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Exposure of Human Peripheral Blood Lymphocytes to Electromagnetic Fields Associated With Cellular Phones Leads to Chromosomal Instability

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Whether exposure to radiation emitted from cellular phones poses a health hazard is at the focus of current debate. We have examined whether in vitro exposure of human peripheral blood lymphocytes (PBL) to continuous 830 MHz electromagnetic fields causes losses and gains of chromosomes (aneuploidy), a major ''somatic mutation'' leading to genomic instability and thereby to cancer. PBL were irradiated at different average absorption rates (SAR) in the range of 1.6–8.8 W/kg for 72 hr in an exposure system based on a parallel plate resonator at temperatures ranging from 34.5–37.5 °C. The averaged SAR and its distribution in the exposed tissue culture flask were determined by combining measurements and numerical analysis based on a finite element simulation code. A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value, demonstrating that this radiation has a genotoxic effect. The SAR dependent aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. Control experiments (i.e., without any RF radiation) carried out in the temperature range of 34.5–38.5 °C showed that elevated temperature is not associated with either the genetic or epigenetic alterations observed following RF radiation—the increased levels of aneuploidy and the modification in replication of the centromeric DNA arrays. These findings indicate that the genotoxic effect of the electromagnetic radiation is elicited via a non-thermal pathway. Moreover, the fact that aneuploidy is a phenomenon known to increase the risk for cancer, should be taken into consideration in future evaluation of exposure guidelines. Bioelectromagnetics 24:82–90, 2003.

Key words: continuous RF fields; nonthermal effects; aneuploidy; centromeric DNA replication; carcinogenesis

INTRODUCTION

The exponential increase in the use of cellular mobile communication over the last few years leads millions in the world's population to be exposed to radiofrequency (RF) electromagnetic radiation. This increased environmental exposure of humans to RF radiation raises questions regarding the biological and health consequences of this exposure, especially its long term effects. In particular, the connection between cancer hazard and exposure to RF radiation is continuously debated [reviewed in Szmigielski, 1996; Rothman et al., 1996; Valberg, 1997; Moulder et al., 1999].

The conflicting evidence arises from difficulties in differentiating between the exposed and unexposed individuals while conducting epidemiological studies relying on small cohorts, as well as from the insufficient elapsed time for the cancer to appear in populations. Thus, a large epidemiological study, which followed cancer morbidity in the whole population of military

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career personnel in Poland during a 15 year period (1971–1985), reported that leukemia and brain tumors are significantly higher in RF exposed personnel compared with unexposed ones [Szmigielski, 1996]. This study was supported by another, conducted on laboratory animals, which presented evidence that exposure to RF radiation leads to an increased risk for lymphoma [Repacholi et al., 1997]. However, there are studies arguing that the increased cancer risk reported in connection with exposure is due to various experimental errors and is not the result of the RF radiation per se [reviewed in Valberg, 1997]. Thus, the question whether exposure to RF radiation leads to cancer, although of grave concern for the general public, still remains a puzzling issue, which is awaiting the arrival of more modern tools to be resolved.

Exploring the genetic effects of RF radiation was previously undertaken using classical and modern genetic tools assessing gene mutation, chromosomal alterations in somatic cells, DNA repair processes, and cell transformation assays [for review see Verschaeve and Maes, 1998; Brusick et al., 1998]. However, many of these studies assayed genetic parameters such as sister chromatid exchange and chromosomal aberrations that were previously shown to be typically associated with ionizing radiation.

One of the most relevant genetic changes associated with the cancerous process is alteration in chromosome complement [aneuploidy; Duesberg et al., 2000].

There is an increasing evidence that in preneoplastic cells, the genetic material undergoes continuous change, resulting in the accumulation of multiple mutations and alterations in the genomic DNA content [Loeb, 1991; Jackson and Loeb, 1998; Duesberg et al., 2000; Loeb and Loeb, 2000]. Among modified genes are those that function in guaranteeing the stability of the genome. Loss in this ability results in a ''mutator phenotype.'' Evidence for a mutator phenotype is the frequent occurrence of aneuploidy displayed in most of human cancers [Lengauer et al., 1998; Pihan and Doxsey, 1999; Duesberg and Rasnick, 2000; Li et al., 2000; Bialy, 2001]. New insights into the analysis of the neoplastic process suggest that aneuploidy is neither the result of gene mutation nor the outcome of some other genomic modification, but constitutes ''the somatic mutation [itself] that makes cancer'' [reviewed in Duesberg and Rasnick, 2000]. Thus, aneuploidy appears to be the cause and not the result of carcinogenesis.

