EFFECT OF SOME ALKYLATING AGENTS AND OF WHOLE-BODY IONIZING IRRADIATION ON THE FORMATION AND REALIZATION OF THE IMMUNOLOGICAL MEMORY

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The secondary response in mice immunized twice with 1×10^6 sheep's erythrocytes was investigated by Jerne's method. The animals were treated with alkylating compounds (phenylalanine mustard, mannomustine, thio-TEPA, and cyclophosphamide) or by γ-ray irradiation either in the period of primary immunization, during revaccination, or in the interval between them. All the agents tested blocked the realization of immunological memory and weakened its formation. The alklylating agents had a stronger effect on memory formation than on the already formed memory. Irradiation was characterized by the opposite picture. The differences observed can be explained on the assumption that resting and proliferating lymphocytes differ in their sensitivity to irradiation and to alkylating compounds.

The features distinguishing the immunodepressive action of various alkylating agents are of great theoretical and practical interest because of the wide use of these compounds in modern medicine. Agents such as whole-body irradiation, phenylalanine mustard, cyclophosphamide, dipin, thio-TEPA, etc., have been shown to act differently on the initial and late phases of the primary immune response [2, 3, 6]. Their ability to induce immunological tolerance if injected in combination with a large dose of antigen also differed significantly [6].

The object of the present investigation was to study the effect of the above agents on the formation of the immunological memory and the secondary immune response.

EXPERIMENTAL METHOD

Adult noninbred mice, mainly males, were immunized intravenously with a small dose (1×10^6) of sheep's erythrocytes on two occasions at an interval of 27-44 days. The number of antibody-forming cells in the spleen 4 days after the second immunization was determined by Jerne's method [8]. The alkylating compounds were injected intraperitoneally, the injection being given either at the time of the first or of the second immunization or during the interval between them.

Phenylalanine mustard was injected in doses of 8 mg/kg daily on 4 successive days, the injections being started either 1 day before the first immunization or 7 days after it, or 1 day before revaccination.

Thio-TEPA was given by a similar scheme, the injections being started either 1 day before the first immunization, 14 days after it, or 1 day before revaccination.

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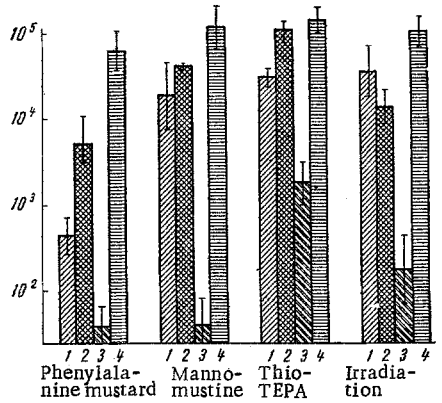

Fig. 1. Effect of alkylating agents and irradiation on formation and realization of the immunological memory. Abscissa, period of injection of compounds (irradiation): 1) with first injection of antigen; 2) in interval between injections of antigen; 3) with second injection of antigen; 4) control. Ordinate, number of antibody-forming cells in the spleen 4 days after second injection of antigen.

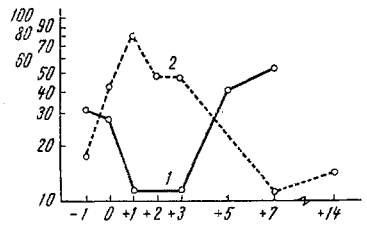

Fig. 2. Comparative sensitivity of different stages of formation of the immunological memory to the action of cyclophosphamide and of whole-body irradiation: 1) mice receiving cyclophosphamide (200 mg/kg); 2) mice irradiated with 500 R. Abscissa, days of irradiation or administration of cyclophosphamide before (–) or after (+) primary immunization; ordinate, number of antibody-forming cells in spleen of experimental animals 4 days after revaccination (in percent of control, logarithmic scale).

Mannomustine was injected in doses of 25 mg/kg daily on 2 successive days, the injections being started either 1 day before the first immunization, 14 days after it, or 1 day before revaccination.

