
Healthy Controls	39	6.4 ± 1.2	4.08 ± 0.99	1.97 ± 0.96	0.38 ± 0.25
Untreated Cancer Patients	42	6.2 ± 1.79	4.27 ± 1.6	1.25 ± 0.71	0.41 ± 0.26
Patients X-irradiated <1000 Rad	9	9.87 ± 4.38[a]	7.11 ± 4.59[a]	1.75 ± 0.80	0.55 ± 0.24
Patients X-irradiated >1000 Rad	48	6.45 ± 3.26	5.07 ± 3.23	0.86 ± 0.59[a]	0.48 ± 0.28

[a]$p<0.05$ compared to untreated patients

Table 4: PMN Functions in untreated patients with solid tumors. The chemotaxis numbers present the average number of migrated PMN per high-power field after counting 20 high-power fields ± standard deviation. The percentage of ingested *Candida albicans* that were killed after 2 hrs incubation with PMN was calculated by determining the viability of 200 intracellular yeast particles. The mean number of phagocytosed yeast particles per 100 PMN is presented ± standard deviation.

Subjects	Number	Chemotaxis	% Intracellular killing of *Candida albicans*	% Phagocytosis of *Candida albicans*
All Head and Neck cancer patients	33	112.0 ± 33.7[a]	14.8 ± 2.39	
Initial diagnosed Head and Neck cancer patients	8	96.7 ± 31.3[a]		
Other solid cancer patients	13	129.0 ± 30.4	10.6 ± 3.84[a]	
All Cancer patients	46	116.0 ± 33.1[a]	13.3 ± 3.5	67.0 ± 14.3
Healthy controls	45	131.4 ±18.2	15.8 ± 7.6	66.4 ±13.5

[a]$p<0.02$ based on paired t-test with healthy controls tested at the same time.

Table 5: PMN chemotaxis in patients with head and neck cancer according to tumor site. The chemotaxis numbers present the mean number of migrated PMN per high-power field after counting 20 high-power fields ± standard deviation.

Tumor site	Number	Chemotaxis
All sites	33	112.0 ± 33.7
Oropharynx	9	106.3 ± 29.8
Larynx	5	108.2 ± 61.7
Salivary gland	4	117.5 ± 19.1
Other sites	15	112.2 ± 32.1

Table 6: Comparison of PMN chemotaxis in cancer patients prior to therapy or after local X-irradiation. Data for PMN from healthy controls (without irradiation) that were tested simultaneously with the other samples are presented in the last row. The chemotaxis numbers present the mean number of migrated PMN per high-power field after counting 20 high-power fields ± standard deviation.

Subjects	Number	<1000 Rad	Number	>1000 Rad
All cancer patients	10	126 ± 36.1	49	93 ± 30.2[a]
Head and Neck cancer patients	5	130 ± 23.2	11	108 ± 30.6
Other Cancer patients	5	122 ± 48.5	38	89 ± 29.0[b]
Untreated Healthy controls	10	129 ± 20.7	49	134 ± 33.8

[a]$p<0.01$ based on paired t-test with simultaneous healthy control samples.

[b]$p<0.01$ based on two sample t-test between irradiated and untreated patients.

Of note, the 5 head and neck cancer patients receiving less than 1000 Rad showed normal chemotaxis (Table 6), which is in contrast to the untreated patients with reduced chemotaxis (Table 4). The lack of pre-therapy evaluations of these 5 patients prevents us from determining whether X-irradiation at doses below 1000 Rad can restore chemotaxis. The effects of X-irradiation on PMN chemotaxis were further corroborated by performing serial evaluations in 7 patients. As demonstrated in Figure 1, 5/7 patients studied serially showed normal chemotaxis prior to X-irradiation. These patients experienced a profound decrease in PMN chemotaxis after X-irradiation $\geq$ 2000 Rad. Four of these patients were evaluated 3-6 months after completion of X-irradiation therapy and demonstrated normalization of their PMN functions.

In the 2 patients that showed initial decreased chemotaxis before X-irradiation, no further reduction in the chemotactic migration was observed after X-irradiation (Figure 1). Both patients had squamous carcinoma, one originating in the head and neck and the other in the lung. The changes in chemotaxis detected in patients receiving X-irradiation were significant and not explained by intra-individual variability. Four healthy subjects evaluated repeatedly showed a more restricted variability and at no time were their values below the control mean of 131±26.

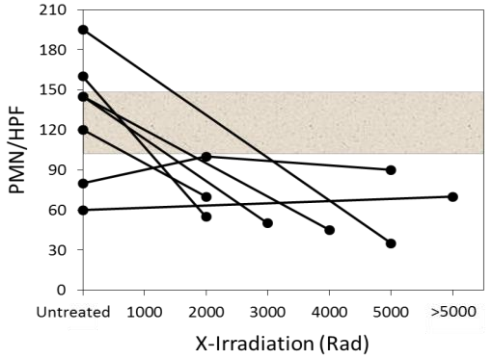

Figure Legend 1: Comparison of PMN chemotaxis before and after local body X-irradiation in 7 patients with a solid tumor. PMN were isolated from the same patients before (Untreated) and 24 hrs after X-irradiation. The numbers in Y-axis present the average number of migrated PMN per high-power field (HPF) after counting 20 high-power fields. The colored area represents the chemotactic range of PMN from healthy subjects.