Aneuploidy results either from chromosome malsegregation or from chromosome fragmentation. It at once alters the dosage of a large number of genes, giving rise to multiple changes in gene expression of both genes whose dosage was altered as well as those that remained in two copies, but whose function depends on two intact copies of other genes. The gross alteration in gene expression accompanying an unbalanced chromosome complement frequently leads to malfunctioning of the chromosome segregating apparatus, making aneuploidy an autocatalytic process, which may continuously destabilize the genome and thus facilitate embarking on the road to cancer. Consequently, aneuploidy accompanied by an abnormal behavior of the chromosome segregating apparatus and modification in gene expression is a hallmark of the preneoplastic phenotype, and as such provides a reliable genetic marker for the identification of agents possessing carcinogenic activity.

Recent studies reveal that there is a correlation between aneuploidy and loss of synchrony in replication timing of homologous DNA counterparts lacking transcriptional ability, such as highly repetitive DNA arrays (satellite DNA) [Litmanovitch et al., 1998]. These arrays, which in man are associated with chromosome mover components (centromeres), replicate synchronously in euploid cells [chromosomally balanced cells; Litmanovitch et al., 1998]. However, an increase in aneuploidy is accompanied by elevation of asynchrony in replication timing of homologous satellite DNA arrays. Thus, asynchronous replication of homologous counterparts of satellite DNA arrays was observed in ovarian cancer tumors [Litmanovitch, 1996], in lymphocytes of patients suffering from familial ovarian cancer [Litmanovitch, 1996; Litmanovitch et al., 1998], and in blood malignancies [Korenstein-Ilan, 2000]. It is not yet clear whether aneuploidy is the cause or the effect of the loss of synchrony in replication timing of homologous centromeres. Whatever the mechanism, a continuous damage in replication timing of centromeres affects the whole mitotic machinery, which is dependent on temporal control of successive events [reviewed in Litmanovitch et al., 1998].

The present study employs aneuploidy and replication assays, based on interphase fluorescence in situ hybridization (FISH), to address the question whether in vitro exposure of human peripheral blood cells to mobile telephone frequencies (continuous 830 MHz) leads to genetic effects that are associated with increased risk for cancer. The FISH replication assay was found to reliably detect replication timing [Mukherjee et al., 1992; Selig et al., 1992; Bickmore and Carothers, 1995; Boggs and Chinault, 1997; Haaf, 1997; Simon et al., 1999], while avoiding the labor intensive classical methodologies. These include thymidine and BudR incorporation [Miller et al., 1973], which themselves influence the course of replication,

followed by Southern blotting [Braunstein et al., 1982] or cell sorting [Hansen et al., 1993]. They necessitate large cell populations and are not sufficiently reproducible [Mukherjee et al., 1992; Simon et al., 1999]. Also, using the FISH replication assay we can simultaneously assess the frequency of losses and gains of the locus identified by the probe used [reviewed in Litmanovitch et al., 1998 and references therein].

MATERIALS AND METHODS

Samples

Peripheral blood samples were obtained with informed consent from ten healthy male volunteers. The blood samples of the first group of five donors were employed for exposure to the continuous wave (CW) 830 MHz RF radiation. Each volunteer donated several samples at different times, each used for a different level of exposure to RF. Blood samples from a second group of five donors were exposed to different temperatures in the range of 34.5–41.0 °C.

Culture Preparation

Heparinized blood was collected from each donor. Three milliliter of peripheral blood cells were added to 62 ml of culture medium (F10) supplemented with 20% fetal calf serum, 3% phytohemagglutinin, 0.2% heparin, and 1% antibiotics (a standard solution of penicillin and streptomycin), in 25 cm^2 culture flasks (TPP, Switzerland). This medium is used in the preparation of phytohemagglutinin stimulated lymphocytes for routine karyotyping analysis [Rooney and Czepulkowsli, 1992].

The samples exposed to RF radiation were inserted into the exposure system, which was later placed in the incubator. The samples were continuously exposed to CW 830 MHz radiation for the duration of the culturing period of 72 hr. The unexposed (sham exposed) sample was placed in the same incubator, at a different height level, with a metal plate between the two samples. The sham exposed samples were also grown uninterrupted for the same period of time. An additional control flask was grown in a different incubator which was set to 37 °C. Following culturing, the samples were harvested for cytogenetic analysis [Rooney and Czepulkowsli, 1992]. No significant differences were found between the two types of control.

