

# Effect of Microwave Irradiation (2450 MHz) on Murine Cytotoxic Lymphocyte and Natural Killer (NK) Cells



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## ABSTRACT

Male Balb/c mice were exposed in an anechoic chamber to 2450 MHz CW microwaves at 5, 10, 15, and 20 mW/cm<sup>2</sup>, 4 hours daily for 4 days, under controlled environmental conditions. T-cell cytotoxicity and natural killer (NK) cell activity were compared in exposed and sham animals. A significant decrease ( $p < 0.001$ ) of cell-mediated cytotoxicity was observed only for a power density of 20 mW/cm<sup>2</sup>. A significant increase ( $p < 0.01$ ) in NK activity was demonstrated following exposures at 15 mW/cm<sup>2</sup>.

## INTRODUCTION

Recent studies have shown that radiofrequency electromagnetic radiation (at high or low power densities) can affect immuno-competent cell systems [Baranski, 1971, 1972; Czarski, 1975; Szmigielski et al. 1975; Smialowicz, 1979; Ta-Fu Huang and Mold, 1980].

Most of the studies cited concentrated on effects on B-cells and showed that microwave exposure produced quantitative changes in B-cell subpopulations [Wiktor-Jedrzejczak, 1977]. Knowledge of cellular immunity and, particularly, of natural-killer (NK) cell activity, is of additional interest in view of the recent development of clinical applications of microwave irradiation in cancer therapy [Kim et al. 1977].

The purpose of this study was to determine consequences of microwave exposure on cell-mediated and NK-cell activity.

## MATERIALS AND METHODS

Balb/c male mice, 2 months old, were irradiated in an anechoic chamber at a frequency of 2450 MHz for 4 hours per day during 4 consecutive days. The animals were destroyed by decapitation 24 hours after the last exposure to radiation. The microwave irradiation system was described in a previous work [Santini, 1982]. Briefly, we have applied microwave radiation with a 1-kW, 2.45-GHz magnetron. Four intensities of microwaves were tested: 5, 10, 15, and 20 mW/cm<sup>2</sup> of body surface. Body surface was calculated from Kayser's [1963] formula

$$S = K \sqrt[3]{P^2}$$

where  $S$  = body surface in square centimeters,  $P$  = animal weight in grams,  $K$  = size geometric constant  $\approx 10$  for mammals. The intensities were established by a Narda powermeter (Narda Model 8221). The SAR was measured by readings of forward and reflected power for any incident radiation.

We have done two experiments for each intensity. In any experiment and for any power density a lot of 20 mice was irradiated; each animal was in an individual polyethylene container with numerous holes for ventilation; the twenty containers were in the anechoic chamber. Sham animals (20 animals) were placed in the anechoic chamber in the same way but with no irradiation. During experiments animals were maintained in darkness, without food or drink; after irradiation they were maintained routinely with food and drink ad libitum. Colonic temperatures of animals were measured at regular intervals; no significant increase was noted in the colonic temperature of microwave or sham exposed mice under the conditions used for our experiments; animal temperature fluctuated around 38°C with a wide overlap for one standard deviation. Our exposures of 5 mW/cm<sup>2</sup>, 10 mW/cm<sup>2</sup>, 15 mW/cm<sup>2</sup>, 20 mW/cm<sup>2</sup> correspond to an SAR of about 5, 7.5, 11, 14 mW/g.

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Table 1 Effect of microwave irradiation on cell mediated cytotoxicity. Results are expressed in cpm of Chrome 51 relargued  $\pm$  SEM or in percentage of target cell lysis ( $3T_3SV_{40}$ ); effector target ratio 50: statistical analysis was performed by Student's t test. For any power density and for any experiment, a lot of 40 mice (20 sham-exposed, 20 exposed) was used

