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Stimulatory Effect of 5-Azacytidine on 7S Antibody Production in Rats (40134)

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The attempts to clarify the inhibitory mechanism and biological activity of 5-azacytidine are not yet complete. The action of 5-azacytidine is polyvalent and several steps in cellular metabolism are affected by this compound (1). Beside remarkable cytostatic effect directed primarily against acute myelogenous leukemia (2), the drug exhibits mutagenic, abortive, antimitotic and immunosuppressive (3) effects resulted from the impaired synthesis of DNA, RNA and proteins (4). 5-Azacytidine diminishes (3) the total hemagglutinin titres in mice immunized with sheep red blood cells (SRBC). It was the aim of this report to follow further the effect of 5-azacytidine on the intensity and character of humoral immune response to SRBC in rats. The diversity of the effect of 5-azacytidine on 7S and 19S antibody production in relation to the dose of drug and the time interval of its administration was studied.

Materials and methods. Female Wistar albino rats 12–14 weeks old were used throughout the experiments. For the analysis of antibody formation, 40% suspension of washed SRBC in saline was injected in two doses of 1 ml intraperitoneally on day 0 (primary immunization) and day 21 (secondary immunization). 5-Azacytidine was administered at a single dose of 2, 6, 16 or 22 mg/kg, intraperitoneally, at various time intervals before or after primary immunization.

At various time intervals after immunization blood samples were obtained by cardial puncture in a light aether narcosis, the sera were collected, heat-inactivated (56° for 30 min) and stored at −25° until examination. For the determination of 2-mercaptoethanol resistant hemagglutinins, aliquots of sera were mixed with an equal volume of 0.2 *M* 2-mercaptoethanol in isotonic phosphate-buffered saline, pH 7.3, and incubated overnight at 4°. Titrations of both total and 2-mercaptoethanol resistant 7S hemagglutinins were performed using a Takátsy microtitrator. One percent SRBC suspension was used as indicator system. Titres were expressed as $\log_2$ of the highest dilution in which hemagglutination was still observed. 19S antibody levels were calculated by subtracting 7S antibody titres from the total ones. The data were submitted to an analysis of variance and the significance of differences between experimental and control groups was determined by Duncan test (Matematical Center of Biological Institutes, Prague 4).

Results. To test the effect of 5-azacytidine on antibody formation, several separate experiments were performed. In the first experimental set animals were treated with 16 mg/kg of the drug shortly before or after primary immunization with SRBC. The secondary challenge with SRBC was carried out on day 21. Blood samples were obtained and assayed for antibody titres as shown in Fig. 1. Levels of 7S hemagglutinins are elevated in all 5-azacytidine treated animals as compared with untreated controls; this effect was higher when 5-azacytidine was administered simultaneously or 6 hr after the primary immunization. Levels of 19S antibodies were generally less affected, showing a slight depression.

In the second experiment, three groups of rats were injected by 5-azacytidine (16 mg/kg) on days 0, 2, or 4, respectively. All animals including untreated controls received two doses of SRBC on days 0 and 21 and were bled on days 5, 10, 21, 28, 35 and 42, as indicated in Fig. 2. Similarly as in the first experiment, 19S antibody levels were only slightly affected by 5-azacytidine. Simultaneous injection of the drug with antigen led to a marked elevation of primary 7S antibody response, as compared with controls. In the

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secondary response the elevation was less pronounced, but still existed. When the drug was administered 48 hr after the antigen, the primary 7S antibody response was practically abrogated and the secondary 7S response was markedly depressed. Application of 5-azacytidine 96 hr after antigen caused only slight inhibition of the 7S antibody production.

