Weak Pulsed Current Narrow-Resonance Stimulation of Antibody Production in Mice

A. A. Podkolzin and V. I. Dontsov

UDC 612.124.017.1.06:615.844].08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 416-418, October 1993
Original article submitted May 21, 1993

Key Words: *antibody production; mice; weak pulsed currents*

Data obtained in recent years demonstrate a high sensitivity of the body to low-intensity factors of various nature, such as a magnetic field of a level below the terrestrial field, weak electromagnetic radiation, exposure to light, weak direct and alternating currents, low (homeopathic) doses of chemical compounds, etc. [2,3,6,7].

It is evident that a lack of "strength" of an agent may be compensated for by the fact that this agent acquires the nature of a signal; in other words, an informative type of bombardment takes place on an intricate system - the body.

One of the best known and most thoroughly studied mechanisms of such agents is the resonance mechanism - the enchancement of certain parameters of a system under the influence of strictly determined signals.

The purpose of this study was to elucidate the possibility of narrow resonance effects of low-intensity factors on a biological object as exemplified by the immune response of mice exposed to weak pulsed currents.

MATERIALS AND METHODS

BALB/c mice weighing 17-19 g were obtained for the experiments from the Stolbovaya nursery of the Russian Academy of Medical Sciences. For immunization erythrocytes of 2-3 outbred rats washed in Hanks solution were intraperitoneally injected in a dose of 5×10^6 cells per mouse in 0.5 ml. A total of twelve experimental series were carried out, each with 15-20 animals; 3-4 animals were used per point.

The immune reaction was assessed by recording plaques in monolayers in glass chambers [5]. For this purpose, washed immune murine splenocytes, $0.5\text{-}1\times10^6$ in 0.05 ml Hanks solution, were mixed with a similar volume of 1% erythrocytes, with which immunization was carried out, and with 0.05 ml of 1:6 diluted rabbit complement adsorbed with rat erythrocytes. The chambers were covered with slides and placed in an incubator at 37°C in an exciccator with 100% humidity. After

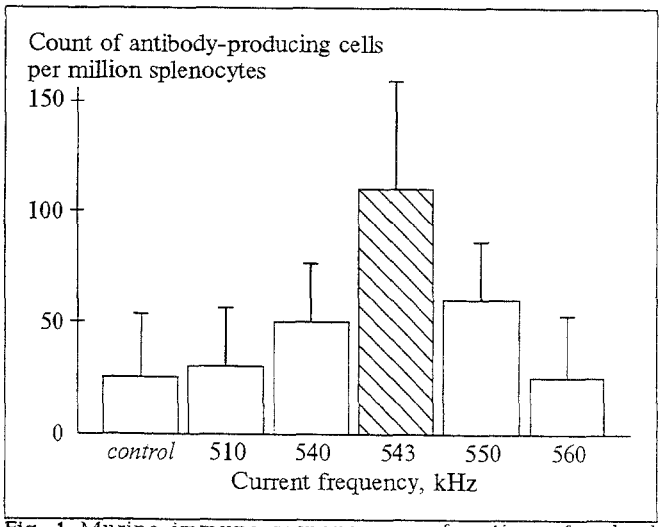

Fig. 1. Murine immune response as a function of pulsed current frequency.

Central Research Laboratory, N. A. Semashko Moscow Medical Stomatological Institute. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0010-1279$12.50

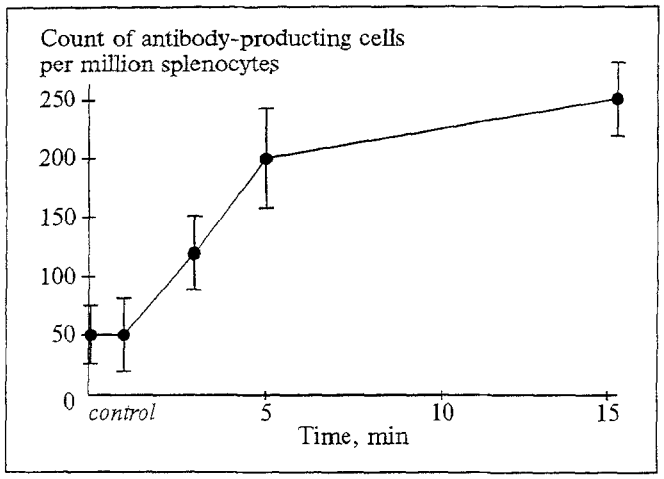

Fig. 2. Murine immune response as a function of stimulation with pulsed currents and time of exposure. Exposure duration 5 min, frequency 543 kHz.