RF Exposure Setup

Figure 1 shows a schematic block diagram of the RF exposure setup. It consists of an exposure cell, an RF generator, and diagnostic means to measure the RF power and the culture temperature. The exposure cell

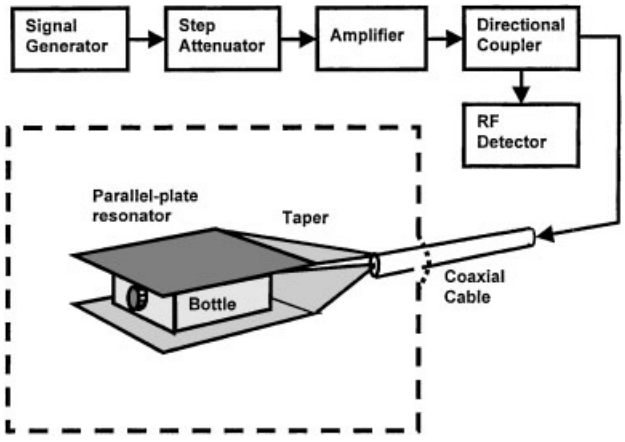

Fig. 1. Schematic block diagram of the RF exposure system.

utilized a standard tissue culture plastic bottle (TPP, 25 cm^2) in which the blood cells were cultured. The bottle was situated within a parallel plate RF resonator fed by a coaxial cable through a tapered transition section. The parallel plate resonator dimensions are 6 cm length, 5 cm width, and 2.4 cm height (i.e., spacing between the plates). The entire exposure cell was installed within an incubator to keep it at the appropriate temperature.

The RF generator unit consists of a CW generator, a digital step attenuator, and an RF amplifier (Mini-Circuits' ZOS-1025, ZFAT 1-2-4, and ZHL-2-8, respectively). The output RF power feeding the exposure cell is coupled by a directional coupler (ZEDC-10-2B) to an RF detector (ACSP-2517NC3). The input RF power to the exposure cell is 1 W or less.

Numerical Dosimetry

The distribution of the electromagnetic radiation inside the exposure cell (including the incubator's wall effect) was computed by an Ansoft HFSS simulation code. The mesh resolution was chosen as ~1/6 of the wavelength in each medium. At 850 MHz, the free space wavelength is ~35 cm, whereas the wavelength in the culture liquid is ~4.1 cm. The relative dielectric constant of the culture medium was found experimentally to be $\varepsilon_T \cong 73–j34$ at 811 MHz and 37 °C.

The HFSS results show that $^2/_3$ of the incident RF power is absorbed by the blood culture, whereas the remainder is dissipated by reflection to the input port and by radiation to the incubator. Figure 2 demonstrates the absorbed power distribution profile at the bottom of the bottle by a 3D contour plot. The accuracy of the HFSS simulation was estimated to be ± 5%. The slight asymmetry in the RF distribution profile is caused by the uneven reflection from the incubator walls (included in the simulation). For a 1 W input power computation,

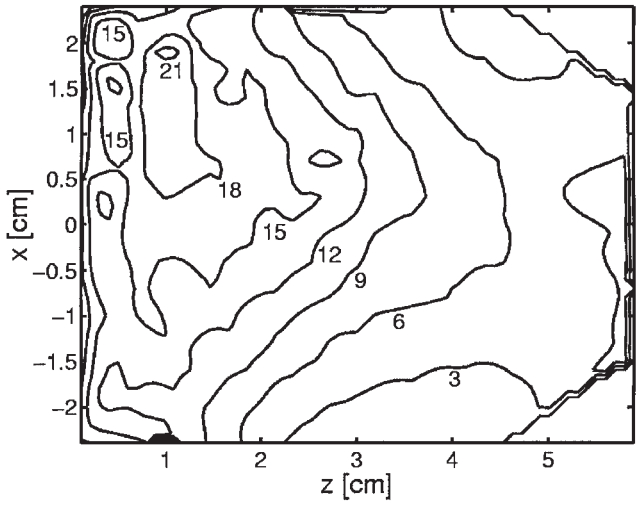

Fig. 2. Contour map of the absorbed power in W/kg at the bottom of the bottle for 1 W input power, based on numerical simulation of the exposed bottle in the incubator. The z-axis represents the longitudinal dimension of the bottle and the x-axis represents the perpendicular dimension.

the maximum power density absorbed in the culture is 24 W/kg, whereas the average power density is 9.4 W/kg. These results also indicate the maximum and average specific absorption rate (SAR) levels, respectively. The SAR levels corresponding to the lower input RF powers applied in the exposure experiments are computed accordingly.