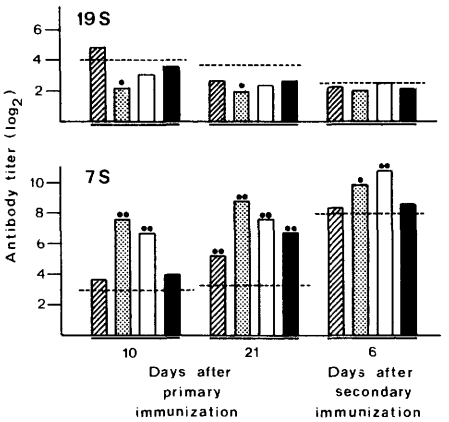

FIG. 1. Modification by 5-azacytidine of hemagglutinin production in rats immunized with SRBC. 5-Azacytidine (16 mg/kg) was administered intraperitoneally to groups of 6 female rats 16 hr before (□), simultaneously (□), 6 hr (□) or 12 hr (■) after primary immunization (1 ml of 40% suspension of SRBC, intraperitoneally). Secondary immunization was carried out on day 21 by the same dose of SRBC. Broken lines: the level of antibodies in untreated controls. Points: significant difference between the experimental group and untreated controls (●: $P < 0.05$, ●●: $P < 0.01$).

The effect of various doses of 5-azacytidine (2, 6 and 22 mg/kg) was further followed. The drug was given intraperitoneally either simultaneously or 48 hr after immunization with SRBC. As it is evident from Fig. 3., two higher doses of the analogue display a similar dual effect on 7S antibody formation as has been shown for the dose of 16 mg/kg. The lowest tested dose of the analogue (2 mg/kg) was in all cases generally less effective (Fig. 3.).

Discussion. The administration of 5-azacytidine results in a remarkable diminution in the number of circulating lymphocytes and of mature myeloid bone marrow cells (5). The largest drop in the synthesis of nucleic acids after 5-azacytidine was observed in the thymus. The drug beside being highly cytostatic in lymphoid leukemia (2, 6) causes an early induction of leukemia in the susceptible strain of inbred AKR mice (7), induces the avian sarcoma virus (8), and is carcinogenic as evidenced by the pulmonary tumour response in strain A mice (9). Also the radioprotective effect (10) of 5-azacytidine in AKR mice and its influence on the proliferation of the stem cells (11) as well as the higher uptake of thymidine into DNA and of orotic acid into RNA in the liver of 5-azacytidine-treated rats (12, 13) cannot be explained by a simple inhibition of cellular proliferation due to 5-azacytidine incorporation (1, 14) into RNA or DNA.

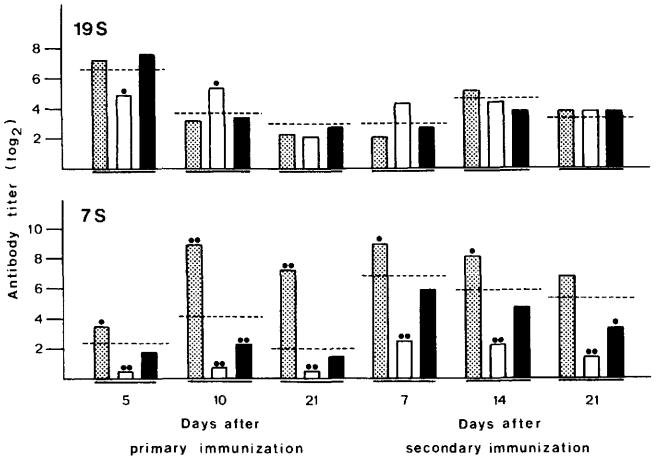

FIG. 2. Modification by 5-azacytidine of hemagglutinin production in rats immunized with SRBC. 5-Azacytidine was administered intraperitoneally to groups of 6–8 female rats simultaneously (□), 48 hr (□) or 96 hr (■) after immunization. Doses and symbols as in Fig. 1.

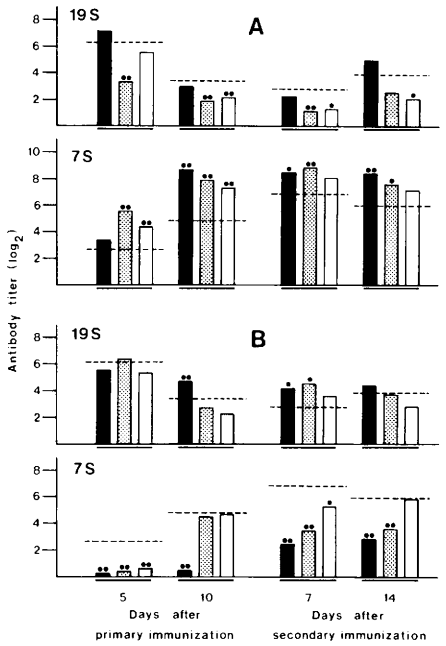

FIG. 3. Effect of various doses of 5-azacytidine on hemagglutinin production in rats immunized with SRBC. 5-Azacytidine was administered intraperitoneally to groups of 7–8 female rats in a dose of 22 (■), 6 (□) or 2 (□) mg/kg, either simultaneously with (part A of the figure) or 48 hr after (part B) primary immunization. Doses of SRBC and symbols as in Fig. 1.

