EFFECT OF COMBINED TREATMENT OF MOUSE VIRUS LEUKEMIA WITH CYCLOPHOSPHAMIDE AND FREUND'S COMPLETE ADJUVANT

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In experiments on leukemia induced in BALB/c mice by Rauscher virus potentiation of the action of cyclophosphamide in inhibiting leukemogenesis was obtained by the use of Freund's complete adjuvant. The best therapeutic effect (a statistically significant increase in the mean life span compared with untreated control animals and with animals treated with cyclophosphamide alone; survival of 60% of mice for not less than 4 months) was obtained by administration of Freund's adjuvant 1 week before a single injection of 250 mg/kg body weight cyclophosphamide given on the 10th day after infection with Rauscher virus.

In recent years attempts to use so-called nonspecific stimulators of immunity such as Freund's complete adjuvant (FCA) for the treatment of leukemias have been described (9, 12]. At the same time, evidence has been obtained that FCA, BCG, and other adjuvants can stimulate carcinogenesis in experimental animals [4, 5, 11]. This evident contradiction has been explained by the authors' investigations on mice with leukemia induced by Rauscher's virus in which administration of FCA in certain combinations with the virus stimulated specific humoral immune responses, and through this stimulation of specific immunity, stimulation of leukemogenesis can take place in a manner resembling the phenomenon of "autopotentiation" of tumor growth [6, 7]. A further study of the mechanism of action of FCA could identify the optimal conditions for its use in conjunction with chemotherapy so as to shift the balance between components of the immune response inhibiting and stimulating tumor growth in favor of effective antileukemic therapy.

The object of the present investigation was to study the effect of administration of Freund's complete adjuvant (FCA) in combinations with cyclophosphamide (CP) in the treatment of leukemia induced in mice by Rauscher virus.

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c mice aged $1\frac{1}{2}$-2 months. Rauscher virus (plasma from leukemic mice of the same line, diluted 1:100) was injected intravenously in a dose of 0.2 ml per mouse. The titer of the Rauscher virus preparations was 10^3-10^4 p.f.u./0.2 ml. The experimental mice were then divided into groups with 20-25 animals in each group; on the 3rd-4th or 10th day after injection of the virus the animals received a single intraperitoneal injection of FCA alone, of CP alone, or of a combination of both at different times after infection. FCA (Difco, USA) was injected in a dose of 0.1 ml, and CP in a dose of 250 mg/kg body weight. The control mice received no treatment. Altogether more than 300 mice were used in the repeated series of experiments. The organs of the dying animals were investigated cytologically and histologically. The mean life span and survival rate of the mice were sub-

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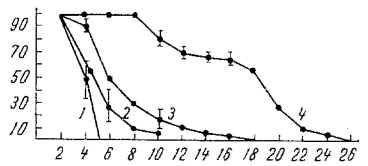

Fig. 1. Effect of treatment of Rauscher leukemia in BALB/c mice by cyclophosphamide combined with Freund's complete adjuvant (mean results of 3 series of experiments). 1) No treatment (control); 2) injection of FCA on 4th day after virus; 3) injection of CP on 10th day; 4) injection of FCA on 4th day and CP on 10th day. Vertical lines represent confidence intervals for 95% level of significance. Abscissa, time after injection of virus (in weeks); ordinate, survival rate (in %).

jected to statistical analysis, only those animals which survived 2 weeks after infection with Rauscher virus being considered in all groups so as to exclude mice dying accidentally after the manipulations associated with the injections or dying in the early period from the toxic action of the preparations. Student's test was used and differences between results were regarded as significant when $P < 0.01$.

EXPERIMENTAL RESULTS

Analysis of the efficacy of the various forms of treatment of Rauscher leukemia on the basis of the life span and survival rate of the mice in the 3 series of experiments (Fig. 1) gave the following results.

Injection of FCA alone on the 4th day after injection of the virus not only did not stimulate development of the leukemia, but led to inhibition of progression of the disease, although admittedly not by a statistically significant degree. At the same time, as previous investigations [6] showed, injection of FCA before injection of Rauscher virus or simultaneously with it led to significant stimulation of leukemogenesis in the BALB/c mice.

A single injection of CP on the 3rd-10th day after Rauscher virus prolonged the mean life span of the animals to 154-208% of the control level. This effect, however, was not statistically significant ($0.05 < P < 0.1$). Fewer than 10% of the mice of these groups survived to the 3rd month after injection of the virus.

Different combinations of CP and FCA led to highly significant prolongation of the mean life span of the mice (from 366 to 534%). However, it must be noted that the simultaneous injection of FCA and CP on the 3rd day after injection of the virus led to a high nonspecific mortality in the early stages; the dying animals exhibited a "devastation syndrome" of the lymphoid organs, usually coupled with pneumonia and enterocolitis. Under these conditions the mice were evidently particularly vulnerable to the cytostatic and immunodepressive action of CP. The best results, frequently confirmed by repeated experiments, were observed in the group receiving CP 1 week after FCA. The mean life span of the mice based on the combined results of the 3 series of experiments was 428% of the life span of the control animals and 244% of that of the mice injected with CP only ($P < 0.01$). Among the mice receiving FCA before CP, 60% of the animals survived for 4 months, whereas the longest period of survival of the control animals did not exceed 5 weeks.

These results demonstrate the efficacy of treatment of Rauscher's leukemia with a combination of FCA and CP. To determine the mechanisms whereby Freund's adjuvant potentiates the inhibitory action of CP on leukemogenesis, at least 2 working hypotheses must be tested experimentally: 1) the phenomenon described is connected principally with the ability of FCA to stimulate the development of immune responses aimed against antigens of the leukemic cells. It has been shown that preliminary sensitization of animals prevents the formation of tolerance by means of CP. The immunodepressive and tolerance-producing action of CP, if injected in the stage of final differentiation of antibody-forming cells, is ineffective and indeed may give the opposite effect: an increase in immunological reactivity to the particular antigen combined with preservation of the cytostatic activity of the compound [3, 8]; 2) the ability of FCA to stimulate proliferation of lymphoreticular tissue cells has a fundamentally important role. It has been shown that ineffectiveness of treatment may be due to predominance of cells incapable of division and resistant to the action of chemotherapeutic agents in the leukemic population [1]. At the same time, it has been shown that "slumbering," undividing leukemic cells are capable, in principle, of embarking once again on the mitotic cycle [12]. Injection of FCA may facilitate the transfer of the mass of resting leukemic cells into the proliferative pool and thus make them sensitive to the action of cyclophosphamide. The possibility cannot be ruled out that a combination of both these factors plays a role in the mechanism of inhibition of leukemogenesis by the FCA–CP combination.

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