2.5 h incubation, the hemolytic plaques in a chamber were counted to determine the count of antibody-producing cells (APC), which was expressed in absolute figures per million immune murine splenocytes.

The mice were exposed to weak currents during the forst hour after immunization using a G6-28 signal generator reproducing signals of various shape and strength in a wide range of frequencies. The standard duration of exposure was 5 min, the electrode voltage was 15-20 mV, and electrodes 6cm^2 in size were placed on the chest and back.

The effects of pulsed currents were compared by the absolute counts of APC and by estimation of the stimulation index (ratio of APC counts in the experiment and intact control). The mean count of APC and the mean square deviation were calculated by comparing the groups using the Student test.

RESULTS

The murine immune response to rat erythrocytes was increased when the antigen was administered in doses of 0.1 to 10×10^6 cells per animal and peaked 4 days after immunization; therefore, in subsequent experiments the dose of 10^6 erythrocytes was used and the response was assessed 4 days after immunization.

A study of the effects of pulsed currents in the 100-1000 kHz range showed the presence of a narrow resonance "window" with a maximum for the frequency 543 kHz. (Fig. 1). A frequency deviation from the preset value within 1-2% markedly reduced the stimulation intensity, which was 5 to 20 times increased in comparison with counts of APC in intact animals in various experiments. Higher stimulation indexes were typical for immunization with low doses of the antigen.

In our experiments the stimulation effects were dose-dependent (Fig. 2) and influenced by the shape of the signal (Fig. 3). Pulsed currents of a single direction, square or triangular, with a rapid increase of current strength, were effective.

Hence, the immune reaction could be markedly stimulated by low-intensity pulsed currents provided that the signal frequency was strictly limited, this being a typical "resonance" mechanism of the effect of physical factors on systems of various types. A resonance type of influence is characterized by the absence of a lower threshold, and even the minimal signals corresponding to the optimal reaction region significantly enhance a particular characteristic or function of a system [1,4]. Such a mechanism may in fact underlie the effects of many low-intensity factors, particularly physical ones, determining their powerful effects on biological systems.

The specific nature of resonating structures of the organism differs widely: electron and ion transport mechanisms underlie virtually every process of energy production and utilization, as well as the regulation of the metabolism, and of a variety of complex physiological functions. Of special importance are regulatory rhythms of the brain, local processes of organ autoregulation, intracellular rhythmic coordination of organelle activity, and, particularly the processes involving membranous structures which determine electrical and chemical gradients, ion transport, and so on.

REFERENCES

1. I. I. Blekhman, *Synchronization in Nature and Technology* [in Russian], Moscow (1981).

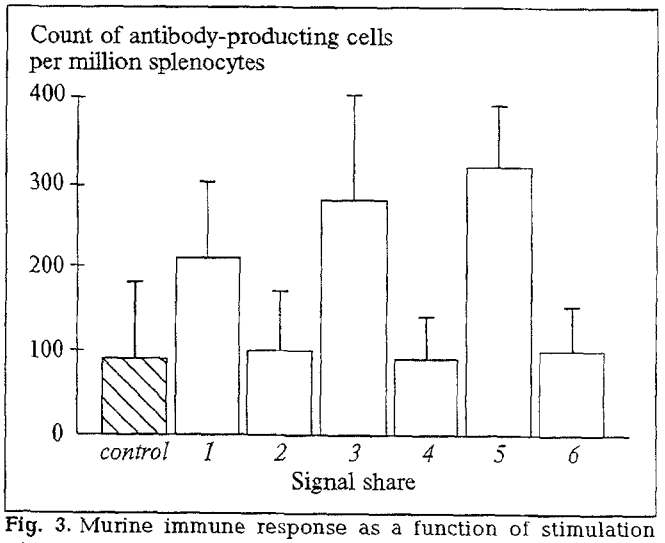

Fig. 3. Murine immune response as a function of stimulation with pulsed currents and signal shape.

