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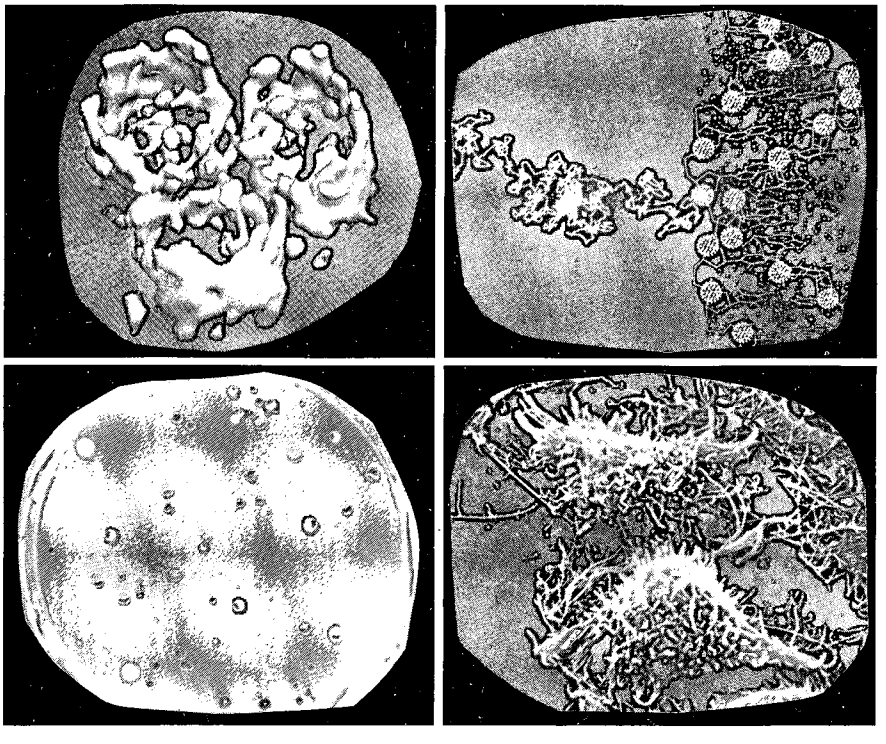

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ABSTRACT: We have investigated whether ELF magnetic fields influence the release of proteins from the cell surface of human lymphocytes. Protein shedding was followed by monitoring the release of fluorescently-labeled monoclonal antibodies directed against CD3(pan T-cell marker) and CD20(pan B-cell marker) cell surface determinants. Preliminary results indicate that T-lymphocytes exhibit an approximate one-fold increase in anti-CD3 antibody release in the presence of a 60 Hz magnetic field (220G, 1.7mV/cm, 60 min, 37°C) compared to isothermal control treatment. In contrast, B-lymphocytes did not show a marked change in anti-CD20 antibody release in the field compared to controls. Such responses suggest that human T- and B-lymphocytes respond differently to ELF fields.

INTRODUCTION. An important way that electromagnetic fields can alter membrane structure and function is to remove proteins for the cell surface [1-3]. Proteins at the cell surface are responsible for recognition of extracellular signals important to cellular function. Two classes of proteins, intrinsic and peripheral, constitute the membrane bilayer (**Figure 1**). Peripheral proteins are loosely bound to the cell membrane, do not span the bilayer, are anchored via cationic and anionic bridges, and are readily water soluble. They are candidates for protein shedding in ELF magnetic fields.

Previously we have shown that ELF fields (220G, 1.0 mV/cm, 60 min, 37C) lead to enhanced protein shedding in rat thymic lymphocytes[4]; these are precursor or immature T lymphocytes. Proteins are released in the 6K-150 KDa range that have a net negative surface charge. This demonstrates that 60Hz fields release endogenous proteins from the cell surface of immature T-cells.

We now asked whether specific peripheral proteins placed on the cell surface of human T- and B-lymphocytes are also released by 60Hz magnetic fields. Results indicate that antibody to CD3 on T-lymphocytes show an approximate one-fold increase in shedding. This increase in protein shedding is consistent with our previous findings for rat thymic lymphocytes. B-lymphocytes, however, did not exhibit a large change (~10%) in release of antibody to CD20 in 60Hz fields compared to isothermal-treated control cells.

METHODS. Monoclonal antibodies were obtained from Becton Dickinson, San Jose, CA. The pan-T-cell monoclonal was fluorescein isothiocynate(FITC)-conjugated and directed against Leu-4 (CD3). The pan-B-cell monoclonal was phycoerythrin(PE)-conjugated and directed against Leu-16 (CD20). Human peripheral blood lymphocytes were isolated using Sigma Ficoll-Hypaque 1077. Monoclonal labeling procedures were as specified by the manufacturer at 4°C. Cells were washed twice in Hepes-buffered balanced salt solution, pH 7.4. Exposures to 60 Hz magnetic fields were as described using special multiring petri dishes to establish a spatially-uniform induced electric field OF 220G, 1.7 mV/cm [5]. Supernatant from cells containing FITC- and PE-conjugated monoclonals were analyzed using a CytoFluor 2350 automated fluorescence plate reader (Millipore, Bedford, MA).