Temperature Measurements of the Samples

The SAR distribution in the medium was inhomogeneous in the culture flask. It possessed a maximum at the flask's area nearest to the coaxial feeding and a minimum at the area furthest from it. Therefore, we made an attempt to measure on line, under steady state conditions of RF irradiation, temperature difference at various points in the flask. It should be taken into consideration that the heat transfer in the medium across the SAR gradient is much faster than the heat transfer to the surrounding environment. Thus, the temperature difference across this SAR gradient (SAR_{diff}) should be proportional to the SAR_{diff}/c_w, where c_w is the specific heat capacity of the aqueous medium. A constant reading was attained (thermal steady state conditions) 4 hr after initiation of exposure to the RF radiation.

For the measurement of temperature difference in the medium, during irradiation, before and after establishment of steady state conditions, we employed an array of four thermistors made adequate for temperature measurements in RF radiation environments by using both RF radiation shielding and high resistance cables. The four probes were inserted into the medium through the flask's cap. Two of the probes were positioned in the region of high SAR, whereas the two other probes were positioned in the area of low SAR. The maximal temperature difference between the probes at the two SAR regions did not exceed 1 °C.

In a different set of temperature measurements we determined, by a calibrated thermocouple, the average temperature of the medium in the bottle, within 4–8 s after the termination of the exposure to RF. During the exposure to RF radiation, the thermocouple (accuracy of ± 0.5 C°) was inserted in the medium at the cap area, which was unexposed to the RF radiation. Immediately after the termination of the exposure, the medium was quickly mixed and the thermocouple was pushed into it. The dependence of temperature rise, relative to the unexposed control, as a function of SAR level is shown in Figure 3 (the data points fitted to a linear function yielded $R^2 = 0.55$). The rise in the temperature can be attributed to the inefficient heat exchange between the exposed sample and the incubator environment in which it was positioned.

In order to prevent the average temperature elevation from exceeding 38.0 °C, the incubator was set to lower temperatures to compensate for the heating, especially when exposing the cells to the high SAR levels. For example, at the highest average SAR of 8.8 W/kg, the temperature of the incubator was set to 33.5 °C (Fig. 3).

Fluorescence In Situ Hybridization (FISH)

We used directly labeled commercial probe (Vysis Inc., Downers Grove, IL) for the centromeric repetitive (α-satellite) DNA arrays of chromosome 17 (CEN17). In situ hybridization, post washing, and detection were performed in accordance with the protocol of Insitus Biotechnologies (Albuquerque, NM),

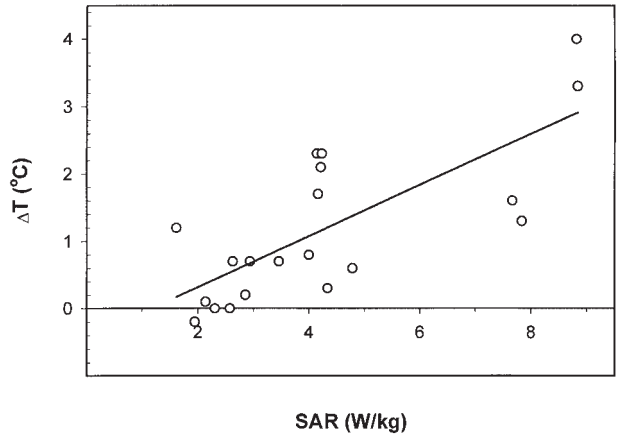

Fig. 3. Dependence of temperature rise, relative to the unexposed control, on specific absorption rate (SAR) levels.

with slight modifications. Five microliter of the probe solution were placed on the targeted area of the sample slides, covered with a 12 mm round silanized coverslip (Insitus Biotechnologies), and then sealed with rubber cement. The slides were placed into a microheating system (True Temp; Robbins Scientific Corp., Sunnydale, CA) at 76 °C and denatured for 6 min at that temperature. Then, the True Temp was turned off, and the slides were allowed to hybridize overnight in the instrument.

Post hybridization wash for probe CEN17 was carried out by immersing the slides in 0.4 × SSC/0.3% NP40 (1 × SSC = 150 mM NaCl, 15 mM sodium citrate) for 2 min followed by 1 min in 2 × SSC/0.1% NP40, both at 76 °C. After draining off excess liquid and brief drying, the slides were treated with 15 µl/test of a solution of antifade containing 6-diamidino-2-phenylindole (DAPI) as counterstain at 3 µg/ml (Vectashield, Vector Labs). Slides were covered with glass coverslips (22 × 60 mm) and stored at −20 °C until analysis (between 1 hr and 2 days).