2. V. I. Dontsov and T. A. Ismailov, *Pat. Fiziol.*, № 5, 65-66 (1984).
3. A. A. Podkolzin and E. P. Markin, *Izvestiya Akad. Nauk SSSR*, **54**, № 10, 40 (1990).
4. E. N. Chirkov and Yu. N. Babaev, *Electromagnetic Nature of Immunity and Cell Differentiation* [in Russian], Akad. Naul SSSR, Inst. Kibernetiki, № 62, 1-18 (1987).
5. A. J. Cunningham, *Nature*, **207**, 1106-1107 (1965).
6. E. Ettlenne, P. Hoeng, and R. Frankel, *Effects of Magnetic Fields on Ca Transport in Isolated Muscle Microsomes: NSWC/URSI Spring Meet. Seatle*, Washington (1979), p. 442.
7. J. Walleczek and R. P. Liburdi, *FEBS Lett.*, **271**, № 1-2, 157-160 (1990).

Immunochemical Identification of Some High-Molecular Proteins in Tumor Nuclear Matrix

L. S. Filatova and I. B. Zbarskii

UDC 616-006-018.1-008.939.6-078.33

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 418-421, October 1993
Original article submitted April 26, 1993

Key Words: *nuclear matrix; fibronectin; hepatomas*

Extraction of isolated cell nuclei with a detergent, 0.14 M NaCl, and 2 M NaCl results in an insuluble residue consisting mainly of nonhistone proteins and retaining the shape and morphological structure of the nucleus. This residue is usually reffered to as the "nuclear matrix" (NM) or "nuclear skeleton."

The NM is not only the structural basis of the nucleus but is also involved in important biological functions, being the site of DNA replication and transcription and of mRNA transport to the nuclear envelope [3].

The NM of tumor cells is characterized by a predominance of the high-molecular protein group [1]. These proteins are virtually unidentified. The major components of this group are pore complex glycoproteins [6], DNA topoisomerases [8], and the MAP-2-like protein p260 [11].

A recent communication from Sauermann's laboratory reports the detection in HeLa cell nucler matrix of fibronectin, a normal component of extracellular matrix heretofore undetected in the nucleus [13]. Fibronectin has also been found in cell nuclei of some hepatomas [5].

N. K. Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow

The findings mentioned above are limited to the immunochemical reaction to fibronectin and do not relate to its fine localization in nuclear structures.

We studied NM isolated from a solid rat hepatoma 27 and ascites mouse hepatoma 22a and compared it to normal rat liver.

MATERIALS AND METHODS

Nuclei from the liver and hepatoma 27 were isolated according to Blobel and Van Potter's method [4] in a modification used in our laboratory [9]. Hepatoma 22a cells transplanted in F_1(CBA×C57Bl$_6$) mice were destroyed by incubation in distilled water and treatment in a Potter-Elvehjem homogenizer. The nuclei were then sedimented at 800 and 20,000 g in 1.8 M sucrose. The NM was prepared by extraction of isolated nuclei with 0.5% Triton X-100, treatment with 100 μg DNase I and RNase each in 5 ml of 20 mM Tris-HCl buffer, pH 7.4, containing 0.2 M sucrose, 2 mM $MgCl_2$, and 3 mM $CaCl_2$. Then the nuclear sap and chromatin were successively extracted with 0.14 M and 2 M NaCl [9].

Preparations of NM were then subjected to electrophoresis in polyacrylamide gel [10]; the pro-

0007-4888/93/0010-1281$12.50