Exposures to 60 Hz magnetic fields were as recently described [5]. A water-cooled solenoid was employed to generate a uniform 220G magnetic field. Inside of the bore of the solenoid, positioned in the middle-third volume, was a secondary waterbath system mechanically isolated from the solenoid. This secondary waterbath housed a multiring acrylic multiring petri dish which was thermoregulated to 37 ± 0.05°C. This multiring plate was positioned perpendicular to the magnetic field so that a spatially uniform induced electric field was established in the ring of the plate where cells were placed. Thus, according to Faraday's Law of Induction [5-7] these cells were exposed to a uniform magnetic (220G) and electric field (1.7mV/cm). Conductivity of the buffer medium was 1.6 S/m, thus the current density was approximately 27 $\mu A/cm^2$. Control treatment of cells consisted of incubating cells in an isothermal waterbath remotely located from the solenoid, at the same time field exposures were conducted. This waterbath was completely shielded in μ-metal to eliminate endogenous 60 Hz magnetic fields (< 5 mG). Immediately following treatment cells were harvested and spun in a high-speed microfuge and the cell-free supernatants collected for fluorescence analyses.

RESULTS Previously we observed that ELF fields lead to increased protein shedding in rat thymic lymphocytes [4], which are immature T-lymphocytes. This suggested that T-lymphocytes may respond in a similar manner and release surface proteins in ELF fields. However, we wished to assess if specific

proteins would be released from the cell surface. Thus we employed FITC-labeled anti-CD3 monoclonal antibodies to label CD3 receptor sites on mature human peripheral blood T lymphocytes.

Figure 2 depicts the results of an experiment in which FITC-labeled T-lymphocytes were treated with a magnetic field or placed in an isothermal waterbath and the supernatant analyzed for the presence of FITC-anti-CD3 antibody. We observed that CD3 antibody was released from sham treated cells, and that this release was significantly increased in the magnetic field. An approximate 73% increase in fluorescence was detected and this corresponds to an increase in CD3 antibody release of 19 to 41 ng/ml. This is based on a standard curve constructed using FITC-anti-CD3 in which fluorescence was correlated to known protein concentration of the monoclonal stock. These results are consistent with results from rat thymic lymphocytes, mentioned above, in which an increase in endogenous protein release was observed.

We have not previously evaluated B-lymphocytes for protein shedding. This was done by performing an analogous experiment as described for T-lymphocytes employing PE-labeled-anti-CD20 antibody. **Figure 3** depicts results from this experiment. Sham-treated B-lymphocytes released anti-CD20 antibody and magnetic field treatment was associated with less protein shedding. The magnetic field resulted in approximately 11% less protein shedding. Although this difference appears statistically significant such a slight change is probably not biologically meaningful.

DISCUSSION. We have evaluated protein shedding previously in erythrocytes exposed to electromagnetic fields[1-3]. Both gel electrophoresis and HPLC detected the enhanced release of endogenous proteins from the cell surface during exposures. Recently, this phenomenon was extended to detect the release of endogenous proteins from the rat thymic lymphocyte using HPLC techniques [4]. The present study reports the first evidence of changes in antibody release from human peripheral blood lymphocytes. Specifically T-lymphocytes respond to magnetic fields by releasing significantly more anti-CD3 antibody than do isothermal control cells. This is consistent with increased release of endogenous proteins from rat thymic lymphocytes [4]. B-lymphocytes do not appear to be markedly influenced (~11% change) by the magnetic fields.

Protein shedding is a natural phenomenon and it is observed in the control, isothermal-treated lymphocytes. Peripheral proteins, as depicted in **Figure 1**, represent the class of proteins which are loosely bound to the cell surface via noncovalent interactions such as hydrogen bonds, anionic/cationic bonds, and polarity/hydrophobic interactions. Possible mechanisms of release must involve chaotropic interference with these interactions. The mechanism of field action for protein shedding is not known, but probably involves movement of charge in the oscillating field which could disrupt noncovalent interactions. We plan further studies to characterize protein shedding using (a) cells maintained in buffers of different conductivity, which should define a dose-response correlated with induced current, and (b) cells exposed in a constant magnetic field at different induced electric fields using our multiring cell culture plate[5-7], which should also define a correlation with induced current.

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FIGURE LEGENDS.