There was no correlation between the effects of X-irradiation on PMN chemotaxis and lymphocyte number. Some patients with pronounced lymphopenia showed normal PMN chemotaxis and vice versa, patients

with normal lymphocyte number, showed defective PMN chemotaxis. Also, there was no correlation between chemotaxis and leukocyte numbers in any of the patient groups investigated (Table 7).

Effect of X-irradiation on PMN phagocytosis and Intracellular Killing of *Candida albicans*

X-irradiation of patients with head and neck cancer with a dose above 1000 Rad significantly impaired PMN intracellular killing of *Candida albicans*, while having no effect on phagocytosis (Table 8-9). Untreated patients with head and neck cancer and patients that received X-irradiation <1000 Rad showed normal intracellular killing of *Candida albicans* (Table 8). Patients with decreased killing capacity prior to X-irradiation did not show further reduction in this function following X-irradiation (data not shown).

Phagocytosis of *Candida albicans* was normal in all untreated and all X-irradiated patients (Table 9). They all showed similar percentage of PMN that have phagocytosed yeast particles and similar number of yeast particles per PMN (Table 9). These data clearly demonstrate that X-irradiation selectively affected only some of the PMN functions.

Table 7: Comparison of blood counts in patients with normal (N) or depressed (D) chemotaxis 24 hrs after X-irradiation. The chemotaxis numbers present the mean number of migrated PMN per high-power field after counting 20 high-power fields ± standard deviation. The numbers of blood cells present mean number (K/l) ± standard deviation.

Subjects		Numbers	Chemotaxis	WBC	PMN	Lymphocytes	Monocytes
Lung Cancer	D[a]	9	77.2±21.2	7.0±4.0	5.4±3.8	0.62±0.31	0.40±0.23
	N[b]	5	128.1±10.6	5.7±2.7	4.7±1.6	0.53±0.48	0.46±0.14
Head and Neck Cancer	D	12	88.0±19.6	7.2±2.4	5.8±2.3	1.14±0.69	0.57±0.3
	N	5	140.3±22.1	7.0±1.2	4.3±1.6	1.58±1.12	0.55±0.3
Breast Cancer	D	11	79.2±23.2	4.3±1.2	3.1±0.7	1.01±0.55	0.29±0.13
Other	D	14	74.7±19.9	6.6±4.4	4.8±4.5	1.08±0.65	0.59±0.32
	N	9	128.6±25.2	8.4±5.2	7.1±4.9	0.90±0.50	0.45±0.16

[a]D = Depressed chemotaxis

[b]N = Normal chemotaxis

Table 8: Intracellular killing of *Candida albicans* by PMN from untreated or X-irradiated head and neck cancer patients. The percentage of ingested *Candida albicans* that were killed after 2 hrs incubation with PMN was calculated by determining the viability of 200 intracellular yeast particles.

Subjects	Number	% Intracellular killing of *Candida albicans*
Healthy Controls	33	15.86 ± 7.25
Untreated Cancer Patients	14	14.8 ± 2.39
Patients X-irradiated <1000 Rad	5	12.3 ± 7.58
Patients X-irradiated >1000 Rad	30	9.16 ± 4.94[a]

[a]$p<0.01$

Table 9: Phagocytosis of *Candida albicans* by PMN from untreated or X-irradiated cancer patients. The mean number of phagocytosed yeast particles per 100 PMN is presented ± standard deviation. The mean number of Candida particle per PMN is also presented.

Subjects	Number	% Phagocytosis of *Candida albicans*	Number of Candida/cells
Healthy Controls	33	66.42 ± 13.51	1.77 ± 0.44
Untreated Cancer Patients	14	67.00 ± 14.33	1.73 ± 0.18
Patients X-irradiated <1000 Rad	6	72.33 ± 10.03	1.83 ± 0.30
Patients X-irradiated >1000 Rad	28	64.82 ± 12.04	1.67 ± 0.17

Conclusion

This study demonstrates selective abnormalities in PMN functions associated with solid cancer. Patients with head and neck cancer show significant depression of PMN chemotaxis, while phagocytosis and intracellular killing of *Candida albicans* are unaffected. In contrast, patients with breast, lung and other solid tumors show decreased intracellular killing of *Candida albicans*, while the 2 other PMN functions are normal. Local body X-irradiation above 1000 Rad induces profound impairment of PMN chemotaxis in most solid cancer patients, except for those suffering from head and neck cancer.

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Conflict of Interest

The authors declare no conflict of interest.

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