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Effect of Fractionated Administration of Antigen on Rosette Formation in Mice

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Rosette formation is shown to be stimulated by administration of the same antigen dose in several injections at short intervals. The effect of fractionated administration of the antigen is mainly due to T lymphocytes, is selectively directed to antigen-binding structures containing disulfide groups, and is realized via the vagus nerve.

Key Words: *effect of fractionated administration of antigen; vagus nerve; rosette formation*

Studies of the early manifestations of the organism's reaction to an antigen have shown that just a few seconds after challenge, rosette formation (RF) in the spleen, bone marrow, and peritoneal suspension of mice drastically increases. A similar *in vitro* effect has been observed after 15-min incubation of cells with the same antigen (sheep red cells - SRC). After incubation of cells without SRC the number of rosette-forming cells (RFC) corresponded to the level of spontaneous RFC. Since the count of early RFC at the peaks of the reaction was several times higher than that of spontaneous RFC, it is obvious that the bulk of the early cells consisted of antigen-activated cells which were able to react to it upon repeated *in vitro* contact long before the beginning of the classical immune response [2]. Hence, we may assume that the immune system is activated by repeated exposure to the antigen *in vivo* in the period of early RF.

In order to verify this hypothesis, we investigated the capacity of immunocompetent organs and tissues to react to repeated injections of antigen soon after the first administration of SRC.

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MATERIALS AND METHODS

Experiments were carried out with 320 CBA mice aged 3 to 4 months divided into 38 groups with at least 7 animals in each. The antigen was SRC injected intravenously or intraperitoneally in doses of 5×10^4 to 5×10^7 per mouse. The principal methodological approach consisted in the following: in contrast to control animals injected the antigen once, the experimental animals were injected it in the same dose and the same volume of 0.9% NaCl but in two or more injections with short (5 to 80 sec) intervals between the injections, without the needle being removed from the vein or peritoneal cavity. Such a method provides for reproducibility of the effect of fractionated administration of an antigen (EFAA) and permits multiple exposure of the organism to the antigen at preset intervals during a single injection procedure. Some of the animals were subjected to sham operation or unilateral vagotomy under Nembutal narcosis (50 mg/kg).

Suspensions of splenocytes, peritoneal exudate, and bone marrow were prepared at various times (30 min, 3 and 5 days) after challenge with SRC.

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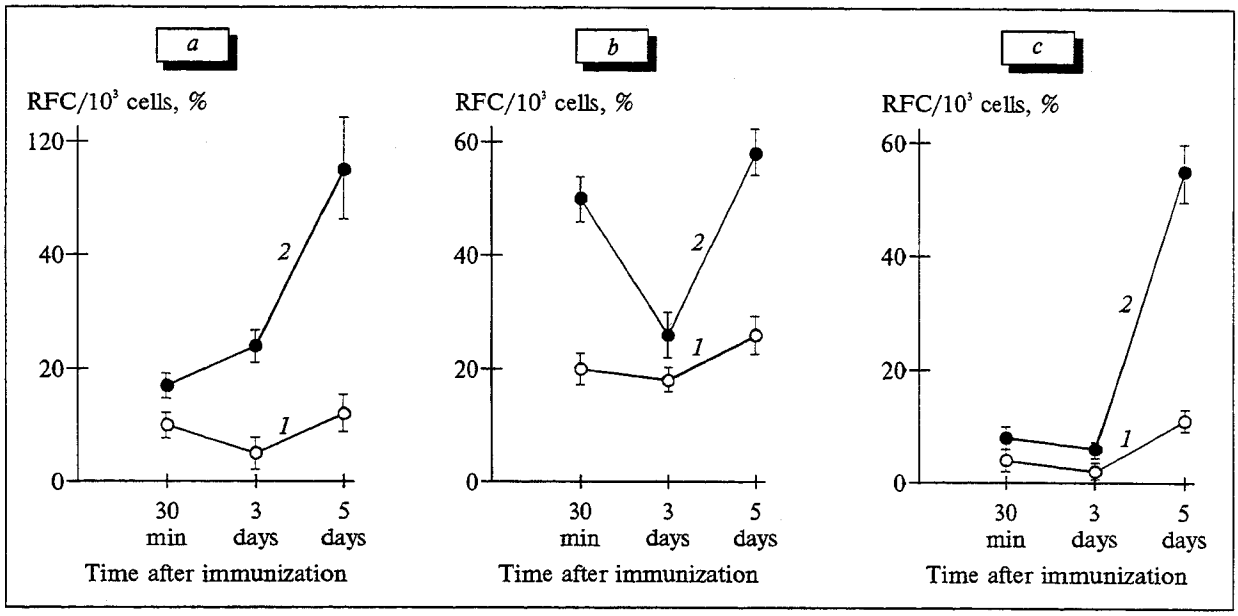

