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MICROWAVES INDUCE AN INCREASE IN THE FREQUENCY OF COMPLEMENT RECEPTOR-BEARING LYMPHOID SPLEEN CELLS IN MICE[1]

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A single 30-min exposure of mice to 2450 MHz microwaves (12 to 15 mW/g body weight) in an environmentally controlled waveguide facility induced a significant increase in the proportion of complement-receptor positive lymphoid cells in the spleen. This effect was further enhanced by repeated (three times) exposures, which in addition produced a significant increase in the proportion of Ig^+ cells. The proportion of θ-positive cells and the total number of spleen cells remained unchanged.

Several studies have shown that low doses (0.5 to 5 mW/cm²) of microwaves affect the immune system both *in vitro* and *in vivo* (1, 2). This includes nonspecific blastoid transformation of lymphoid cells irradiated *in vitro* (1) and an increase in the proportion of blast-like lymphoid cells in the peripheral blood of rabbits exposed *in vivo* (2). Bone marrow from guinea pigs exposed to microwaves daily for 2 weeks subsequently showed a disturbance of the rhythm of diurnal mitoses (2). Mice exposed for 6 to 12 weeks and subsequently immunized with SRBC showed an increase in IgM-secreting cells as compared with nonexposed immunized mice (2). Recently, a new microwave dosimetry system (3) has been developed which characterizes the exposure conditions, not in terms of field density (in mW/cm²) to which an animal was exposed, but in terms of amount of energy actually absorbed by the animal as the absorbed dose rate (in mW/g body weight). Simultaneously, an environmentally-controlled waveguide facility has been developed (4) in which on-line measurements of forward, reflected, and transmitted power allow the calculation of precise absorbed dose rate. With rapid developments of new, sophisticated methods to determine the immune capacity of animals, as well as improvements in microwave dosimetry, we were encouraged to evaluate the potential effects of microwaves on the immune system. The problem identified for this study was the effect of microwaves on the quantitative relationships between different lymphoid cell populations.

MATERIALS AND METHODS

Irradiation. The exposure of animals to 2450 MHz-amplitude modulated 12 Hz microwaves was performed with a forward power of 0.6 watts in an environmentally-controlled waveguide facility (4). A Hewlett-Packard (Palo Alto, Calif.) 8616A signal generator with a 491C amplifier tuned to 2450 MHz ± 500 Hz, using a tuneable coherent synchronizer model 251 (Sage Laboratories, Natick, Mass.), was used as a microwave source. A single mouse was exposed at a time in a position "head to tail" to the irradiation source, restrained in a plastic (polystyrene) holder, which was previously found not to absorb or reflect microwaves. Weight of the animals was measured before exposure, and rectal temperature was determined before and after exposure with the use of an electric model 46 TUC telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). During exposure, a constant temperature of 25 ± 0.5°C and relative humidity of 50 ± 5% were maintained with a constant airflow of 38 liters/min inside the waveguide. The exposure duration was 30 min and during exposure, the forward, reflected, and transmitted power was measured with power meters. From these measurements and the weight of the animals, the absorbed dose rate was calculated for each mouse (for each exposure separately) by using an IBM computer. Since the facility used allowed exposure of only one mouse at a single time, it was only possible to expose nine mice a day. Experiments were repeated at least two times and the results were evaluated together, resulting in a standard error usually far below 10%. In the study of the effects of a single exposure to microwaves, the average absorbed dose rate ($\bar{x}$ ± SD) for the 1st series of experiments was 14.4 ± 1.3 mW/g and for the 2nd series, 12.7 ± 0.7 mW/g. The rectal temperature difference (before and after exposure), respectively, ranged from −0.1°C to −0.5°C. In the study of the effects of a triple exposure to microwaves, the average absorbed dose rates (mean ± SD) for animals of the 1st series were 15.6 ± 1.2 mW/g (1st exposure); 15.5 ± 0.9 mW/g (2nd exposure); and 13.3 ± 0.6 mW/g (3rd exposure). For animals of the 2nd series, the average absorbed dose rates were 13.9 ± 0.9 mW/g (1st exposure); 12.8 ± 1.2 mW/g (2nd exposure); and 12.3 ± 2.0 mW/g (3rd exposure). Rectal temperature difference (before and after exposure) were: 1st series, 1st exposure,

