AD-A076 768 GEORGETOWN UNIV WASHINGTON DC LOMBARDI CANCER CENTER F/G 6/18
EFFECT OF MICROWAVES ON THE IMMUNE SYSTEM.(U)
SEP 79 K SULEK , W WIKTOR-JEDRZEJCZAK N00014-78-C-0278
UNCLASSIFIED TR-1 NL

1 OF 1
AD A076768

END
DATE FILMED
12-79
DDC

OFFICE OF NAVAL RESEARCH

Contract N00014-78-C-0278

Task No. NR201-337

(9) TECHNICAL REPORT NO. 1

(6) EFFECT OF MICROWAVES ON THE IMMUNE SYSTEM

(5) LEVEL II

(14) TR-1

By

(10) Kazimierz Sulek, M.D., Ph.D.,
Wieslaw Wiktor-Jedrzejczak, M.D., Ph.D.,
Allan H. Smith Ph.D.,
[redacted]
Philip L. Calcagno M.D.

Immunologic Oncology Division
Lombardi Cancer Center
and the
Department of Pediatrics
Georgetown University School of Medicine
3900 Reservoir Rd., N.W.
Washington, D.C. 20007

(12) 10

(11) 15 Sep[redacted]79

The research reported here has been supported under
(15) ONR Contract N00014-78-C-0278 with funds provided by
the Navy Medical Research and Development Command

EFFECT OF MICROWAVES ON THE IMMUNE SYSTEM

A. ABSTRACT

Over the past year we have been involved in the evaluation of the effects of microwave exposure on various population of immune cells, with particular reference to lymphocytes bearing complement receptors (CR+). From our previous observations, it is known that microwave exposure increases the frequency of CR+ spleen cells in exposed mice as compared to sham-exposed mice. In ~~the studies reported here~~ this report, the threshold level of absorbed energy required to trigger the inductive events was determined. It was found that a minimum of a single 15 minute exposure (0.6 W, 11.8 W/kg, 10.6 J/g) or a single 30 minute exposure (0.3 W, 5.0 W/kg, 9.07 J/g) induced a significant increase of CR+ cells on day three which peaked on day six. It was also found that once the critical threshold level of absorbed energy was obtained, the induced response was maximum. In evaluating the effect of sub-threshold doses it was found that the absorption of multiple subthreshold quantities of microwave energies was cumulative, inducing and increase in CR+ cells, providing the exposures occurred within one hour of one another.

In a subsequent series of experiments, evidence was accumulated to suggest that susceptibility to the inductive increase in splenic CR+ cells, following exposure to 2450 MHz microwaves, is under the control of a gene (s) closely linked to genes on chromosome 17 of the mouse that are near the mouse major histocompatibility complex known as H-2. All responsive strains of mice tested were of the particular $H\text{-}2^k$ type, while mice of the $H\text{-}2^a$, $H\text{-}2^b$, $H\text{-}2^d$, and $H\text{-}2^l$ haplotypes, were refractory to the microwave induced increases in CR+ cell.

In attempting to evaluate the mechanisms responsible for these changes, we examined the role of endotoxin, which might be released from the microwave irradiated intestinal tract, and the role of hydrocortisone, as the animals are stressed during the microwave exposure. It was found that endotoxin produced an increase in CR+ cells in strains of mice (non $H\text{-}2^k$) which failed to respond to microwaves, and microwave exposure induced an increase in CR+ strains of mice unable

to respond to endotoxin, indicating that it is unlikely that endotoxin plays a major role in this event. It was also shown that the microwave induced increases in CR+ cells were not mimicked by the intraperitoneal injection of hydrocortisone, suggesting that this hormone is unlikely to be responsible for the observations.

These studies indicate that microwaves do induce changes in cell surface receptors, and that susceptibility to these changes is under genetic control and that it is unlikely that endotoxin and cortisone play a significant role in these increases.

B. INTRODUCTION

The following technical report summarizes the first year's progress on ONR Contract N00014-78-C-0278 the "Effect of Microwaves on Various Cell Types Within the Immune System". The growing interest of scientists in the biological effects of microwave irradiation is derived from its rapidly expanding practical application in the military and industrial sectors. Despite its increasing use, little is known about the biological effects of microwave radiation on the immune system. Since this system plays an important role in the homeostasis of living organisms, especially in defense against infectious agents, knowledge of the biological effects of low dose microwave exposure on the immune system would be valuable. During recent years we have developed a system to examine some of the immunologic consequences of exposing mice to various types of radiofrequency radiation. We have shown that near field exposure to 2450 MHz microwaves at a forward power of 0.6W induced marked increases in the complement receptor positive (CR+) and Fc receptor positive (FcR+) spleen cells. Little effect on the number and functional characteristics of thymus derived (T) cells was found following single or multiple exposures and we therefore decided to determine the threshold conditions needed to trigger the increase in splenic CR+ cells in more detail, and determine whether multiple subthreshold doses of microwaves were cumulative.