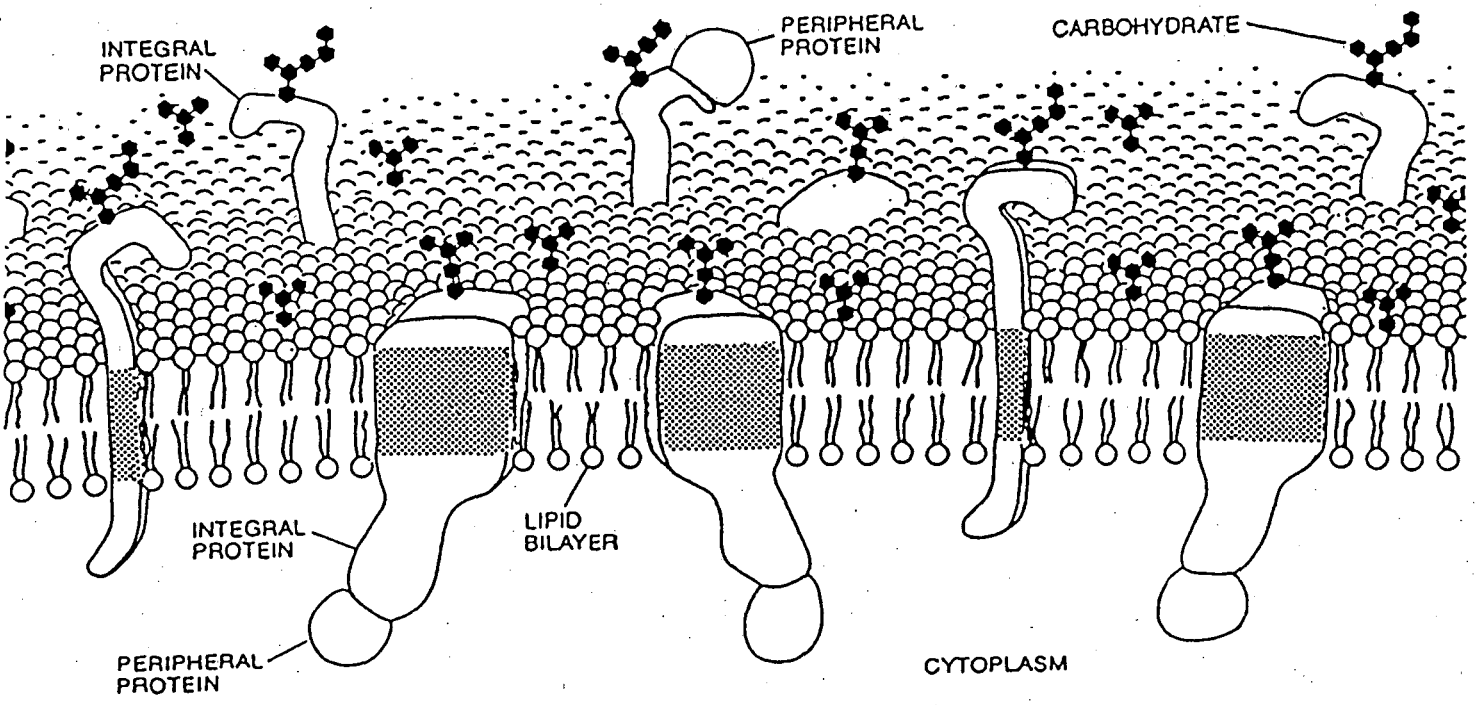
Figure 1. Schematic Representation of Peripheral and Intrinsic Proteins in the Cell Membrane.

Figure 2. Protein Shedding of Anti-CD3 Antibody From Human T Lymphocytes. Mean ± S.E. (n=12).

Figure 3. Protein Shedding of Anti-CD20 Antibody From Human B Lymphocytes. Mean ± S.E. (n=12).

Figure 1

PERIPHERAL PROTEINS ARE LIKELY CANDIDATES FOR PROTEIN SHEDDING



PERIPHERAL Proteins, Unlike INTRINSIC Proteins

- ❑ Do Not Span the Bilayer
- ❑ Are Bound Via Cationic/Anionic Bridges
- ❑ Are Released By Mild Choatropic Agents That Do Not Disrupt the Bilayer
- ❑ Are Readily Water-Soluble and Not Associated With Membrane Lipids