Fig. 1. Manifestation of the effect of fractionated administration of antigen on the time course of rosette formation in the spleen (*a*), peritoneal suspension (*b*), and bone marrow (*c*) of mice after intravenous injection of SRC (5×10^7 per mouse) one time (*1*) or in two equal portions at a 10–sec interval (*2*).

The samples were washed three times in Hanks' solution with 0.001 M HEPES, pH 7.2, by centrifugation in silicon-coated tubes. The resultant suspensions were incubated for 15 min at 37°C with equal volumes of a 3% suspension of SRC (5 to 20 per cell) and stirred, and a mobile preparation was made, with the slide supported with a ring of mineral oil inside which the examined cells were placed with the use of a phase-contrast microscope (×1000), 1000 cells were examined, and the total number of RFC was counted. Treatment of the RFC with 2-mercaptoethanol (0.1 M) for 2 h at 4°C allowed the cells to be divided into those sensitive and insensitive to this agent [8]. For identification of the type of RFC cells, the adhering cells were first removed [7] and the rest of the cells were differentiated into T- and B-lymphocyte-enriched cells using monoclonal anti-Thy 1.2 "G" antibodies and rabbit complement [9]. The counts of RFC were assessed for each of the cell populations using the above manipulations. The data were statistically processed using Student's *t* test. The arithmetic mean values with 95% confidence intervals are presented.

RESULTS

Rosette formation served as the test of antigen-induced activation of the immune system. This test is convenient for detecting the actual fact of exposure and for studying its resolving capacity due to the generalized and highly sensitive process, the heterogeneity of RFC in origin and function, and their early manifestation after immunization [2].

The results of the first experiment confirmed an earlier hypothesis about the possibility of activating the function of the immune system due to the reentry of antigenic information in the organism within an extremely short period after the first challenge. Fractionated administration of the antigen intensively stimulated the reaction and changed its time course (Fig. 1).

Assessment of the effect of the ratio of the antigen dose (1:3, 1:1, 3:1) injected in two portions showed that injection in equal portions was optimal, and this mode was used in further experiments.

Study of the intervals between injections of SRC (5×10^6) in 2 portions (Fig. 2, *a*) showed that intervals equal to the peaks of early rosette formation (10-20 sec and 30 min) were optimal. Disappearance of EFAA at an interval of 80 sec corresponded to a decline in the count of early RFC 2 min after immunization [2]. During administration of the antigen in 1, 2, 3, 4, or 5 portions the maximal stimulation (seven-fold) was recorded with the SRC dose divided into 3 portions.

The equal reproducibility of EFAA permitted us to replace intravenous injections of SRC with intraperitoneal injections. During a study of this effect at 30-min intervals an additional injection was not found to be a physiologically significant factor for the formation of the subsequent immune response. Stimulation of rosette formation after reinjection of SRC after 30 min was due to EFAA.

Comparison of EFAA during injection of SRC in different doses at optimal intervals between the portions showed a clear-cut reduction in the count

of RFC after a single injection with a lower SRC dose. Using different variants of EFAA, we managed to attain an equal level of rosette formation in response to doses differing by 10 and 100 times (Fig. 2, *b*).

Study of RFC induced by EFAA using 2-mercaptoethanol (destruction of S-S bonds by their reduction to SH groups) showed (Fig. 3, *a, b*) that fractionated injection of the antigen selectively induced activation of mercaptoethanol-sensitive cells in all the tissues tested. In the spleen, where EFAA was more evident, we compared RFC counts corresponding to the adherent and T- and B-lymphocyte-rich cells (Fig. 3, *c*). One can see that EFAA was expressed in an intensification of rosette formation in all cell types, but particularly in T lymphocytes, where the count of RFC was 12 times increased. Moreover, the ratio of RFC was altered. In the control 73.2% of RFC belonged to cells rich in B lymphocytes, whereas in mice injected the same RFC dose in 2 portions 68.1% of cells were T-RFC.

Hence, the increase of RFC content caused by EFAA was mainly due to T lymphocytes binding SRC on account of proteins containing S-S groups, to which IgM and IgM-like molecules are known to belong.