Cytogenetic Evaluation

For the analysis of aneuploidy, 200 cells were blindly scored from each sample. In each scored cell the copy number (number of FISH signals) of CEN17 was determined.

For the analysis of replication timing, 100 cells, each containing two hybridization signals, were scored. Accordingly, an α-satellite array in the course of replication reveals two differently shaped configurations depending on its replication status [Selig et al., 1992]. The cells were divided into two categories: a single dot and a large beaded signal (singlet; S) representing an unreplicated sequence and a doubled dot and elongated rod like beaded signal (doublet; D), indicating that the sequence has already replicated. Thus, in a population of replicating cells, out of the total population of cells with two hybridization signals, the frequency (%) of cells containing two dissimilar (asynchronous) signals (%SD), represents the level of asynchrony in replication.

Statistical Analysis

Statistical significance was analyzed by two tailed Student's *t*-test.

RESULTS

Effect of Exposure on Loss and Gain of Chromosome 17

The change in the aneuploidy of chromosome 17 following exposure to different levels of SAR in the range of 1.6–8.8 W/kg is given in Figure 4a. An increase in the aneuploidy level is observed upon elevation of the SAR level. The dependence of aneuploidy of chromosome 17 on the SAR level was fitted to a polynomial of the second order ($R^2 = 0.35$).

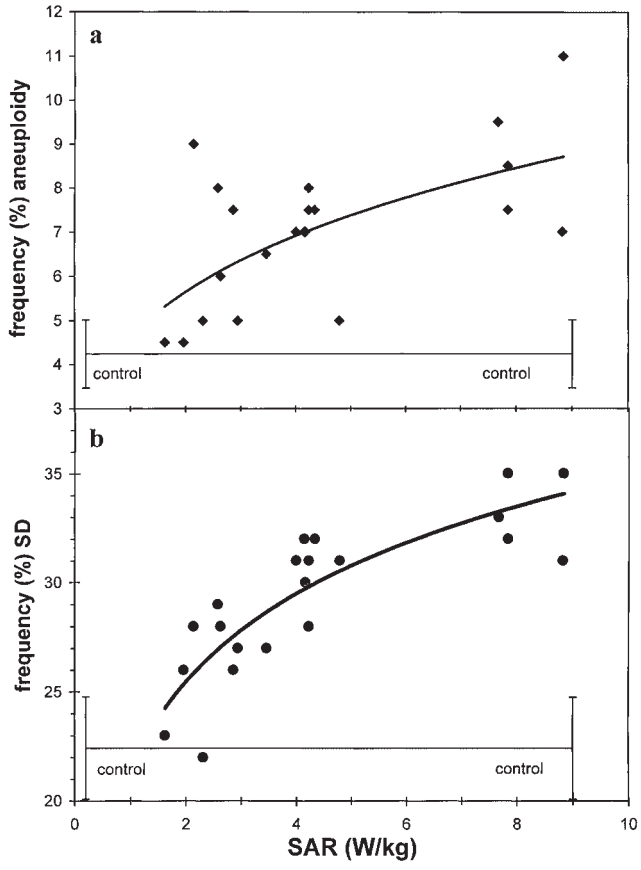

Fig. 4. Dependence on exposure to RF of human peripheral blood cells in the specific absorption rate (SAR) range of 1.6–8.8 W/kg of chromosome 17 aneuploidy (**a**) and frequency of asynchronous replication of α-satellite DNA of chromosome 17 (%SD) (**b**). Each point represents a single experiment carried out at the appropriate average SAR per flask.

In order to compare aneuploidy changes at the different levels of exposure, the samples were subdivided into four groups according to their average SAR level of exposure. Since the exposure was not evenly distributed over the whole range, the samples were grouped and subdivided into four levels of exposure: 1.6–2.3 (2.0 ± 0.3 W/kg; mean ± standard deviation; n = 4), 2.6–3.5 (2.9 ± 0.3 W/kg; n = 5), 4.0–4.8 (4.3 ± 0.2 W/kg; n = 7), and 7.8–8.8 W/kg (8.2 ± 0.6 W/kg; n = 5) which were represented as 1st to 4th levels, respectively. The dependence of aneuploidy of chromosome 17 on the four exposure levels is shown in Figure 5a. No significant increase in aneuploidy was observed following exposure to the 1st level of exposure as compared to control ($P > 0.4$). However, exposure to