5-Azacytidine considerably affects antibody response of rats to SRBC, particularly its 7S component (Figs. 1, 2 and 3). The action of the drug depends practically entirely on the time interval between immunization and drug administration. When the drug was given 2 days after primary immunization a profound inhibition of 7S hemagglutinin synthesis persisting even after the secondary challenge with the antigen was observed. Our results are comparable to those of Goldin's group (3, 15) where 5-azacytidine diminished the total hemagglutinin titres in mice immunized with SRBC. However, much higher doses of the drug and different time intervals between immunization and drug treatment were used by those authors; the lowest dose of 5-azacytidine (20 mg/kg) applied one day before or after immunization had practically no effect on total hemagglutinin titres to SRBC in mice (15).

When 5-azacytidine was given simultaneously with the first dose of antigen a considerable enhancement of 7S antibody production was obtained in our system (Figs. 2 and 3). The stimulatory effect of 5-azacytidine was observed using a wide-range concentrations of the drug (2–22 mg/kg). However, the intensity of both the inhibitory as well as stimulatory effect of 5-azacytidine was closely related to the amount of administered drug. Only, when the highest dose of the drug (22 mg/kg) was used, the stimulation of 7S antibody production was delayed (Fig. 3) reaching maximum on day 10 after immunization. It is evident that the stimulation of 7S antibody synthesis following 5-azacytidine administration is not preferentially related to low doses of the drug.

Similar enhancing effect on antibody production was already described for 6-mercaptopurine in mice immunized with low doses of bovine gamma globulin or with SRBC (16, 17). In the present experiments both enhancing and suppressing effects of 5-azacytidine on 7S antibody titres persist for many days and are not affected by the secondary challenge with antigen. Moreover, the level of 19S antibodies is less affected by 5-azacytidine than 7S antibodies. The observed changes in 19S antibody levels are inversely related to changes in 7S antibodies and could be due to a lack of negative feedback mechanism operating between 7S and 19S antibodies (18). Predominant effects of some immunosuppressive drugs on 7S antibody production with relatively intact 19S antibody production have also been described by other authors (19, 20). Further, Galanaud et al. (21) have recently shown that the effect of azathioprine on the immune response is closely related to the type of antigenic stimulus.

Thus, the direct influence of 5-azacytidine on antibody synthesis is less probable and rather the modification of the cell proliferation and/or regulatory interactions among various types of immunocompetent cells seem to be involved. Although B cells are believed to be more sensitive to immunosuppressants than T cells (20–24), Röllinghoff *et al.* (25) found that T cell-mediated cellular immunity was more affected by azathioprine than B cell-mediated one. The time-dependent stimulation of immune response by 5-azacytidine could also be mediated by a selective elimination of suppressor T cell population since another drug, namely cyclophosphamide, interferes with the suppressor T cells as has

been found out by several authors (26–28).

Our results do not allow us to make conclusions about the mechanism of the effect of 5-azacytidine on the immune response of rats to SRBC. It seems, however, that a complex influence on the immune system should be expected, depending on the time interval between immunization and drug administration. It is supposed that the dual effect of 5-azacytidine is mediated by and reflects the interference of the analogue with the synthesis and/or function of various types of RNA molecules (4, 29, 30) including regulatory ones.

Summary. 5-Azacytidine administered to rats simultaneously with immunization by sheep red blood cells results in the stimulation of 7S antibody formation while its administration 48 hr after immunization causes a marked depression of their synthesis. The response of 7S antibody level to 5-azacytidine depends on the time interval of drug administration while the intensity of the effect is proportional to the dose level of analogue.

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