C. RESEARCH DESIGN

The attached manuscripts, currently submitted for publication contain the detailed data. In this section the results will be summarized as they relate to the contract structure.

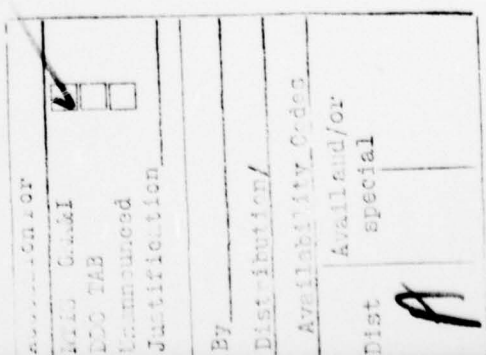
The work accomplished thus far has been in collaboration with researchers at the Bureau of Radiologic Health, Food and Drug Administration, Rockville, MD and the researchers at the Naval Medical Research Institute, Bethesda, MD. The research goals of this contract were to proceed through a number of areas of interest.

1. Identification of quantitative changes in the cell populations using cell surface markers for the study of differentiated cells.
2. Definition of threshold exposure conditions for the appearance of any previously identified effects.
3. Examination and definition of the mechanism responsible for these effects.
4. Genetic predisposition for the potentially harmful or beneficial effect.
5. Methods for the prevention and/or enhancement of effect of microwave exposure on cells within the immune system.

D. RESULTS

1. Identification of quantitative changes in the cell populations of the lymphoid system.

In previous experiments it had been shown that a single exposure of CBA/J mice to 2450 MHz microwave produced a significant increase (up to 40%) in the frequency of both CR+ cells and FcR+ cells. Using the combined immunofluorescence-rosette technique, we observed that the majority of microwave induced CR+ cells,



also stained positive for surface immunoglobulin, and are therefore by definition B cells (derived from bone marrow precursors). It was subsequently found that these microwave induced increases in CR+ cells, take place not only in the splenic lymphoid population, but also in peripheral blood, peyer's patches and lymph nodes, but not in the bone marrow or thymus of adult exposed mice.

2. Definition of threshold exposure conditions for the appearance of the Microwave induced effects in the immune system.

Using the induction of CR+ cells in the spleen as a marker of biologic effect of microwaves allowed us to evaluate the critical threshold level of absorbed energy required for the induction of these cells. When a threshold of 9 to 10 J/g was obtained, using either variation of exposure duration or increased forward power, the induced response was maximal. Multiple subthreshold exposures were cumulative provided that exposures occurred within one hour of another. This effect was independent of absorbed dose rate or weight of the exposed animal as well as the spatial orientation of the animal toward the irradiation source.

3. Examination and definition of the underlying mechanisms for microwave effects in the immune system.

In our previous studies, it was found that cell proliferation was not directly involved in the generation of microwave induced increases in CR+ cells in the spleens of exposed mice. It was therefore suggested that the increase in CR+ cells was a purely maturational effect. It was also suggested, that such events may have been produced indirectly by the endotoxin release from damaged intestinal tissue, induced by the microwave exposure, rather than a direct effect of microwaves on the cell population. To study this problem, we utilized certain mutant strains of mice, genetically unable to respond to endotoxin (CBA/N, C_3H/hej), and were able to show

that these strains of mice responded well to microwave in terms of increasing CR+ cells in the spleen, but not to endotoxin. This would indicate that endotoxin plays a minor role in the generation of CR+ cells. It was subsequently suggested that internal release of corticosteroids, due to the stressful conditions of exposure, caused the increase in CR+ cells. This hypothesis was evaluated by injecting animals with various doses of hydrocortisone, and showing that this material in no way mimicked the effect of microwave exposure.

We are unable at this time to describe the mechanism of action of microwaves on the various cell population. We do not know whether this is a direct action on the cell, or a response mediated via some circulating factor, however we propose to continue our research in this particular area. Knowledge of the mechanism of action would be useful in designing safety standards and protective reagents.