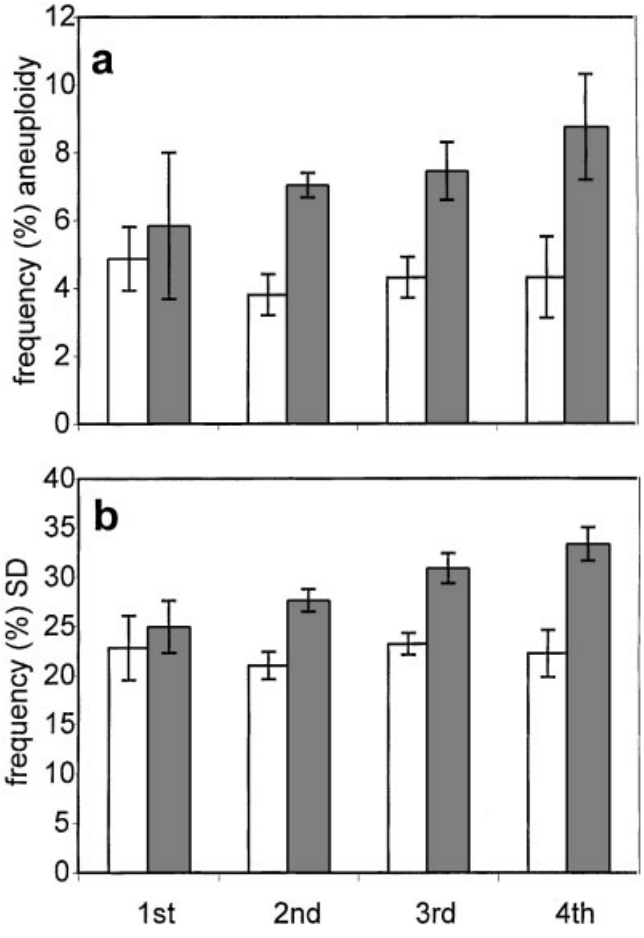

Fig. 5. Mean and standard deviation of the level of aneuploidy of chromosome 17 (**a**) and asynchronous replication of α-satellite DNA of chromosome 17 (%SD) (**b**) in human peripheral blood cells following exposure to four different levels of average specific absorption rate (SAR) of 2.0 ± 0.3 (n = 4), 2.9 ± 0.3 (n = 5), 4.3 ± 0.2 (n = 7), and 8.2 ± 0.6 (n = 5) W/kg (1st to 4th levels, respectively). Empty columns, controls; full columns, exposed samples.

the 2nd level resulted in a 65% increase of aneuploidy as compared to control ($P < 0.004$). Exposure to the 3rd level yielded an increase of 70% ($P < 0.002$), whereas exposure to the 4th level led to a 100% increase in the level of aneuploidy ($P < 0.00002$).

Effect of Exposure on the Temporal Order of Replication of the Centromere of Chromosome 17

The frequency of cells showing asynchronous replication (%SD) of CENT17 following exposure to RF increased in a similar manner as that of the aneuploidy frequency (compare Fig. 4a and 4b). The dependence of %SD on the SAR level was fitted to a logarithmic function ($R^2 = 0.55$).

The frequency of SD following exposure to the 1st SAR level was not significantly different from control ($P > 0.3$). However, exposure to the 2nd level of SAR brought about a 29% increase in the level of SD ($P < 0.00005$). The exposure to the 3rd level resulted in a 32% increase relative to control ($P < 0.00001$). The largest effect on SD was obtained following exposure to the 4th level of SAR, where an increase of 49% ($P < 0.00004$) was attained.

Effect of Temperature on the Aneuploidy and the Temporal Order of Replication of the Centromere of Chromosome 17

Since exposure to increasing SAR levels is accompanied by elevation in the sample temperature (Fig. 3), we determined the effect of this increase on both aneuploidy and the level of replication asynchrony of CENT17 in the absence of RF radiation. The PBL samples were exposed to different temperatures in the range of 34.5–41.0 °C for 72 hr. Both the aneuploidy level and the level of temporal replication were found to be unaffected by the temperature variation in the range of 34.5 to 38.5 °C ($4.4 \pm 0.8\%$ and $21.5 \pm 2.4\%$, respectively; Fig. 6). However, elevation of the temperature to 40 °C led to an increased level of $7.8 \pm 0.3\%$ in aneuploidy ($P < 10^{-11}$), while further increase to 41 °C had no additional effect ($8.1 \pm 0.7\%$). Interestingly, there were no differences in the frequency of asynchronous replication in any of the temperatures in the range tested.