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TABLE I

The effect of a single exposure to 2450 MHz microwaves on the total cell number and the propotions of θ^+ and Ig^+ cells in murine spleens

Day Post Exposure	No. of Animals Per Group	Total Spleen Cell No. ($\times 10^6$) Control/Exposed ($\bar{x} \pm$ S.E.)	% Ig-Positive Control/Exposed ($\bar{x} \pm$ S.E.)	% Theta-Positive Control/Exposed ($\bar{x} \pm$ S.E.)
3	6	163.7 ± 12.5 / 160.5 ± 10.7	47.4 ± 2.7 / 46.7 ± 3.1	32.9 ± 2.0 / 35.5 ± 2.5
6	6	160.4 ± 15.4 / 167.6 ± 14.8	51.6 ± 2.9 / 53.9 ± 2.7	37.8 ± 1.7 / 34.7 ± 2.3
9	6	167.2 ± 10.8 / 159.9 ± 9.7	50.8 ± 3.3 / 50.8 ± 1.7	33.1 ± 2.4 / 33.3 ± 3.2

−0.06°C; 2nd exposure, +0.02°C; and 3rd exposure, −0.09°C. During the 2nd series: 1st exposure, −0.1°C; 2nd exposure, −0.2°C; and 3rd exposure, +0.05°C. Sham-exposed animals served as controls. They were handled in the same manner as microwave-exposed animals, except the power was not turned on.

Cell suspensions. At desired days after exposure to microwaves, mice were sacrificed by cervical dislocation, their spleens were removed, and spleen cells were obtained by gentle teasing with a rubber policeman into Hanks' balanced salt solution (HBSS). Cell aggregates were disrupted by passing the cell suspension through a 26-gauge needle. The cell suspension was then washed with RPMI 1640, resuspended in 5 ml of this medium, and cell counts were determined with a Fisher Autocytometer (Fisher Scientific, Silver Spring, Md.).

Surface markers. The proportion of cells bearing surface immunoglobulin (Ig^+)[4] was determined as previously described (5) by immunofluorescence using fluorescent-labeled rabbit anti-mouse-κ (Fl-anti-κ). The proportion of cells bearing θ antigen on their surface (θ^+) was determined by an indirect method (6). The sum of θ^+ and Ig^+ cells in the spleen was detected by treating the cells first with anti-θ (anti-Thy 1.2) serum and then developed with Fl-anti-κ. The number of θ^+ cells was estimated by subtracting the number of cells stained with Fl-anti-κ alone from those stained by anti-θ plus Fl-anti-κ. The proportion of complement receptor bearing (CR^+) cells was determined by the method of Bianco *et al.* (7), as previously described (8). Briefly, spleen cells were incubated with EAC reagent for 45 min in 37°C and proportions of cells forming rosettes (five or more erythrocytes attached to lymphoid cells) were determined. EAC reagent was made of sheep red blood cells (E), 19S human anti-sheep red blood cell antibody (A), and fresh mouse serum as source of complement (C). EAC reagent contained 0.01 M EDTA (9) to prevent binding of EAC complexes by granulocytes or macrophages. Net percentage of increase of CR^+ cells in microwave-exposed mice was calculated as:

$$\frac{\%\ CR^+ \text{ in microwave-exposed} - \%\ CR^+ \text{ in sham-exposed}}{\%\ CR^+ \text{ in sham-exposed}} \times 100.$$

RESULTS

The effects of a single exposure of mice by 2450 MHz microwaves on the total spleen cell number and proportions of Ig^+ and θ^+ cells are shown in Table I. No significant changes were observed in the proportions of Ig^+ and θ^+ cells at days 3, 6, and 9 after a single exposure, as compared to sham-exposed animals. The total number of cells per spleen also remained unchanged.

Changes in the proportion of CR^+ cells after a single exposure are shown in Figure 1*a*. There was a statistically significant increase in the proportion of CR^+ cells on day 6 after exposure (a net increase of 33.5%), whereas the frequency of CR^+ on days 3 and 9 after exposure did not differ significantly, as compared to values obtained with sham-exposed mice.