The vagus nerve is known to contribute to changes in the redistribution of blood leukocytes [3], it has been reported to be sensitive to antigen challenge [4], and some immunostimulants of the regulatory systems are known to mediate their effects through the vagus nerve. For these reasons we speculated that this nerve may participate in the realization of EFAA and confirmed this experimentally. Preliminary unilateral vagotomy suppressed EFAA: the reactions of mice administered the total dose of antigen in a single injection or in several portions were the same.

These data suggest that EFAA is a result of interaction between the immune and nervous systems induced by the antigen. The similarity between the initial enhancement of EFAA after fractionated injection of the antigen in more and more portions and the phenomenon of summation of the traces of excitation in the neuromuscular synapse known in physiology appears to be no accident. The further decline of EFAA following fractionation of the antigen dose into still more portions (more than 3) may be explained by the fading amplitude of the neuronal reaction for rhythmic activation [1].

EFAA is not quite understood. It is evident that a repeated or rhythmic challenge at short-term intervals, needed for the reproduction of the effect, involves not just the mechanism of the peripheral nervous system. The priority of the immune system in the reaction to antigenic information forces us to make two assumptions: either immunocompetent organs and tissues contain cells which are capable of repeatedly or rhythmically reacting to rhythmic challenge with SRC at intervals of several seconds or minutes, or a new share of the respective cell population is involved with every new portion of antigen. These assumptions are to

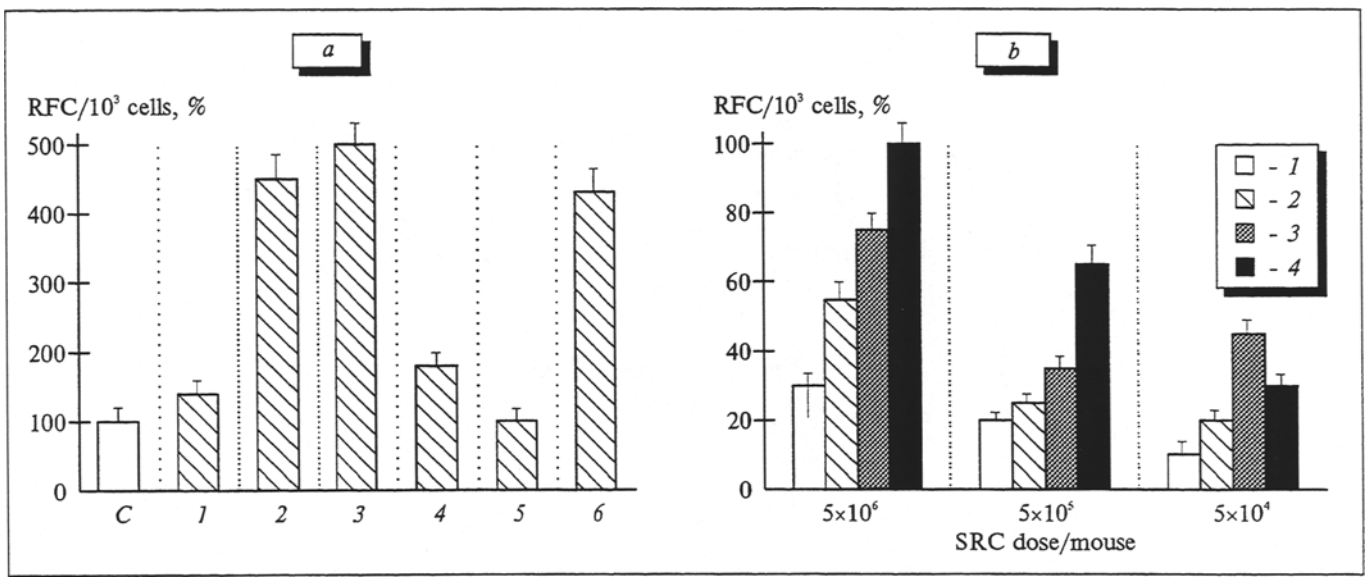

Fig. 2. Manifestation of the effect of fractionated administration of antigen injected by different methods. *a*) role of intervals between portions of SRC for the manifestation of EFAA with intravenous injection of antigen (5×10^6/mouse) in two portions with 5-sec (*1*), 10-sec (*2*), 20-sec (*3*), 40-sec (*4*), 80-sec (*5*), and 30-min (*6*) intervals between the portions. Control (*C*): mice administered the total SRC dose in a single injection. *b*) EFAA for intraperitoneal injection of SRC in various doses: in a single dose (*1*); in 3 portions with 10-sec intervals (*2*); in 2 portions with 30-min intervals (*3*); in 6 portions: the first 3 portions at 10-sec intervals and 30 min later the remaining 3 portions at 10-sec intervals (*4*).

