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Lymphomas in Eμ-*Pim1* Transgenic Mice Exposed to Pulsed 900 MHz Electromagnetic Fields

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Whether radiofrequency (RF) fields are carcinogenic is controversial; epidemiological data have been inconclusive and animal tests limited. The aim of the present study was to determine whether long-term exposure to pulse-modulated RF fields similar to those used in digital mobile telecommunications would increase the incidence of lymphoma in Eμ-*Pim1* transgenic mice, which are moderately predisposed to develop lymphoma spontaneously. One hundred female Eμ-*Pim1* mice were sham-exposed and 101 were exposed for two 30-min periods per day for up to 18 months to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms. Incident power densities were 2.6–13 W/m^2 and specific absorption rates were 0.008–4.2 W/kg, averaging 0.13–1.4 W/kg. Lymphoma risk was found to be significantly higher in the exposed mice than in the controls (OR = 2.4, P = 0.006, 95% CI = 1.3–4.5). Follicular lymphomas were the major contributor to the increased tumor incidence. Thus long-term intermittent exposure to RF fields can enhance the probability that mice carrying a lymphomagenic oncogene will develop lymphomas. We suggest that such genetically cancer-prone mice provide an experimental system for more detailed assessment of dose–response relationships for risk of cancer after RF-field exposure. © 1997 by Radiation Research Society

INTRODUCTION

Concern has been expressed for a number of years that exposure to radiofrequency (RF) fields emanating from telecommunications devices, heating equipment and radar and television transmitters may increase the incidence of cancer in humans. Epidemiological studies have not indicated an increased cancer risk, but the methodology and exposure assessments are generally considered to have been suboptimal (*1–3*).

The mechanisms presently known by which normal cells are transformed into neoplastic cells involve alterations to the structure of somatic cell DNA such as point mutations, translocations, deletions, amplifications and retroviral provirus insertions (*4, 5*). Experiments reviewed for the World Health Organization (*2*) and for the National Radiological Protection Board of the UK (*1*) did not demonstrate convincingly any direct damage to DNA after acute or chronic exposure of biological systems to RF fields. In particular, when temperatures were maintained within normal physiological limits, no evidence for induction of DNA breaks or chromosome aberrations was found. On the other hand, two recent studies have suggested that RF fields can affect DNA. In the first, Sarkar *et al.* (*6*) found evidence of an alteration in the length of a DNA microsatellite sequence in brain and testis cells of mice exposed to 2.45 GHz fields at a specific power absorption rate (SAR) of 1.2 W/kg for 2 h/day for up to 200 days. In the second, Lai and Singh (*7*) reported the occurrence of single-strand breaks in rat brain DNA shortly after the animals had been exposed for 2 h to pulsed or continuous-wave 2.45 GHz fields with SARs of 0.6 or 1.2 W/kg. Until these results and their interpretation are confirmed, doubt will remain as to whether RF fields can induce any of the types of genetic change in cells that lead to malignancy.

A number of studies in experimental animals have sought to determine directly whether RF fields can affect the development of cancer. Szmigielski *et al.* (*8*) and Szudzinski *et al.* (*9*) reported that chronic exposure of mice to RF fields (2.45 GHz, SAR 2–8 W/kg, 2 h/day, 5–6 days per week for up to 12 months) accelerated the development of metastatic colonies from transplanted sarcoma cells and increased the incidence of primary mammary tumors in predisposed animals and of skin tumors induced with 3,4-benzopyrene. Further work by this group (*10*) found that similar exposures increased the number of hepatomas, sarcomas and skin tumors in mice treated with chemical carcinogens. On the other hand, Wu *et al.* (*11*) were unable to demonstrate significant enhancement of colon carcinogenesis by

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dimethylhydrazine in mice chronically exposed to 2.45 GHz fields, and two other studies of transplanted melanoma (*12*) and brain tumors (*13*) in mice likewise failed to find significant effects of 2.45 GHz or 915 MHz fields, respectively. Furthermore, a large study of rats exposed for 21.5 h/day for 2 years to 2.45 GHz fields pulsed at 800 Hz and producing SARs of 0.15–0.4 W/kg did not show any alterations in over 150 parameters of health and longevity (*14*). No single type of tumor was increased in frequency to a statistically significant extent in the exposed animals. The overall incidence of malignancies was raised significantly, but the authors of the study (*14*) questioned the biological significance of this finding because the higher incidence levels of specific malignancies were similar to those reported previously for unexposed rats of the strain used.

The overall conclusion from the studies published so far is that uncertainty persists as to whether exposure to RF fields can influence the process of carcinogenesis. One way of attempting to resolve this issue is to perform further tests under carefully controlled conditions using large numbers of animals with a genetic predisposition to develop tumors, the incidence of which is greatly increased by weakly carcinogenic influences. Transgenic mice expressing an activated *Pim1* oncogene in their lymphoid cells seemed to fulfill these criteria for malignant lymphoma (*15*, *16*). We therefore performed a study designed to test whether long-term exposure of Eμ-*Pim1* mice to pulse-modulated 900 MHz fields can increase the incidence of lymphoma. The pulse modulation and the frequency were selected to correspond to those of the recently introduced digital system of cellular mobile telecommunications. This paper describes the experimental system and the results, which show a moderate but statistically significant increase in lymphomas in the exposed animals.