Treatment power density (mW/cm <sup>2</sup> )	Experiment 1			Experiment 2		
	cpm Chrome 51 relargued	% Lysis	Significance	cpm Chrome 51 relargued	% Lysis	Significance
5 Sham-exposed	4467 $\pm$ 200	42	NS	4351 $\pm$ 98	39.9	NS
5 Exposed	4030 $\pm$ 63	40		4216 $\pm$ 93	38.2	
10 Sham-exposed	4512 $\pm$ 53.4	29.5	NS	4534 $\pm$ 72	31.14	NS
10 Exposed	4498 $\pm$ 32	30.2		4381 $\pm$ 51	29.52	
15 Sham-exposed	5210 $\pm$ 30	34.2	NS	5364 $\pm$ 78	36.72	NS
15 Exposed	5528 $\pm$ 35	34.05		5472 $\pm$ 186	40.85	
20 Sham-exposed	4781 $\pm$ 122	43.4	S $p < 0.001$	4485 $\pm$ 154	28.38	S $p < 0.001$
20 Exposed	3250 $\pm$ 63	10.5		3230 $\pm$ 76	1.76	

Table 2 Effect of microwave irradiation on NK activity. As in Table 1 but target cells were human melanoma cells

Treatment power density (mW/cm <sup>2</sup> )	Experiment 1			Experiment 2		
	cpm Chrome 51 relargued	% Lysis	Significance	cpm Chrome 51 relargued	% Lysis	Significance
5 Sham-exposed	3388 $\pm$ 60	34.5	NS	3742 $\pm$ 70	35.08	NS
5 Exposed	3551 $\pm$ 72	36		3785 $\pm$ 81	35.46	
10 Sham-exposed	4193 $\pm$ 21	22.5	NS	4246 $\pm$ 83	23.3	NS
10 Exposed	4542 $\pm$ 61	30.5		4542 $\pm$ 34	31.5	
15 Sham-exposed	4938 $\pm$ 96	21.08	S $p < 0.01$	4978 $\pm$ 78	25.8	S $p < 0.01$
15 Exposed	6666 $\pm$ 93	38.5		7122 $\pm$ 181	42.06	
20 Sham-exposed	3172 $\pm$ 74	18.1	NS	3130 $\pm$ 57	25.05	NS
20 Exposed	3131 $\pm$ 142	17.3		2865 $\pm$ 135	16.5	

Spleen lymphocytes were prepared as described in a previous paper [Deschaux et al. 1982]. Cytotoxicity of T lymphocytes was studied with the Cr<sup>51</sup> release assay. The target cells were  $3T_3SV_{40}$  for testing T cytotoxic cells [Glaser, 1979], and melanoma cells (melanome B16) for NK cells [Deschaux et al. 1982].

## RESULTS

### 1. Effect of Microwave Irradiation on Cell Mediated Cytotoxicity

The results are shown in Table 1, in which we have reported data of two experiments. The results are expressed as mean of cpm of Chrome 51 relargued (mean of 20 mice per irradiation for any lot of animals), and in percentage of lysis. The statistical analysis was done on cpm relargued with Student t test. A marked decrease of cell-mediated cytotoxicity against  $3T_3SV_{40}$  target cells was observed after an exposure of the animals to microwave irradiation. This result was observed only at a power density of 20 mW/cm<sup>2</sup>. At other power densities no variation from control values was observed.

### 2. Effect of Microwave Irradiation on NK Activity

The results are shown in Table 2; they are expressed in the same way as in paragraph 1. We observed a significant increase in natural killer activity against melanoma cells after irradiation at a power density of 15 mW/cm<sup>2</sup>.

## DISCUSSION

In a previous study [Ivancic et al. 1980] we did not affect the immune animals. Here, the cytotoxic cell-mediated cytotoxicity. Different authors [Wiktor-Huang et al. 1980] have shown that macrophages, and granulocytes result of interactions on target cells by microwaves on cytotoxicity.

The target structures of immune T lymphocytes. Cytotoxicity is necessary for the target surface [Kaplan and Calleja 1980]. Unlike cytotoxic T lymphocyte sensitization. We hypothesize that our data is not related to the target structure.

The mechanism of cell-mediated cytotoxicity seems to be related to the delivery of cytotoxic granules precisely to the target cell. Interferon which participates in the process to possess a remarkable degranulation. Our data do not allow us to propose a mechanism [Bardos et al. 1980; Deschaux et al. 1980]. Deschaux et al. 1980; Deschaux et al. 1980]. In vivo, hormones or thymic epithelial cells but not on target cells would be transformed and disappeared and it seems that T cytotoxic cells.