In another series of studies, groups of CBA/J adult male mice were exposed to 2450 MHz microwaves for 30 min daily, on 3 days, separated by 3-day intervals, in the same conditions as described above. Subgroups of six animals each were sacrificed and assayed on days 3, 6, and 9 after the last exposure. As seen in Table II, there were no significant changes in total spleen cell number and the proportion of θ^+ cells on any of these days. A significant increase in the proportion of Ig^+ cells was noted on day 6 after the last exposure, whereas on days 3 and 9 the proportion of Ig^+ cells, although higher in exposed animals, did not differ significantly from sham-exposed animals. In contrast, the proportion of CR^+ cells, as seen in Figure 1*b*, was significantly increased on each (3, 6, and 9) day after the last exposure, being highly significant on day 6 ($p < 0.001$).

The increase in the frequency of CR^+ cells after exposure to microwaves was not secondary to microwave-induced increase in body temperature. Moreover, the temperature inside the waveguide was maintained at 25°C with a constant airflow of 38 l/min, which caused the body temperature after exposure to be slightly lower than before exposure, as confirmed by measurements of rectal temperature (see *Materials and Methods* section).

DISCUSSION

Despite the fact that it has been proposed that CR may serve as an activation structure for B cells (10) and that CR^+ B cells respond mostly to T-dependent antigens (11), it is difficult at present to assess the biologic significance of the increase in CR^+ lymphoid cells. According to Hämmerling *et al.* (12), CR represents a marker of a maturational stage of B lymphocytes occurring in the ontogenetic development between the Ig^+Ia^+ cell and the plasma cell. The same authors were able to induce an increase in CR^+ cells with a short-term incubation (150 min) of a fraction of spleen cells with *Escherichia coli* lipopolysaccharide (LPS) (12). This effect was interpreted by them as a consequence of maturation of the $Ig^+Ia^+CR^-$ cell already present in the spleen into the $Ig^+Ia^+CR^+$ cell. On the other hand, the increase in the proportion of CR^+ cells is evident after several days after exposure of mice to microwaves. This allows sufficient time for the involvement of cell proliferation and would correspond with earlier reports (1, 2). However, since there was no quantitative increase in the total spleen cell number, present data argue against the role of nonspecific cell proliferation in the increase of the frequency of CR^+ cells.

Various mechanisms should be considered as possibly responsible for the increase in the proportion of CR^+ cells after exposure to microwaves: i) Direct or indirect stimulation of conversion of CR^- precursor cells into CR^+ cells; ii) inhibition

[4] Abbreviations used in this paper: Ig^+, surface immunoglobulin; Fl-anti-κ, fluorescent-labeled rabbit anti-mouse-κ serum; θ, θ antigen; anti-Thy 1.2, antiserum against θ antigen; CR^+, complement receptor.