MATERIALS AND METHODS

Mice

The characteristics of the ppG64 strain of Eμ-*Pim1* transgenic mice have been described (*15–17*). Virgin, hemizygous-transgenic females and nontransgenic C57BL/6NTac females were purchased from GenPharm International (Mountain View, CA). From an original mixed genetic background derived from two mouse strains (C57BL/LiA and CBA), the transgenic mice used here were the product of the fourth successive backcross with the inbred wild-type C57BL/6NTac strain and were therefore expected to be >90% C57BL in genetic composition. The specific-pathogen-free (SPF) animals were air-freighted to Australia at 4–6 weeks of age, transferred to an SPF facility, ear-clipped for identification and distributed randomly into two groups. After 10 days' conditioning to their new environment and diet, they were entered into the study. The animal experimentation was approved by the Animal Experimentation Ethics Committee of the Institute of Medical and Veterinary Science, Adelaide, South Australia, and conducted in accordance with its guidelines.

Study Design

The strain of *Pim1* transgenic mice used here has been reported to develop lymphoma to an incidence of 5–10% in the first 10 months of life (*15*, *17*). Information provided by the commercial supplier of these mice indicated that by 18 months the incidence of lymphoid tumors reaches about 15%, a level that is well suited as a baseline against which to detect moderately carcinogenic influences. Statistical calculations showed that the use of approximately 100 animals per exposure group in an 18-month study would allow the detection of as little as a doubling of lymphoma incidence with 95% confidence. The study was designed as a blinded trial. The mice and the samples taken from them for pathological analysis were coded to ensure they would be assessed without knowledge of their derivation with respect to RF-field exposure. The code was broken only after statistical analysis of the results had been completed.

Animal Husbandry

The animals were maintained in a disinfected facility kept at positive pressure by a supply of filtered air at the rate of 15–20 room volumes per hour. Animal care staff entered through an air-lock and exchanged their clothing for sterile overalls, gloves, masks, hats and boots. Air temperature was maintained at 22 ± 2°C. The lights were on from 0600 h to 1800 h each day.

From the initiation of the study, the mice were housed in groups of five in 180 × 150 × 300-mm filter-top transparent polycarbonate cages (Tecniplast, Buguggiate, Italy) in which the steel-grille lid had been replaced by a perforated glass lid, the food pellets were placed on the floor, and the glass water bottle was end-mounted distal to the ground plane of the RF-field source to minimize perturbations to the RF field. The sawdust bedding, food pellets (Joint Stock Ration II from Milling Industries Stockfeeds, Murray Bridge, South Australia), water (acidified with 4 m*M* HCl) and equipment were sterilized before transfer into the facility. Twice weekly, the cages were cleaned and fresh food pellets and water were provided. The mice were weighed weekly and the data recorded on a computer system that would sound an alert if an individual weight differed from the previous value by more than 10%. To ensure equal average exposures to the RF fields, cages were moved clockwise to the next position after cleaning. All mice were inspected closely during the weekly weighing. They were also observed daily and disturbed to check their mobility. When any showed dyspnea, reduced mobility, weight loss, a local swelling or any other clinical abnormality, they were designated for closer monitoring and submitted for pathological assessment when the abnormality was judged to be life-threatening or causing significant distress.

Pathology

Animals were normally submitted live for pathological assessment and killed by anesthetic overdose. Any mouse found dead in the cage was placed on ice or refrigerated at 4°C and subsequently submitted on ice to the pathology laboratory. A full necropsy was performed. Samples of thymus, lymph nodes (if enlarged), spleen, liver, lung, kidney, adrenal, large and small bowel, urogenital system, eyes, brain and any tissue appearing abnormal at autopsy were immersion-fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 3 μm and stained with hematoxylin and eosin. Histological assessment of lymphomas and any other pathology was then performed. Lymphomas were diagnosed and classified predominantly on morphological criteria (e.g. see ref. *18*). Any mice that were clinically healthy after 18 months of exposure or sham exposure were counted as survivors and discarded without further investigation.

Representative cases of lymphoma were immunophenotyped with the aim of determining their cell lineage of origin. Samples of enlarged lymphoid organs were dispersed mechanically into single-cell suspensions in RPMI-1640 culture medium (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) containing 10% dimethyl sulfoxide and slow-frozen in 1-ml cryotubes (Nunc, Denmark) for storage in liquid nitrogen. Accumulated batches of these frozen cells were subsequently thawed and tested for the presence of lymphoid tumor cells expressing T- or B-lineage cell surface markers by standard methods of immunofluorescence staining. The reagents used were fluorochrome-conjugated antibodies against CD45R(B220) (clone RA3-6B2 from Caltag, South San Francisco, CA), immunoglobulin (sheep anti-mouse immunoglobulin from Silenus, Hawthorn, Victoria, Australia), Thy1, CD4 and CD8

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(clones 30-H12, GK1.5 and 53-6.7, respectively, from Becton Dickinson, San Jose, CA). Staining was assessed by fluorescence microscopy.

Monitoring of SPF Status

Wild-type female C57BL/6NTac mice (from GenPharm) were used as sentinels distributed randomly among the Eμ-*Pim1* animals in the exposed and sham-exposed groups. Each month, one sentinel from each group was sent to the pathogen testing service of the Central Veterinary Laboratory (Adelaide, South Australia). The mice were examined there for a broad range of pathogenic viruses, chlamydia, mycoplasma, bacteria and parasites by serological assays, culture tests, gross autopsy examination, direct microscopy and histology. Apart from occasional detection of a questionably pathogenic protozoan (trichomonad), the mice remained free of known infectious disease organisms through the study period.