Cyclophosphamide was injected as a single dose of 200 mg/kg (the times of the injection are shown below).

The animals were irradiated from a cobalt source in a single dose of 500 R. In the experiments of series I irradiation was given either a few hours before the first immunization, 7 days after it, or a few hours before revaccination. In the experiments of series II the times of irradiation varied.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of the various agents on the formation of the immunological memory and on its realization (the secondary response) was compared. The results are given in Fig. 1, which shows that all the agents tested have the strongest depressant effect on realization of the immunological memory: phenylalanine mustard, mannomustine, and irradiation completely suppressed the secondary response,* while thio-TEPA weakened it considerably. All the agents likewise depressed the formation of the immunological memory, but less severely; phenylalanine mustard had the strongest action, irradiation the weakest. Administration of phenylalanine mustard, mannomustine, or irradiation in the interval between the two injections of antigen weakened the secondary response, while thio-TEPA under the same conditions had no effect.

If the action of the same agents on the formation of the immunological memory and on the memory when already formed is compared, it will be clear that the process of formation of the memory was more vulnerable to the alkylating compounds (phenylalanine mustard, thio-TEPA) and less vulnerable to the action of irradiation. This is confirmed by the results of the experiments of series II, in which the effect of cyclophosphamide and whole-body irradiation on the various stages of formation of the immunological memory is compared (Fig. 2).

It will be clear from Fig. 2 that cyclophosphamide caused the strongest suppression of formation of the immunological memory if injected 1-3 days after primary immunization; injection of cyclophosphamide at other times was less effective. Irradiation, on the other hand, partially prevented the formation of the immunological memory if given just before the primary immunization, had almost no effect 1 day after immunization, and considerably weakened the immunological memory if carried out 1-2 weeks after primary immunization.

The immunological memory, if tested relative to the production of IgM-antibodies during the secondary response, is known to appear in mice [13, 14] 1-2 days after primary immunization with sheep's erythrocytes and to attain its full development after 5-9 days. The results of the experiments of series II can thus be expressed as follows: the process formation of the immunological memory is relatively radioresistant, but it is sensitive to cyclophosphamide; conversely, the cells carrying the mature memory are radiosensitive

*Cyclophosphamide had a similar action [3].

but resistant to cyclophosphamide. Analysis of Fig. 1 suggests that the above remarks about cyclophosphamide can be applied to some extent also to the other alkylating agents tested.

Lower radiosensitivity of the stage of formation of the immunological memory than of the memory when formed has also been observed previously by other workers [9, 11]. No clear explanation of this fact has yet been given. The suggestion has been made [9] that antigen persisting after the first injection may resensitize intact lymphocytes still remaining intact after irradiation; in the later stages no antigen is present and the memory cannot be restored after irradiation. This hypothesis evidently cannot explain differences either between the action of irradiation before and after the primary injection of antigen (Fig. 2) or in the action of irradiation and the alkylating compounds on the immunological memory.

Another hypothesis [11], which postulates proliferative activity of the cells during formation of the immunological memory, appears more promising. Proliferating blast cells are more radioresistant than small lymphocytes [10, 12], which carry mature immunological memory [4]. The blast cells are evidently relatively more sensitive to the action of alkylating agents than to irradiation, as is confirmed by morphological [7] and immunological [2, 6] observations. The biophysical basis of these differences may be differences in the action of alkylating compounds on the chromosomal apparatus of the cell: there is some evidence [1] that these compounds, unlike irradiation, give rise mainly to latent, persistent changes which are subsequently either brought to light in the S-phase of the cell cycle or repaired. If the cell population is proliferating at the time of action of the alkylating agent, the probability of fixation of potential lesions of the chromosomal apparatus is substantially increased.

The differences between the action of alkylating compounds and irradiation on immune processes observed above must not obscure the properties which are common to these agents (for example, the preferential effect on the secondary immune response compared with the process of memory formation), or the substantial differences between the action of individual alkylating compounds. This aspect of the problem has been considered by the authors elsewhere [2, 3, 5, 6].

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