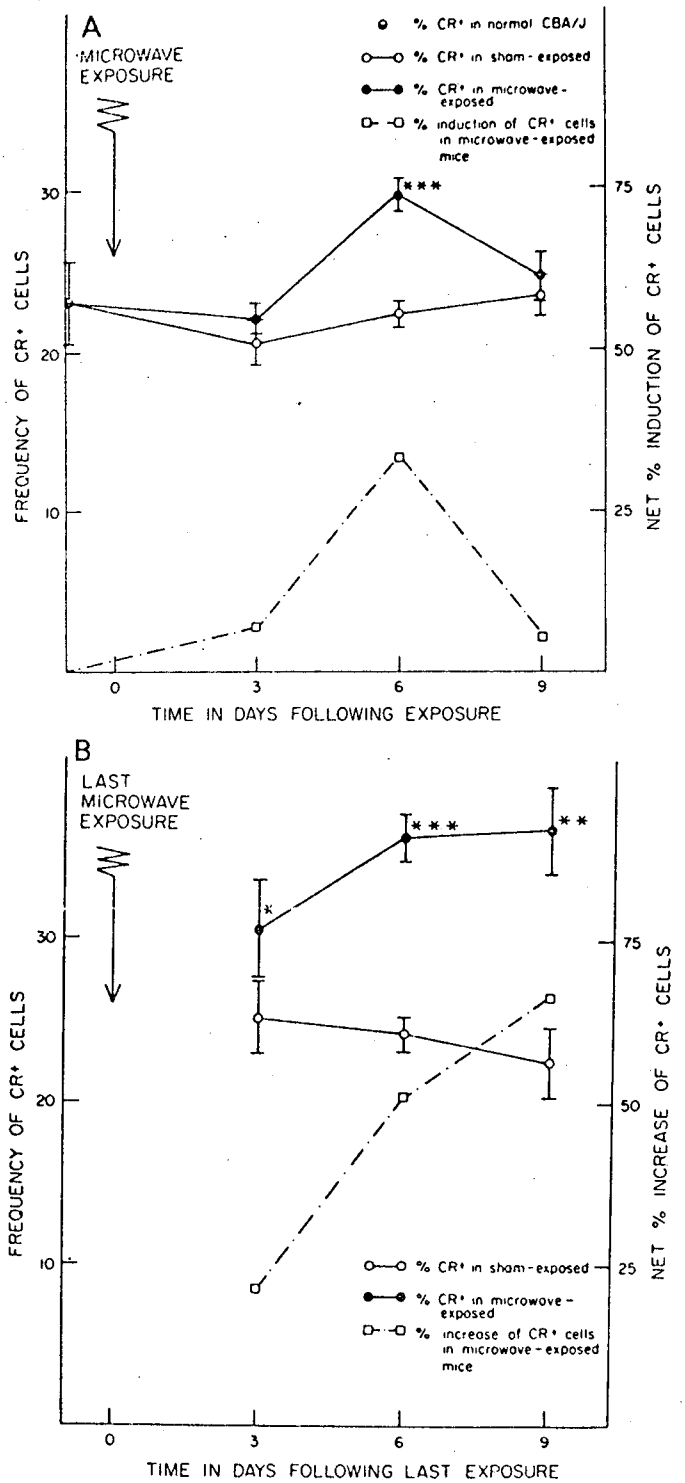

Figure 1. Effect of a single (*A*) and a triple (*B*) exposure to 2450 MHz microwaves on the proportion of CR^+ lymphoid cells in mouse (CBA/J) spleens. * Significant $p < 0.05$; ** significant $p < 0.01$; *** significant $p < 0.001$.

of further maturation of CR^+ cells into CR^- plasma cells; therefore, a subsequent accumulation of B cells in the CR^+ stage; iii) it cannot be excluded that there are cell-free CRs present in circulation which may be specifically or nonspecifically adsorbed to spleen cell surface membranes altered by interaction with microwaves in a manner independent of actual functional characteristics of the cell; and iv) there may be a direct effect on the B cell membrane which triggers CR expression.

It is known that macrophages in mouse spleen also possess CR (9). The formation of EAC rosettes by macrophages, in contrast to lymphocytes, is Ca^{++} dependent and, since the EAC reagent used for the detection of CR^+ cells in the present study contained EDTA, the increase of CR^+ cells in our data is not due to macrophages, but represents changes in the lymphoid cell subpopulation.

Although there are reports that at least some CR^+ cells found in murine spleen cells are T cells (θ^+) (13, 14), our own experience (to be published elsewhere), as well as data published by Gelfand *et al.* (15), suggest that 98% of EAC rosettes in mouse spleen are Ig^+ and θ^-. Also, a lack of increase in the proportion of θ^+ cells and concomitant significant increase in Ig^+ cells 6 days after triple exposure to microwaves suggest that the effect observed in these studies involves B cell CR^+ population.

It is impossible at present to relate the observed effect to safety limits currently in force (10 mW/cm² in the U.S.A.), since safety limits were introduced originally for far-field situations, whereas in our system animals were exposed in near-field conditions for only 30 min one or three times. There are unique properties of microwave penetration in animal bodies where the wavelength shortens and the field may be disturbed by a number of reflections during penetration from one tissue to the other; e.g., from the fat tissue to the muscles, with possible formation of standing waves (16, 17). Nonetheless, the observed increase in the proportion of CR^+ cells was consistent and reproducible.

Further studies on the mechanism underlying the increase in the mouse CR^+ spleen cell population and on changes in the function of the immune system associated with observed effects of exposure to microwave radiation are in progress.

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REFERENCES

1. Stodolnik-Baranska, W. 1967. Lymphoblastoid transformation of lymphocytes *in vitro* after microwave irradiation. Nature 214:102.