The Exposure Facility

Exposed and sham-exposed mice were housed in separate, adjacent rooms. The exposure room was 2.6 m long, 2.2 m wide and 2.45 m high, the other room 2.6 × 1.8 × 2.45 m. The rooms were lined individually with overlapping sheets of 1-mm-thick aluminum, which gave a shielding effectiveness of 40 dB at 900 MHz. Air-conditioning ducts were screened, and the doorway was fitted with metal fingers to achieve a conductive seal with the aluminum sheet covering the door. Each room was designed to contain a vertical ground plane, 2.5 m wide and 2.2 m high, running parallel to the 2.6-m-long wall, with a one-quarter-wave monopole antenna located at the center of the ground plane. Twenty lucite stands (150 × 300 mm) for mouse cages were mounted perpendicular to the ground plane in a circular array with the center of each stand 0.65 m from the antenna. The far field of the quarter-wave antenna, acting on the ground plane as a half-wave antenna, was located beyond a distance of $2D^2/\lambda$ = 165 mm. All exposures of the mice therefore occurred in the far field.

The monopole antenna was fed by a 900 MHz 70-W amplifier to produce an RF field that was modulated at a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms. The duty cycle of the transmitter was 0.13, giving an average power output of 9.1 W. The amplifier was under computer control and the power output was monitored while the antenna was energized. Animals were exposed daily for 30 min preceding lights on at 0600 h and 30 min before lights off, 12 h later, in the evening, when the mice were expected to be in their most active state. The sham-exposure room was set up identically so that the animal care staff could not discriminate between the two groups of mice, but the antenna in that room was not energized.

RF-Field Dosimetry

The RF fields were measured with a broadband meter (model 8616, Narda Microwave Corp., Hauppauge, NY) and an isotropic electric field probe (Narda 8662B), the calibration of which was verified at the Australian Radiation Laboratory before and after use. Measurement of the RF power levels at each of the 20 mouse cage positions were made while the other 19 cages were present with their complement of five mice, food and full water bottle. The root mean square RF power density (corrected for the probe calibration factor) was measured at 10 mm from the ground plane and at the distal end of each mouse cage stand (300 mm). The values at these various positions ranged from 2.6 to 13 W/m^2. Numerous measurements of the field distribution inside the room were conducted to assess the interference patterns produced by reflections from the aluminum walls. While significant variations could be detected in the room, the variation in the vicinity of the animal cages was within the range of values given above.

The SAR evaluation for a single mouse was determined experimentally because there was a substantial range of body weights and fat content among the mice used in the present study that did not fit the standard mouse models of the Dosimetry Handbook (*19*). The accuracy of these measurements was estimated at ±1.6 dB (*20*). Measurements of the electric fields induced by RF fields were made in three phantoms representing small, medium and large mice. Two tissue-equivalent gels were used in constructing the phantoms using the following complex dielectric constants as a guide:

Average human tissue at 900 MHz: $\varepsilon_\tau' = 34.3$, $\varepsilon_\tau'' = 21.3$ (from ref. *19*).
Fat at 900 MHz and 37°C: $\varepsilon_\tau' = 9.94$,
$\varepsilon_\tau'' = 3.46$ (from C. Gabriel, unpublished data).

The gels were contained within thin plastic shells of dimensions determined from outline tracings of mice weighing 26, 34 and 64 g. The two larger body shells (excluding the head) were lined with fat-equivalent gel to account for approximately 30% of the total body mass, and the remaining space was filled with human tissue-equivalent gel. The small mouse phantom contained no "fat."

A miniature isotropic E-field probe with 1.5-mm dipoles (Narda 8021) was inserted into the phantoms to measure the internal electric fields in V^2/m^2. Linearity and isotropicity of the probe response at 900 MHz were verified. Using the procedure of Hill (*21*), the enhancement factor for responses in gels relative to those in air was determined to be 2.42. All measurements were conducted in a shielded semi-anechoic room. A coaxial-to-waveguide adapter was used to generate a continuous-wave exposure field at 900 MHz. The waveguide flange was WG-4 with internal dimensions of 124 × 248 mm. The mouse phantoms were placed on the bore-sight of the aperture at a distance of 0.7 m, which was in the far field by the $2D^2/\lambda$ criterion, and the phantom and the adapter were oriented to produce E, H or K polarization relative to the long axis of the phantom. The incident power flux density ($S = E^2/377$ W/m^2) was measured with the Narda probe at the position occupied by the phantom.

Midline measurements were made at 0.25, 0.5 and 0.75 along the length of the phantom by inserting the Narda probe through predrilled holes along the top of the shell. The SAR was calculated for each point, using $SAR = \sigma E^2/\rho$, where $\sigma = 1.066$ *S*/m and $\rho = 1000$ kg/m^3. These measurements were averaged to arrive at the whole-body average SAR for the phantom and then divided by the measured power flux density.

Empirical calculations of the SAR values using spheroidal models for various weight groups of five mice were derived from the Radiofrequency Dosimetry Handbook (*19*). Estimates of the equivalent whole-body SAR values, at 900 MHz for E-polarization and for five mice at variable orientation in a close-packed group, are given in Table II.

Statistical Methods

Evaluation of end points such as lymphoma occurrence and time to occurrence in the mice was performed using logistic regression (which allows adjustment for related factors such as age and weight of the animals) and survival analysis. If exposure is the only variable used in a logistic regression model, the results are analogous to those of a χ^2 test for a 2 × 2 table. Cause-specific incidence of disease was analyzed using a competing risks model which accounts for mice dying of causes other than lymphoma (renal disease, etc.). The incidence of specific disease such as lymphoblastic lymphoma can then easily be adjusted for mice developing non-lymphoblastic lymphoma. The method of Pepe (*22*) allowed for such comparisons without requiring the competing causes of death to be independent. Comparisons of disease occurrence were performed using the conditional binomial exact test (*23*), which, while being analogous to the standard χ^2 test for large samples, is more powerful in analyzing 2 × 2 tables when frequencies are low.