This different response in the destruction of cancer cells.

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## DISCUSSION

In a previous study [Ivanoff et al. 1979], we demonstrated that microwave exposure at 1 mW/cm<sup>2</sup> did not affect the immune response and that exposure at more than 20 mW/cm<sup>2</sup> led to the death of animals. Here, the cytotoxic activity of T and NK cells was modified, showing a decrease of cell-mediated cytotoxicity at 20 mW/cm<sup>2</sup> and an increase of natural killer activity at 15 mW/cm<sup>2</sup>. Different authors [Wiktor-Jedrzejczak et al. 1977; Mayers et al. 1973; Szmigielski et al. 1975; Ta-Fu Huang et al. 1980] have suggested that microwaves exert effects on T and B lymphocyte functions, macrophages, and granulocytes. The final immunological expression of microwave effects may be the result of interactions on multiple blood cells. The data from our experiments show the action of microwaves on cytotoxic cells (cytotoxic T cells and NK cells).

The target structures recognized by NK cells are different in nature from those recognized by immune T lymphocytes. Cytotoxic T cells react preferentially against histocompatible target cells and it is necessary for the targets to have molecules of the major histocompatibility complex on their surface [Kaplan and Callewaert, 1980]. NK cells demonstrate no known restriction to this complex. Unlike cytotoxic T lymphocytes, the activity of NK cells also appears to be independent of antigenic sensitization. We hypothesize that the effect of microwave irradiation on T cells and NK cells shown by our data is not related to the recognition of the target structures.

The mechanism of cellular lysis exerted by NK cells is beginning to be known. The phenomenon of cytolysis seems to be associated with active cellular secretion of possibly cytotoxic substances delivered precisely to the contact area. On contact with the target, the NK cell is capable of releasing interferon which participates in the regulation of the activity of these cells. The NK system seems thus to possess a remarkable degree of autoregulation capacity in which interferon plays a crucial role. Our data do not allow us to predict precisely where the effect of microwaves is exerted. Many authors [Bardos et al. 1980; Deschaux et al. 1982] have suggested that pre-NK cells can be considered as pre-lymphocytes T. In vivo pre-lymphocyte T differentiates into T lymphocyte by the action of thymic hormones or thymic epithelial cells on contact [Deschaux, 1980], shown by T cell markers on T cytotoxic cells but not on NK cells. Microwaves could act on pre-lymphocytes T; in a first step these cells would be transformed into NK cells. At a power of 20 mW/cm<sup>2</sup> this effect on NK cells disappeared and it seems that the irradiation could act on the differentiation of pre-lymphocytes T into T cytotoxic cells.

This different response between NK cells and T cytotoxic cells, two populations of cells involved in the destruction of cancer cells, is important in view of the use of microwaves in cancer therapy.

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## Ridged Waveguide Microwave Heating

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### ABSTRACT

This paper contains an analysis of a single pass applicator consisting of two waveguide configurations placed about 1 cm apart. The variation of the attenuation coefficient is illustrated as a function of the distance for the standard heating

### INTRODUCTION

A microwave heating system which is a waveguide structure with a uniform field. Different configurations of travelling-wave type applicators for microwave heating systems are discussed. The wave energy within the process is absorbed by the applicator.

A conventional rectangular travelling-wave applicator for sheet heating has been used. This is achieved through the use of tapered ridges [1]. However, when the process is done in a slotted rectangular waveguide, the power is absorbed in the first pass applicator for treating high-temperature sheets. This is convenient for treating sheet materials.

The present work is concerned with double ridges placed symmetrically for the heating of thin dielectric sheets. The specific power absorption coefficient, specific power absorption, and the absorbed power are investigated as a function of ridge height.

### MATHEMATICAL ANALYSIS

The available information on the propagation of the dominant TE-mode in a ridged rectangular waveguide is used. The effect of tapering the ridges on the wave number and the absorption coefficient and the absorbed power are evaluated for the configuration shown in Fig. 1.

Practically, the process is done in the center of the applicator. For uniform heating within the material must be maintained. The component of maximum amplitude

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