TABLE II

The effect of three exposures to 2450 MHz microwaves on the total cell number and the proportions of theta⁺ and Ig⁺ cells in murine spleens

Day Post Last Exposure	No. of Animals Per Group	Total Spleen Cell No. (× 10^6) Control/Exposed (x ± S.E.)	% Ig-Positive Control/Exposed (x ± S.E.)	% Theta-Positive Control/Exposed (x ± S.E.)
3	6	158.7 ± 14.3 / 162.2 ± 10.9	48.7 ± 2.3 / 52.7 ± 2.7	36.1 ± 2.6 / 39.0 ± 2.6
6	6	167.4 ± 9.1 / 161.7 ± 12.7	48.9 ± 1.4 / 59.4 ± 2.4	35.5 ± 1.4 / 30.9 ± 1.8
9	6	164.7 ± 8.7 / 167.1 ± 17.8	46.2 ± 1.5 / 52.5 ± 2.3	34.3 ± 1.6 / 34.4 ± 3.6

2. Czerski, P. 1975. Microwave effects on the blood-forming system with particular reference to the lymphocyte. Ann N.Y. Acad. Sci. 247:232.
3. Youmans, H. D., and H. S. Ho. 1975. Development of dosimetry for RF and microwave radiation-I: Dosimetric quantities for RF and electromagnetic fields. Health Phys. 29:313.
4. Ho, H. S., E. I. Ginns, and C. L. Christman. 1975. Environmentally controlled waveguide irradiation facility. IEEE Trans. Microwave Theor. Techn., MTT21:837.
5. Scher, I., A. Ahmed, D. M. Strong, A. D. Steinberg, and W. E. Paul. 1975. X-linked B-lymphocyte immune defect in CBA/HN mice. I. Studies of the function and composition of spleen cells. J. Exp. Med. 141:788.
6. Spear, P. G., A. L. Wang, U. Rutishauser, and G. M. Edelman. 1973. Characterization of splenic lymphoid-cells in fetal and newborn mice. J. Exp. Med. 138:557.
7. Bianco, C., R. Patrick, and V. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. J. Exp. Med. 132:702.
8. Strong, D. M., J. N. Woody, M. A. Facktor, A. Ahmed, and K. W. Sell. 1975. Immunological responsiveness of frozen-thawed human lymphocytes. Clin. Exp. Immunol. 21:442.
9. Lay, W. H., and V. Nussenzweig. 1969. Ca^{++}-dependent binding of antigen-19 S antibody complexes to macrophages. J. Immunol. 102:1172.
10. Dukor, P., and K. U. Hartmann. 1973. Hypothesis-bound C_3 as second signal for B-cell activation. Cell. Immunol. 7:349.
11. Lewis, G. K., R. Ranken, D. E. Nitecki, and J. W. Goodman. 1976. Murine B-cell subpopulations responsive to T-dependent and T-independent antigens. J. Exp. Med. 144:382.
12. Hämmerling, U., A. F. Chin, and J. Abbott. 1976. The ontogeny of murine B lymphocytes. II. The sequence of B cell differentiation from Ig^- precursors to plasma cells. J. Immunol. In press.
13. Arnaiz-Villena, A., M. I. C. Gyöngyössy, and J. H. L. Playfair. 1974. Rosette formation by mouse lymphocytes. II. T-cell specificities in a CRL subpopulation. Clin. Exp. Immunol. 18:177.
14. Arnaiz-Villena, A., and F. C. Hay. 1975. Complement receptor lymphocytes. Analysis of immunoglobulin on their surface and further evidence of heterogeneity. Immunology 28:719.
15. Gelfand, M. C., G. J. Elfenbein, M. M. Frank, and W. E. Paul. 1974. Ontogeny of B-lymphocytes. 2. Relative rates of appearance of lymphocytes bearing surface immunoglobulin and complement receptors. J. Exp. Med. 139:1125.
16. Baranski, S., and P. Czerski. 1976. Biological Effects of Microwaves, Dowben, Hutchison & Ross, Stroudsburg. P. 234.
17. Johnson, C. C., and A. W. Guy. 1972. Nonionizing electromagnetic wave effects in biological materials and systems. Proc. IEEE 60:692.