RESULTS

Dosimetry of RF Energy Absorption by Mice

The SAR values measured for an individual mouse ranged from 0.0078 to 4.2 W/kg. The lower value was the product of the measured H-polarization SAR of 0.003 (W/kg)/(W/m^2) for a small mouse (Table I), and the minimum power density exposure of 2.6 W/m^2. The upper value

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TABLE I
Average Whole-Body SAR per Incident Power Flux Density for Each Polarization (E, H and K) for Small, Medium and Large Mouse Phantoms

Mouse phantom	SAR [(W/kg)/(W/m^2)] E	H	K
Large	0.31	0.011	0.056
Medium	0.32	0.009	0.037
Small	0.24	0.003	0.029

Note. Small, medium and large phantoms represented mice of 26, 34 and 62 g, respectively.

TABLE II
Values of Whole-Body SAR for Exposure to an Average Power Density of 10 W/m^2, for Groups of Five Mice, as Determined from Durney *et al.* (*19*)

Total body mass (g)	SAR (W/kg)
5 × 26	1.09
5 × 32	0.92
5 × 62	0.49

applies to the E-polarization SAR of 0.32 (W/kg)/(W/m^2) for a medium-size mouse during its exposure to the maximum power density of 13 W/m^2. Our estimate for the range of SAR values applying to animals in groups of five comes from adjusting the values shown in Table II according to the measured maximum and minimum power densities. This yielded an SAR range of 0.13–1.4 W/kg.

Mouse Body Weight

As the experiment progressed, the mice showed a tendency to obesity. Allowance for this was made in the estimation of SAR values. While the body weight of 1-year-old virgin females of common inbred strains such as C57BL/6J is 20–25 g in our experience (see also, e.g., ref. *24*), the *Pim1* mice at 1 year averaged 36.3 ± 7.6 g (n = 69) in the exposed group and 35.7 ± 6.2 g (n = 82) in the sham-exposed group. The mean for 95 non-transgenic control mice of the same age was 39.1 ± 6.5 g. Hence the accumulation of weight was not affected by RF-field exposure and was not caused by the transgene, but was a characteristic of the C57BL/6NTac mouse strain that provided the genetic background for the *Pim1* transgene.

Diseases Found

Over the 18-month course of this exposure study, the mice developed several abnormalities at varying frequency. Some of these had not been reported previously in *Pim1* mice. The numbers of animals from the exposed and sham-exposed groups found in the major diagnostic categories are shown in Table III.

Renal disease. A lethal renal disease occurred. It first appeared in a few terminally ill animals at 5–8 months of age, reaching a cumulative incidence in both the RF-exposed and the sham-exposed groups of about 10% at 19 months of age, when the experiment was completed. It was the sole cause of terminal illness in 7–8% of the animals. At autopsy, these mice often showed anasarca, the subcutaneous connective tissues having a gelatinous and shiny appearance. Both kidneys were pale and enlarged. In histological sections of these kidneys, most, if not all, glomeruli were abnormal. The most striking change was ballooning of the glomerular capillaries, which were filled with amorphous eosinophilic material (Fig. 1). This disease was also detected histologically in variably milder form in a number of the animals that were killed with other predominant diseases. The substantial incidence of renal pathology seemed to be a product of transgene action, since we saw only a single case in a group of 197 non-transgenic female C57BL/6NTac mice housed in the same SPF facility for 19 months (our unpublished observations).

Lymphoblastic lymphoma. The predominant malignant disease found in the transgenic mice up to about 10 months of age was thymic lymphoblastic lymphoma, as expected from previous studies of mice expressing the *Pim1* proto-oncogene (*15*, *16*). Cells obtained from several representative tumors tested for surface markers by immunofluorescence stained strongly for the T-cell markers Thy1, CD8 and/or CD4. Mice developing this tumor were recognizable only at a late stage of their disease when they suffered respiratory distress. The terminal stage developed too rapidly for the tumor to be detected by the weekly weighing regimen. As a result, the first three cases were mice found dead in the cage, although a diagnosis was still made from the histological appearance of the tissues. Subsequently, the mice were examined more frequently to identify cases

TABLE III
Cases of Lymphoma and Other Diseases among Eμ-*Pim1* Mice Exposed or Sham-Exposed to 900 MHz Fields

Group	n	Lymphoma: Lymphoblastic	Lymphoma: Nonlymphoblastic	Lymphoma: Total	Renal disease[a]: Alone	Renal disease[a]: Total	Other disease[b]	Undiagnosable[c]
Control	100	3	19	22	7	11	8	7
Exposed	101	6	37	43	8	10	12	7

[a]Terminal glomerulopathy; some of these mice also had lymphoma.
[b]Other deaths due to miscellaneous causes, including dehydration, injuries, hepatoma and amyloidosis.
[c]Mice found dead, with tissues too autolyzed for pathological evaluation.