Figure 2

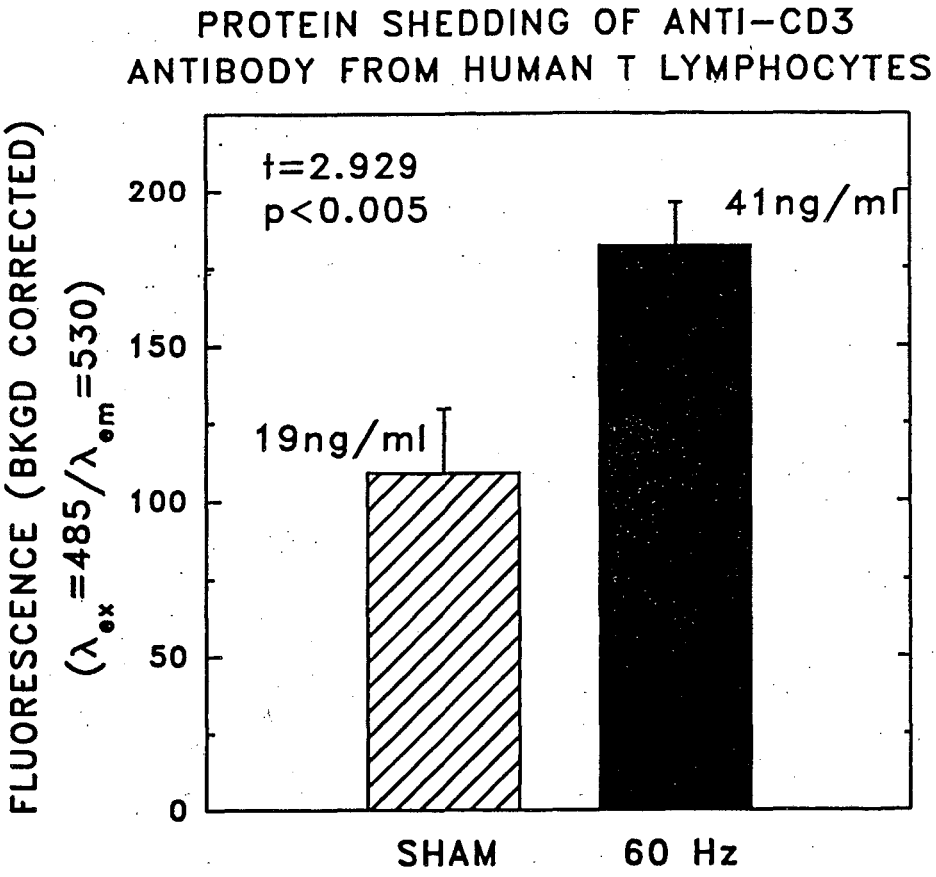
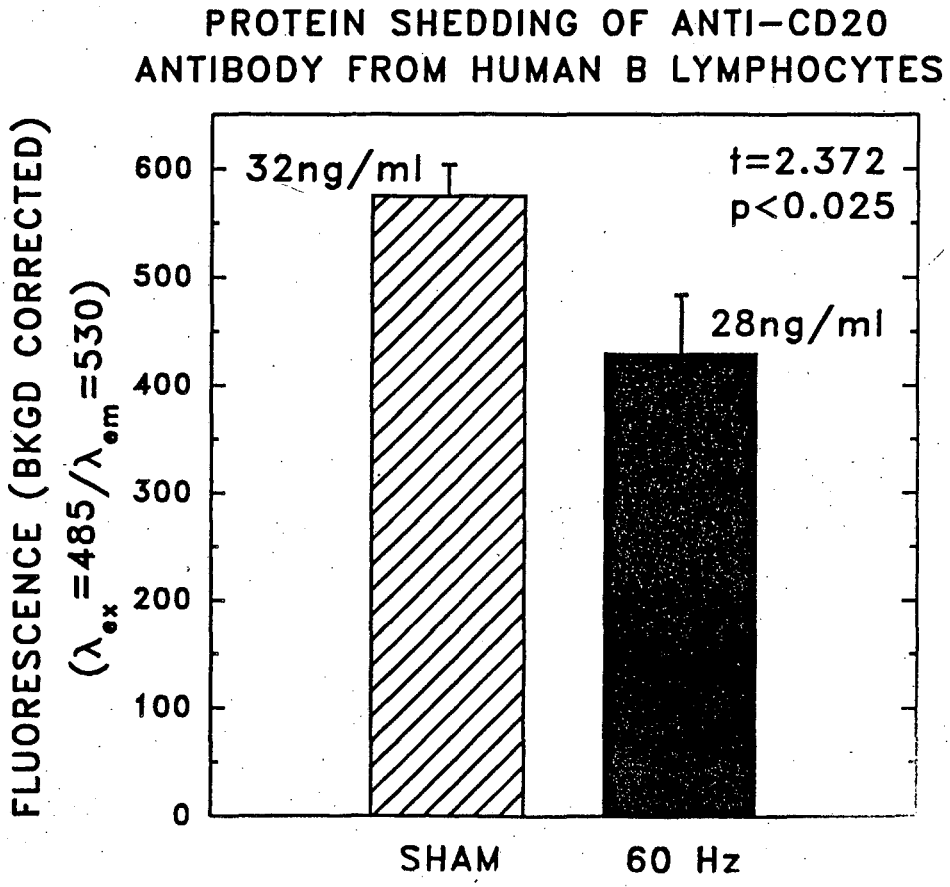


Figure 3



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