4. Genetic predisposition for microwave effects on the immune system.

It would be useful to know if the observed effects were under genetic control, which might indicate that certain individuals would be more or less susceptible to microwave induced changes, based on their genetic type. Using various inbred, mutant-inbred, and congenic strains of mice, it was found that sensitivity to microwave induced CR+ cell increases is under genetic control. In particular, the H-2 (histocompatibility) type of the exposed mice was shown to play an important role. This suggests that genes on chromosome number 17, close to those of the mouse major histocompatibility complex (H-2) are responsible for determining animal susceptible to the microwave induced events. All of the $H\text{-}2^{k}$ strains of mice were susceptible, while most other strains tested were not.

Further studies aimed at the more precise definition of genes controlling the response to microwave exposure will be done, including backcross breeding of F1 and F2 hybrid animals between responding and non-responder strains, and an evaluation of the segregation of genes determining positive responses in these mice. This kind of study will tell us how many genes are involved in the control and will allow for preliminary mapping.

CONCLUSIONS FROM THE COMPLETED RESEARCH

The research completed thus far indicates that single doses of microwaves produce quantitative changes in the various cell populations within the hematopoetic system, particularly in the B cell population. We have defined threshold exposure conditions for the appearance of these changes which are at least partially under genetic control. Although the exact cellular mechanism responsible for these effects is still poorly understood, considerable progress has been made in excluding such possibilities as the intermediary action of endotoxin or corticosteroids. These studies are the first that utilized detailed and sophisticated immunological technology in microwave biomedical research. Simultaneously, they represent some of the pioneer work in the area of the radiobiology of microwaves. Further continuation of these studies should lead to the understanding of mechanisms responsible for the effects observed, their classifications in terms in potential health hazards and eventually the development of methods to prevent or alter susceptibility.

PAPERS AND ABSTRACTS

K. Sulek, A. Ahmed, C. Schlagel, W. Wiktor-Jedrzejczak, H. Ho, W.M. Leach, Microwave radiation and immune response. 4th FDA Science Symp. Proc. (in press).

K.Sulek, C.J. Schlagel, W. Wiktor-Jedrzejczak, H.S. Ho, W.M. Leach, A. Ahmed, J.N. Woody, Biologic effects of microwave exposure. I. Threshold conditions of the increase in complement receptor positive (CR+) mouse spleen cells following exposure to 2450 MHz microwaves. (Submitted for publication).

C.J. Schlagel, K. Sulek, H.S. Ho, W.M. Leach, A. Ahmed, J.N. Woody, Biologic effects of microwave exposure. II. Studies on the Genetic control of susceptibility to microwave induced increases in complement receptor positive (CR+) mouse spleen cells following exposure to 2450 MHz microwaves. (manuscript in preparation).

C.J. Schlagel, K. Sulek, A. Ahmed, H. Ho, W. Leach, Kinetics and mechanisms of the induction of an increase in complement receptor positive (CR+) mouse spleen cells following a single exposure to 2450 MHz microwaves. URSI Bioelectromagnetic Symp. Seattle, 1979, Abstract. P. 478.

C.J. Schlagel, T. Folks, J. Woody, W. Leach, K. Sulek, Enhancement of natural killer cell activity in mice after exposure to microwave radiation. FASEB Annual Meeting, Dallas 1979, Fed. Proc., 38, Abstract 3625 (1979).

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: 1	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): Effect of Microwaves on the Immune System	5. TYPE OF REPORT & PERIOD COVERED: Interim (annual)
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Kazimierz Sulek, M.D., Ph.D., Wieslaw Wiktor-Jedrzejczak, M.D., Ph.D. Allan H. Smith, Ph.D. and Philip L. Calcagno, MD	8. CONTRACT OR GRANT NUMBER(s): N00014-78-C-0278
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Immunologic Oncology Division, Lombardi Cancer Center & Dept. of Pediatrics, Georgetown University, Washington, D.C. 20007	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: Task No. NR201-337
11. CONTROLLING OFFICE NAME AND ADDRESS: Office of Naval Research, Biophysics (Code 444), 800 N. Quincy Street, Arlington, VA 22217	12. REPORT DATE: 15 September 1979
	13. NUMBER OF PAGES: 8
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report)
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

See enclosed list.

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Microwave, immunology, mouse model, compliment receptors, lymphocytes.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Over the past year this contract has focused on the evaluation of the effects of microwave exposure on various populations of immune cells, with particular reference to lymphocytes bearing complement receptors (CR+). The findings were

(1) a threshold level for absorbed microwaves energy required to increase the frequency of CR+ cells was determined.

(2) once this threshold was reached, no further increase in microwave energy increases the number of CR+ cells.

(over)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Enter

(3) multiple subthreshold exposures could induce an increase in CR+ cells;

(4) the sensitivity to microwave induced CR+ increase was under the influence of H-2 genes;

(5) the mechanisms for the increase in CR+ cells is not related to endotoxin release or hydrocortisone increase.