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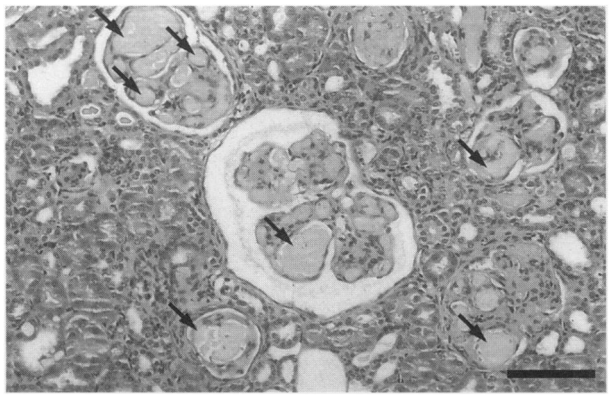

FIG. 1. Histological appearance of the distinctive renal disease in Eμ-*Pim1* mice. The photomicrograph of a hematoxylin and eosin-stained section of a kidney from such a mouse killed when terminally ill shows dilated glomerular capillaries (some examples marked with arrows) filled with amorphous eosinophilic material (scale bar, 100 μm).

before death. In this disease, masses of uniform lymphoblasts replaced most of the normal lymphocytes in the thymus and formed major deposits in the spleen, lymph nodes, lungs, liver, kidneys and bone marrow. An example is shown in Fig. 2. Of the 201 transgenic animals in this study, 9 were diagnosed with lymphoblastic lymphoma (3 in the control group and 6 in the exposed group). Only one of these occurred beyond 1 year of age.

Non-lymphoblastic lymphoma. From 10 months of age onward, some of the mice started to become ill with lymphomas that were different from the lymphoblastic tumors found in the younger animals. The new cases continued to appear through to the end of the experiment, at which time they had reached a total of 56, with 19 in the control group and 37 in the exposed group. Attempts to immunophenotype such tumors using cell suspensions gave inconclusive results, possibly because the tumor cells did not survive the dispersion and freeze–thawing procedure. These mice did not present with dyspnea and a large thymus, but commonly with readily palpable splenomegaly, or with swelling in the ventral neck region due to enlargement of the cervical lymph nodes. Histologically, none of these was lymphoblastic. Most showed follicular lymphoma in the spleen (Fig. 3), some lymph nodes, the lungs and, to varying extent, the liver. A number had histiocytic morphology, some with giant multinucleate cells scattered among the histiocytic sarcoma cells. Of the four remaining cases, two had diffuse large-cell lymphoma and two had small-cell lymphoma. Eight representative cases of follicular lymphoma and two of histiocytic sarcoma were assessed independently by Dr. T. N. Fredrickson (Registry of Experimental Cancers, National Cancer Institute, Bethesda, MD) and confirmed as lymphomas of probable follicular center B-cell origin and of histiocytic sarcoma, respectively.

Miscellaneous diseases and deaths. The SPF status of the facility was maintained throughout the study, so there were no outbreaks of infectious disease. A total of 20 mice killed with abnormal clinical signs were found to have no histological evidence of lymphoma. Two had hepatoma, 1 had amyloidosis, 2 had signs of central nervous system disorder, 2 appeared dehydrated and 7 had wounds or signs of local infection secondary to trauma. In the remaining 6, no cause of illness could be discerned. In 14 additional cases, no specific diagnosis could be made because the animals had been found dead in the cage and their tissues were too autolyzed for histopathological assessment. The miscellaneous and undiagnosable cases occurred in approximately equal numbers in the exposed and sham-exposed groups (Table III).

Statistical Analysis

The increase in the proportion of mice contracting a lymphoma of any type in the RF-field-exposed group from 22% to 43% was found to be significant ($P < 0.001$) by the conditional binomial exact test. A multivariate analysis using logistic regression was also performed to test the significance of this difference after adjusting for any differences in age and body weight. In this analysis, an additional adjustment was made for mice dying from causes other than lymphoma. Taking into account all competing risks to survival, the total lymphoma incidence in the exposed group was found to be over twice that found in the controls. The odds ratio was 2.42 at $P = 0.006$ with a 95% confidence interval of 1.3–4.5.

The crude proportions of mice contracting thymic lymphoblastic lymphoma in the control and exposed groups were 3 and 6%, respectively. Because the number of cases was small, this difference was not significant by the conditional binomial exact test ($P = 0.38$). Multivariate logistic regression analysis, and competing risks analysis adjusting for all other causes of death yielded a nonsignificant difference for lymphoblastic tumors between the exposure groups ($P = 0.95$ and $P = 0.33$, respectively).

The crude cumulative incidence of non-lymphoblastic lymphomas was 19% in the control mice and 37% in the exposed animals. This was significant by the binomial exact test at a confidence level of 99.8%. When adjusted for age and weight, the excess incidence in the group exposed to RF fields was found to be 2.7-fold with a 95% confidence interval of 1.4–5.4 ($P = 0.002$). After adjustment for competing risks, the difference in the time to appearance of non-lymphoblastic lymphoma was also highly significantly different. The increase in the probability of lymphoma with age is shown in Fig. 4. The probability that the faster rate of appearance of these tumors in the exposed mice was due to chance was calculated to be 0.014.