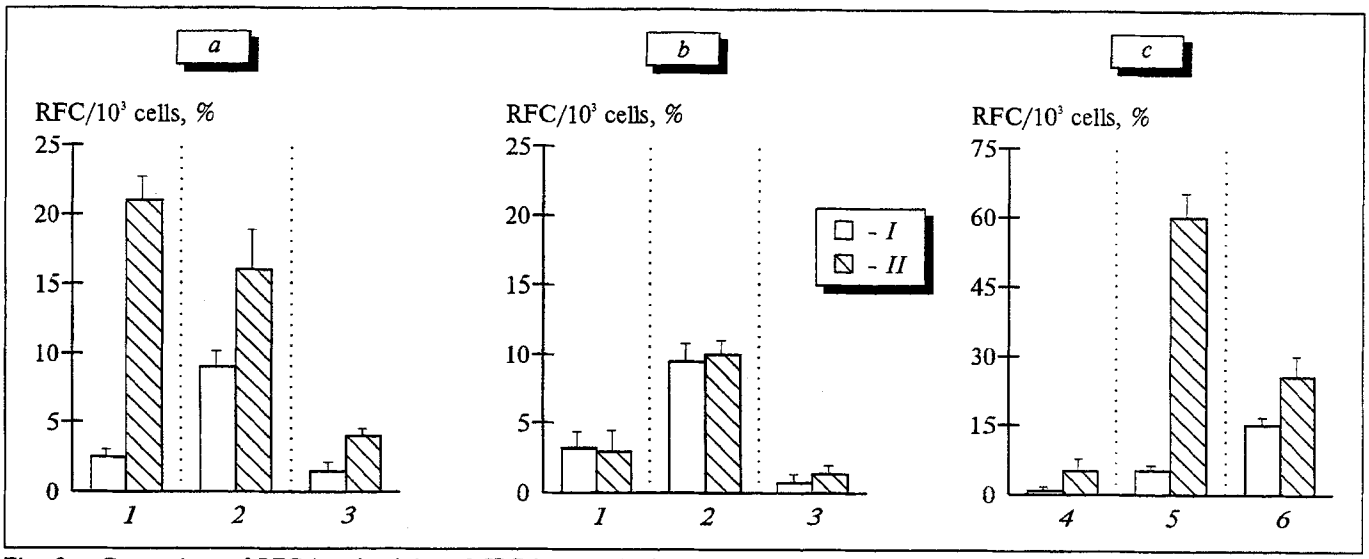

Fig. 3. Comparison of RFC in mice injected SRC in a single dose (*I*) or in 2 portions at a 10–sec interval (*II*). Effect of fractionated administration of antigen vis–a–vis the counts of RFC sensitive (*a*) and resistant (*b*) to mercaptoethanol in splenocytes (*1*), peritoneal exudate (*2*), and bone marrow cells (*3*) of mice 3 days after intravenous injection of SRC (5×10^7/mouse). EFAA and the content of RFC on day 5 after injection of SRC (5×10^6/mouse) in the splenocytes (*c*) divided into adherent cells (*4*), T lymphocytes (*5*), and cells rich in B lymphocytes (*6*).

a certain extent based on the data on the close similarity between the immune and nervous systems responding to rhythmic information by rhythmic changes in the electric potential of the cell membrane, on the changing electric potential of lymphocytes in response to an exposure [5], on the ability to respond to rhythmic exposure with rhythmic reactions which is characteristic not only of neurons [6], and on the increasing number of RFC among cells repeatedly (*in vitro*) exposed to the antigen within a very short period [2].

Hence, the described effect of fractionated administration of antigen is evidently due to repeated or rhythmic antigen challenge at short-term intervals (seconds or minutes) and is realized through the peripheral nervous system. The essence of EFAA consists in intensification of the reaction to the antigen by administration of the same antigen dose not all at once, but in several portions.

Situations giving rise to EFAA may not be confined to experiments. They may occur, for example, during autoimmune processes and in case of methodological errors during injection of immunogenic substrates.

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