DISCUSSION

The exposure of PBL to CW 830 MHz radiation of increasing average SAR levels in the range of 1.6–8.8 W/kg resulted in an increase in the losses and gains of chromosome 17 (Figs. 4a and 5a). The averaged SAR and its distribution in the exposed tissue culture flask were determined by combining measurements and numerical analysis based on finite elements. The analysis shows a 2.5 fold ratio between the maximal and the average SAR level in the exposed cells. Thus, the inhomogeneous exposure does not allow for the determination of the exact SAR threshold leading to aneuploidy, but rather confines it to an average SAR value of 2.9 ± 0.35 W/kg. This SAR range is higher than the restricted ICNIRP guideline of occupational whole body average exposure of 0.4 W/kg but lower than the localized exposure limit for the head and trunk of 10 W/kg [ICNIRP guidelines, 1998].

Exposure guideline limits set for RF radiation, assume that biological effects solely result from tissue heating [ICNIRP guidelines, 1998]. The possibility of RF induced effects via nonthermal pathways is a highly

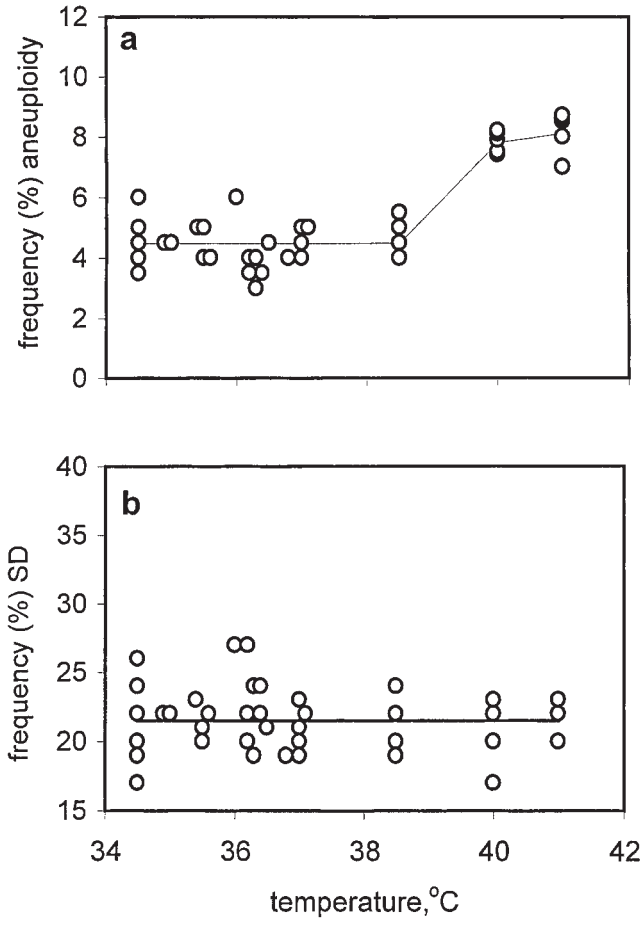

Fig. 6. The level of aneuploidy of chromosome 17 (**a**) and asynchronous replication of α-satellite DNA of chromosome 17 (%SD) (**b**) in peripheral blood cells following culturing at different temperatures in the range 34.5–41.0 °C.

controversial and debated question. In order to determine whether the observed increase of aneuploidy originates from sample heating, we studied the sole effect of temperature on the level of aneuploidy of chromosome 17. No effect of temperature elevation in the range of 34.5–38.5 °C on the level of aneuploidy was observed. However, at higher temperatures of 40–41.0 °C a significant increase (about 80%) in the aneuploidy level was observed. This finding is in agreement with a previous report [Prabhakara and Murthy, 1995] that demonstrated, based on metaphase analysis, an increase in aneuploidy from a control level of 3.6% to the level of 12.7% in human lymphocytes exposed to temperature of 43 °C.

However, since in our RF exposure experiments the temperature did not exceed, even locally, 38.0 °C, it must be concluded that the increase in aneuploidy observed by us is attributable to a nonthermal effect of the RF radiation. The nonthermal origin of our observed genetic effects is further supported by the finding that exposure of the PBL to RF radiation resulted in a SAR dependent increase in the level of the asynchronous replication of the homologous centromeres of chromosome 17, though the level of asynchrony was found to be independent of temperature elevation in the range of 34.5–41.0 °C.