DISCUSSION

In the present study we sought to determine whether oncogene-transgenic mice could be used to detect a carcinogenic effect of exposure to RF fields. Mice of the Eμ-*Pim1* transgenic strain employed here express the *Pim1*

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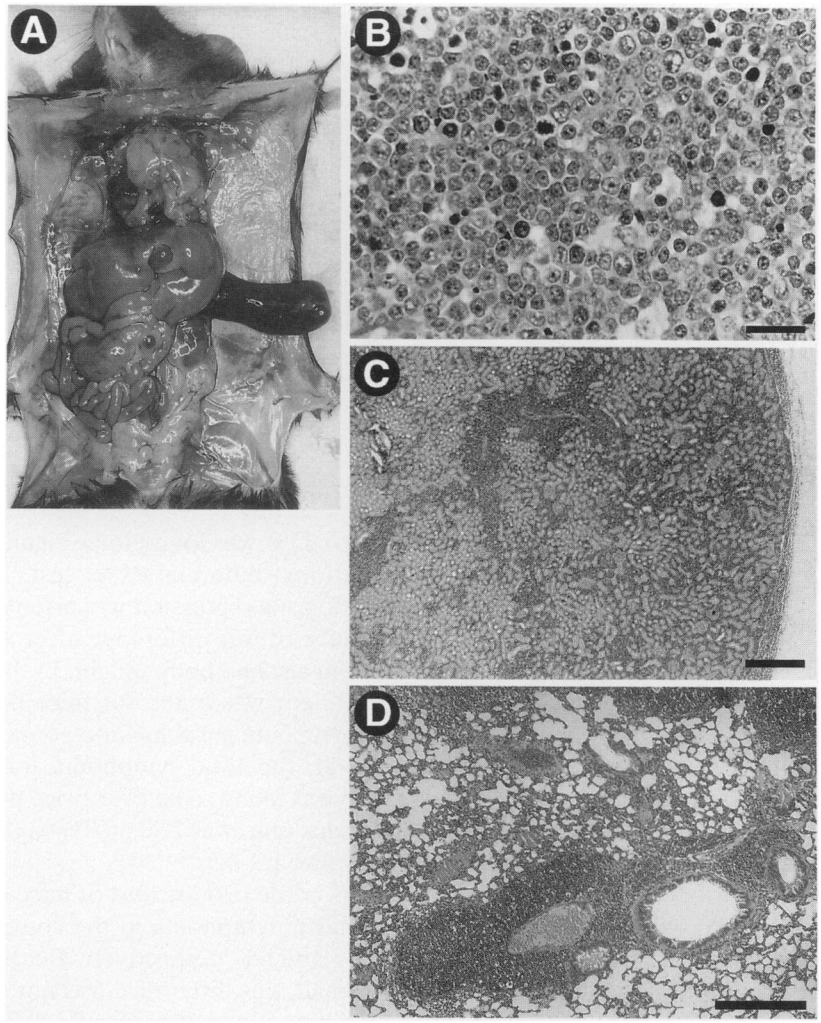

FIG. 2. A typical case of advanced lymphoblastic lymphoma of thymic origin in an Eμ-*Pim1* mouse. The panels show (A) a postmortem dissection exposing greatly enlarged thymus, spleen and lymph nodes, and hematoxylin and eosin-stained sections revealing (B) a mass of lymphoblasts, with frequent mitotic figures and some pycnotic tumor cells, filling the enlarged thymus (scale bar, 20 μm), (C) extensive infiltration by lymphoblasts (darkly stained regions) of the cortex of the kidney (scale bar, 400 μm), and (D) periarterial tumor nodules and diffuse infiltration of alveolar septa by lymphoblasts in the lung (scale bar, 200 μm).

oncogene in their lymphoid cells and have a modest propensity to contract malignant lymphoma spontaneously. Previous reports had indicated that they are specifically predisposed to develop thymic T-cell lymphoblastic lymphoma (*15*, *17*), although one case of follicular lymphoma was also recorded (*15*). However, those reports did not document the fate of the mice that survived beyond about 9 months of age. In the present study, lymphoblastic lymphoma occurred in 3–6% of the mice, but we also found that about 10% of the animals developed a terminal renal disease from 6 months of age onward, and 20–40% developed non-lymphoblastic lymphomas after 10 months and up to 19 months, when the study was terminated. The predominant tumor type in this category was follicular lymphoma, amounting to about 80% of the non-lymphoblastic lymphoma cases. Follicular lymphoma is a neoplasm derived from the germinal center B lymphocytes of lymphoid tissue and is a common lymphoid malignancy in humans (*25*, *26*). Of the remaining non-lymphoblastic tumors in the *Pim1* mice, all but two (which were hepatomas) were found predominantly in lymphoid tissues and were therefore counted as lymphomas. Some of them were diagnosed histologically as histiocytic sarcoma. They were likely to be of either B-cell or macrophage origin.

The incidence of lymphoma was higher in the RF-field-exposed *Pim1* mice than in the sham-exposed animals. For lymphoblastic lymphomas, the 2-fold increase in frequency was not statistically significant because the number of cases of that type of lymphoma was small. On the other hand, the increased incidence of all types of lymphoma and of non-lymphoblastic lymphoma was highly significant. With a lymphoma incidence of about 20% in the sham-exposed animals, groups of 100 mice were sufficient to obtain statistical significance from a 2-fold or greater increase in lym-

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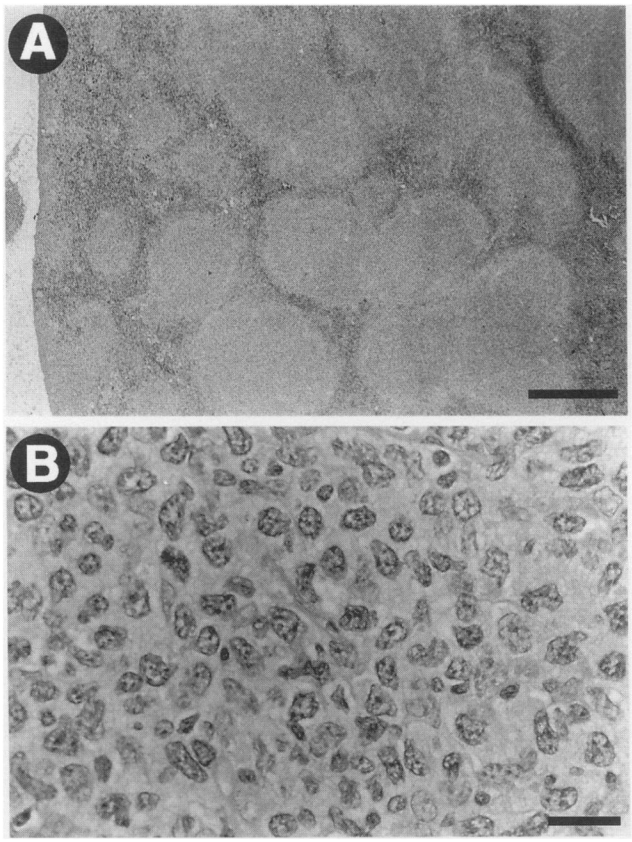

FIG. 3. A representative case of follicular lymphoma in an Eμ-*Pim1* mouse. Photomicrographs of hematoxylin and eosin-stained tissue sections show (A) a low-power view of the spleen in which numerous large tumor nodules have replaced the normal small islands of white pulp (scale bar, 500 μm), and (B) a high-power view of the tumor cells (scale bar, 20 μm).

phomas despite the competing risks of renal failure and incidental abnormalities, which were not altered by RF-field exposure. In the event, we found that RF-field exposure was associated with an overall increase of 2.4-fold in the risk of developing lymphoma. The statistical probability that the apparent increase was due to chance was calculated to be less than 1%.

By what mechanism can RF fields perturb biological systems? Unlike ionizing radiation or ultraviolet light, the photon energy of RF fields is much too low to break chemical bonds directly. However, RF fields induce electric fields that result in the flow of ions and rotation of asymmetric charged molecules (dipoles). This increase in linear and rotational energy is rapidly dissipated by molecular collisions, which generate heat. The field-induced molecular rotation is known as dielectric dispersion and is maximal for a given dipole at a characteristic relaxation frequency. At 900 MHz, the dominant relaxation phenomenon (in which there is a rapid change in the dielectric constant and conductivity of the absorbing tissue) is the δ-dispersion, which results from the relaxation of bound water, amino acids and charged side chains in proteins (*2*). The δ-dispersion and, to a lesser extent, the other relaxation phenomena are responsible for the eventual heating of tissue after absorption of RF energy. Under the conditions used in the present study, the thermal load induced in an exposed mouse would be small relative to the heat generated by normal metabolic activity. Only the SAR values at the upper end of the range measured here would add significantly to the resting metabolic rate in the mouse of 7–15 W/kg (*27*). Some investigators suggested earlier that resonant excitation of particular molecules such as DNA may lead to specific biological effects independent of heating (*28*), but subsequent tests of whether resonant absorption occurs in DNA gave negative results (*29*, *30*). Others have postulated that an effect on the molecular interactions responsible for transducing mitogenic signals from the cell surface may enable RF fields to influence cellular processes leading to malignancy (*31*, *32*), but the evidence for such a mechanism is not compelling.

A number of previous efforts to discern effects of RF exposure on lymphoid cells *in vitro* have been documented. An early report of an RF-field-induced increase in lymphoblastic transformation (*33*) was not confirmed by subsequent studies (*34–36*). Some evidence that RF fields can induce an alteration in antibody binding to mouse B-cell surface immunoglobulin (*37*) and inhibition of T-cell cytotoxic activity (*38*) has been described, but this has not been confirmed or extended using the more meaningful and sophisticated assays available today. In other reports, tests for effects of pulse-modulated RF fields on the capping of mouse B-cell surface immunoglobulin (*39*) or on DNA or protein synthesis in mitogen-activated lymphocytes *in vitro* (*40*) have yielded negative results. Thus the limited literature available on the subject does not seem to offer a mechanism by which RF-field exposure, either directly, or indirectly through effects on immune competence, could increase the incidence of lymphoid malignancy.

The activated *Pim1* oncogene in the Eμ-*Pim1* mouse does not act alone to transform lymphocytes to the malignant state. The lymphomas arise in a stochastic fashion as they do in other strains of oncogene-transgenic mice (*41*), and the current view is that acquisition of malignancy requires multiple somatic mutations which activate cooperating sets of oncogenes and genes that prolong cell survival, as well as inactivating tumor suppressor genes (see reviews in refs. *42* and *43*). In the case of the *Pim1* mouse the lymphocytes start their existence one step toward malignancy but must undergo mutation in endogenous genes before one of the cells can initiate a lymphoma. Lymphomas accelerated by chemical carcinogens in *Pim1* mice were found by Breuer *et al.* (*16*) to over-express the *Myc* gene, which has proliferation-promoting activity. There is no convincing evidence that RF fields can induce mutation or activate genes directly, but if such fields can cause an increase in gene expression, perhaps as a result of transient low-level warming of exposed tissues, then they might increase the likelihood of spontaneous mutation in the precancerous *Pim1*-expressing lymphocytes by stimulating cell proliferation.