The possibility that biological effects following exposure to the microwave region of the electromagnetic spectrum under very low exposure intensity, are elicited via a nonthermal mechanism has been debated in the literature [Foster, 2000]. However, a recent report shows that prolonged exposure to extremely low intensity microwave fields (SAR of 0.001 W/kg) can induce heat shock proteins (HSP) in the soil nematode *Caenorhabditis elegans* via a nonthermal mechanism [de Pomerai et al., 2000a,b].

The mechanism by which the RF radiation induces loss and gain of chromosomes, probably does not involve a direct effect of the radiation on structural alteration of DNA due to the high energetic expenditure of bond breakage. However, it may affect the function of proteins that regulate the pathway of chromosome segregation on the one hand and DNA replication on the other. That heat shock proteins (HSP) are induced via a nonthermal pathway following exposure to both ELF [Lin et al., 1997; Lin et al., 1998; Tsurita et al., 1999] and microwave [de Pomerai et al., 2000a,b] is in accordance with the notion that HSP are nonspecific stress proteins which are induced by many kinds of environmental changes and act as molecular chaperones which are involved in the defense mechanism against proteitoxic stresses such as heat and chemicals [Ohtsuka and Hata, 2000]. Indeed there are reports suggesting that nonthermal exposure to microwaves affects protein structural rearrangements [Porcelli et al., 1997; La Cara et al., 1999; Bohr and Bohr, 2000a,b], which in a cell can be repaired by HSP.

The possible significance of our study emerges when comparing the RF induced changes in the frequency of aneuploidy in our in vitro exposed PBL with those observed in PBL of patients suffering from hematological malignancies. The reported level of aneuploidy of chromosome 17 of patients suffering from hematological malignancies [Korenstein-Ilan, 2000] is twice the value obtained at the highest SAR level of our exposure ($16.0 \pm 5.0\%$ and $8.7 \pm 1.6\%$, respectively). It should be stated that the aneuploidy observed in cancer arises from a continuous defect in the segregating apparatus and thus is not chromosome specific. Therefore, it might be speculated that the RF induced alteration of aneuploidy is likewise nonchromosome specific and not restricted to chromosome 17.

If one accepts the notion that aneuploidy is an autocatalytic process leading to the transition from a pre-neoplastic phenotype into a neoplastic one [Duesberg and Rasnick, 2000], then the elevation of aneuploidy to a level that is on one hand twice than the control and on the other hand half the value typical of hematological malignancies, may reflect a situation which constitutes a risk for cancer. This suggestion is further supported by a recent epidemiological study [Lalic et al., 2001], where the genotoxic effects of occupational exposure to ionizing and nonionizing radiation were investigated in 25 physicians and nurses working in hospitals and in 20 individuals working at radio relay stations. Chromosomal aberration analysis of peripheral blood lymphocytes showed that the number of chromosome aberrations in people exposed to ionizing and radio frequency radiation were almost equally increased compared to those of unexposed subjects.

The RF induction of improper chromosome segregation was accompanied by increase in the abnormal (asynchronous) replication of the homologous centromeric arrays. Though the level of asynchronous replication timing of CENT17 following exposure to the highest SAR was 1.5 fold higher than in controls, it had a close value to the one observed in patients suffering from hematological malignancies [Korenstein-Ilan, 2000] ($33.2 \pm 1.9\%$ and $37.5 \pm 4.4\%$, respectively). This again suggests that the RF induced elevation in the asynchronous replication pattern of the centromeric DNA arrays can be considered to be associated with an epigenotype of cancer.

CONCLUSIONS

Our results indicate that human cells exposed to RF radiation acquire premalignant genotypes associated with elevated levels of aneuploidy and abnormalities in replication mode as expressed in asynchrony in the replication timing of homologous chromosomal regions associated with chromosome segregation. These findings support the view that exposure to RF radiation of average SAR values of 2.6–8.8 W/kg may lead, through a nonthermal pathway, to a carcinogenic activity. Our study does not elucidate the specific primary mechanism by which radiation interacts with the cell and alters its genetic material. However, it does demonstrate that exposure to RF radiation results in a gross somatic mutation leading to major modulation in gene expression which may be amplified by epigenetic mechanism of gene expression reflected by the asynchrony in the replication timing of the homologous DNA loci. Our work shows that aneuploidy was generated by the exposure to the electromagnetic radiation. As, according to the aneuploidy theory, random chromosome number mutations evolving autocatalytically from the status of aneuploidy open the road to cancer, our results suggest the exposure to RF radiation at a SAR value of 2.9 W/Kg and above pose a risk for cancer.

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