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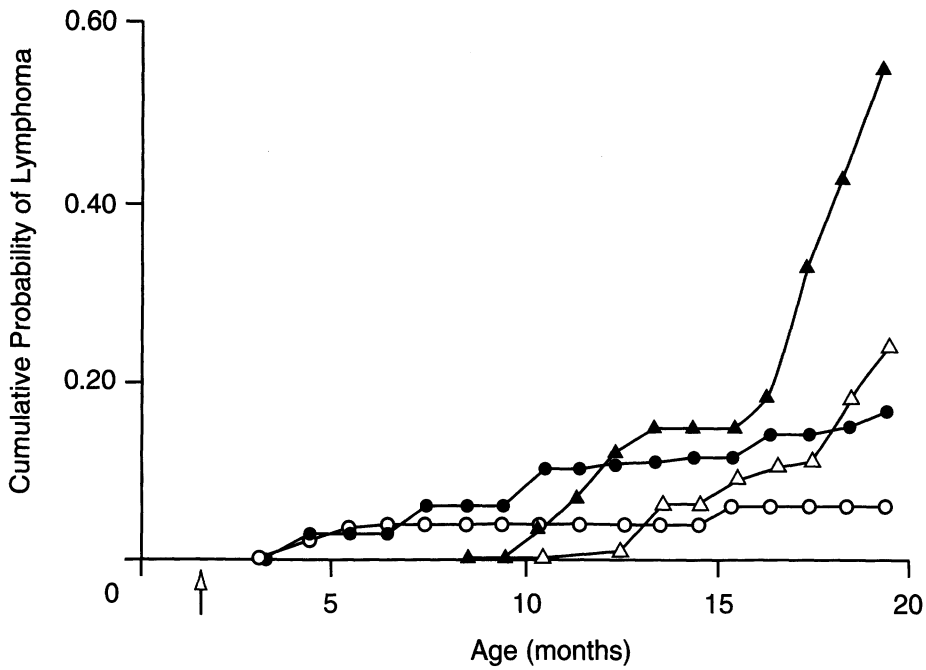

FIG. 4. Cumulative probability of development of lymphoma with age in Eμ-*Pim1* mice. (●, ○) Lymphoblastic lymphoma and (▲, △) non-lymphoblastic lymphoma in RF-field-exposed (●, ▲) and sham-exposed (○, △) animals. The cumulative probability values were calculated by adjusting the crude incidence of lymphoma for losses of mice to other causes such as other tumors, renal disease, incidental injuries and undiagnosed terminal illness (see Table III).

Stimulated cell proliferation after tissue damage has been proposed by Ames *et al.* (*44*, *45*) to account for the tumorigenic effects of high doses of non-mutagenic chemicals in tests of carcinogens in rodents. By analogy, a small enhancement of proliferation on a daily basis by RF-field exposure might suffice to increase the rate of initiation of lymphoma by the factor observed here in the *Pim1* mice.

While the increase in the incidence of lymphoma found here was highly significant statistically, and the exposure conditions were designed to mimic the fields generated by a digital mobile telephone, the implications of the study for the risk of carcinogenesis in humans are unclear. It is difficult to extrapolate directly from mice to humans due to differences in their absorption of energy from RF fields. The mice were exposed approximately 0.65 m from the radiating antenna, i.e. in its far field, where the magnetic and electric field vectors are orthogonal. By contrast, the head of a human using a cellular telephone is in the near field, where the magnetic and electric field strengths do not have a constant relationship. Further, 900 MHz RF energy is absorbed almost uniformly throughout the mouse, whereas in humans it is absorbed in a non-uniform manner in the skin and underlying muscle, and the eye, with little penetration to deeper tissues (*46*, *47*). The RF energy absorbed by the *Pim1* mice during their exposure ranged from 0.008 W/kg up to 4.2 W/kg. This estimate took into account their varying orientation to the incident RF field and the varying incident power density as they moved around the cage, their change in body mass with age and their tendency to rest as a close-packed group. Since the variation is so wide, it is not possible to determine what SAR or SAR range was responsible for causing the increased incidence of lymphoma. However, on the basis of studies reported previously, one would expect that the higher SARs would have done so. It seems important in light of the present results to determine the relationship between exposure dose and lymphoma incidence. One way to reduce the uncertainty of SAR values would be to restrict the movement of the mice during their exposure, such as by placing them in a tube having a fixed orientation to the field. For 30-min exposure periods this would be a feasible option for use in future studies.

There is a need to replicate and extend this study to test whether the tumor-prone transgenic mouse is a reproducible system for assaying biological effects of RF fields. The *Pim1* mouse model used here is somewhat complicated by its propensity to develop at least two types of lymphoid tumor and an unusual renal disease. Other mice carrying an activated oncogene or an inactivated tumor suppressor gene have the potential to be useful in testing whether the provocative findings described here have some more general validity. Transgenic mice bearing an activated *Abl* (*48*) or cyclin D1 (*49*) oncogene, or mice with a deleted *Rb* (*50*) or *p53* tumor suppressor gene (*51–53*), for example, develop various tumors and could be candidates for such testing.

The *Pim1* mouse would be expected to respond to carcinogenic agents with an increase in lymphomas because it expresses an activated oncogene selectively in its lymphoid cells. Hence we would not interpret the results as indicating

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that RF-field exposure would be specifically lymphomagenic in normal animals. Other types of cancer might be induced either more or less easily in other tumor-prone animals. No humans are presently known to carry an activated *Pim1* gene, but some individuals inherit mutations in other genes, such as *p53* in the Li-Fraumeni syndrome (*54*), that predispose them to develop cancer, and these individuals may comprise a subpopulation at special risk from agents that would pose an otherwise insignificant risk of cancer. That is not to imply that any humans at all are necessarily at increased risk of cancer as a consequence of exposure to RF fields. No single experiment on animals can allow such a conclusion. Rather, we believe the study reported here indicates a need for further research. Tumorigenesis in genetically predisposed mice may provide a useful assay for interactions between RF fields and biological systems. With the current rapid expansion in the use of RF fields for telecommunications, a reliable assay is required to enable a better assessment of the limits to safe levels of human exposure.

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