ABILITY OF ANIMALS TO GIVE A HUMORAL IMMUNE RESPONSE AND ITS EFFECT ON DEVELOPMENT OF EXPERIMENTAL LEUKEMIAS

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Experiments on AKR and C57BL mice of different ages, using experimental models of transplantable Gross (first generation), Pujman, and spontaneous leukemia of AKR mice, showed that the ability of mice to produce humoral antibodies is inversely proportional to their susceptibility to inoculation of syngeneic leukemic cells and also to the incidence of spontaneous leukemia. The difference in ability to form an immune response to heterologous antigen were due to the physiological processes taking place in the aging organism. The results indicate that a failure of one component of immunity does not necessarily lead to an increased risk of tumor development in vivo.

KEY WORDS: leukemia; immunity; age differences.

One possible way of studying the role of immunity in the pathogenesis of tumor growth is a study of the principles governing tumor development depending on the state of humoral and cellular immunity against the background of which the pathological process develops. Laboratory animals of different age groups provide a natural model for the study of these problems.

In this investigation the development of leukemia was studied in mice with different humoral immune responses to injection of sheep's red cells. Differences in ability to form an immune response to heterologous antigen under these circumstances were due, not to any particular extremal factors, but to physiological processes taking place in the aging organism.

EXPERIMENTAL METHOD

Experiments were carried out on AKR (400) and C57BL (240) mice of different age groups. Primary G Gross (first generation) and Pujman (La) transplantable leukemias and spontaneous leukemia of AKR mice (Gross) were used as the models. The last form of leukemia developed spontaneously in 80-85% of cases by the age of 1 year in animals of this strain bred by the writers. Leukemias were transplanted by intraperitoneal injection of $5 \cdot 10^5$-$5 \cdot 10^6$ syngeneic leukemically transformed cells into the mice. The presence of a transplanted and spontaneous leukemia in the animals was diagnosed on the basis of clinical features, hematological changes, and the results of morphological investigations of the killed or dying mice. Their life span and the times of development of the disease were compared. Ability of the mice to form a humoral immune response was assessed from the number of antibody-forming cells (AFCs) in the spleens of the experimental animals [5] and from the height of the serum hemolysin titer.

EXPERIMENTAL RESULTS

The study of the ability of intact mice of both strains to give an immune response to injection of sheep's red cells showed that with age this diminished both in AKR mice, a strain highly susceptible to leukemia, and C57BL mice, with low susceptibility (Table 1).

As the results in Table 1 show, the decrease in number of AFCs in response to antigenic stimulation began in AKR mice at the age of 8 months i.e., in the preleukemic period. The fact that this phenomenon is

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TABLE 1. Number of AFCs in AKR and C57BL Mice of Different Age Groups (calculated per 10^6 nucleated spleen cells; M ± m)

Strain of mice	Number of AFCs in animals of different ages					
	2 months	4 months	6 months	8 months	10 months	12 months
AKR	1350±83	1350±148	1450±105	780±115	330±69	—
C57BL	249±27	—	235±49	219±42	272±35	63±9

TABLE 2. Development of Spontaneous Leukemia in AKR Mice with Strong and Weak Immune Response to Injection of Sheep's Red Cells

Experiment No.	Total % of mice dying from leukemia	% of mice dying from leukemia whose hemolysin level was	
		Above 1:160	Below 1:160
1	55	67	33
2	50	62	0
3	64	71	50
4	57	100	40
5	50	75	37
6	70	80	43
7	52	60	40

independent of the initial phase of leukemia development was demonstrated by the writers earlier [2]. Depression of the humoral immune response to sheep's red cells was observed later in life in the C57BL mice (at 12 months).

Transplantation of syngeneic leukemically transformed cells into intact AKR and C57BL mice, with strong (animals aged 2 months) and weak (C57BL mice aged 12 months and AKR mice aged 9-10 months) immune response to sheep's red cells revealed differences in their survival. Among both AKR and C57BL mice, the old animals, i.e., mice with a reduced humoral response to injection of sheep's red cells, died from transplanted leukemia at later times. For instance, whereas the survival period of female AKR mice aged 2 months with transplanted Gross leukemia was 25 ± 1.2 days in one experiment, animals aged 9 months in the same experiment died after 41 days ($P < 0.05$), and the survival of C57BL mice with Pujman leukemia was 8 ± 0.09 days (aged 2 months) and 9 ± 0.2 days (aged 12 months) respectively ($P < 0.05$).

Frequent repetition of the experiments in accordance with the same scheme showed high reproducibility of the results. In all eight series young sexually mature animals died earlier from transplanted leukemia. However, the survival period of AKR mice with transplanted Gross leukemia varied considerably in different experiments. This phenomenon was evidently connected with differences in the level of malignancy of the leukemically transformed cells injected into the animals, for a different donor of leukemia was used for each series. This may also explain the differences in the survival period of AKR and C57BL mice with transplantable syngeneic leukemias.

The decrease with age in the ability of AKR and C57BL mice to give a humoral immune response to heterologous antigen thus coincided with their increased resistance to transplantation of syngeneic Gross and Pujman leukemias.

Results of a similar character also were obtained by the use of Gross spontaneous leukemia as the model. These experiments showed (Table 2) that mice retaining higher ability to give a humoral immune response to heterologous antigen until the preleukemic age died from spontaneous leukemia sooner and in a higher percentage of cases (period of observations up to 10 months inclusive). In all seven series of this experiment, performed at different times on an adequate number of animals, the differences in the incidence of spontaneous leukemia among mice with strong and weak humoral immune response were statistically significant.

The reciprocal relationship thus revealed between the ability of AKR and C57BL mice to produce humoral antibodies and their susceptibility to transplantation of syngeneic leukemically transformed cells and

also the incidence of spontaneous leukemia among them is evidence that failure of one component of immunity does not always lead to an increased risk of tumor development in vivo. The results of these experiments are in harmony with the view that relations between immunity and carcinogenesis are complex and not always consistent in character [1, 3, 4, 6, 7].

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PARTICIPATION OF ANTIBODIES SYNTHESIZED IN VITRO IN ROSETTE FORMATION

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The possibility of rosette formation by cells binding antibodies synthesized in vitro was investigated. Within the period of the rosette-formation test at 37°C and with prolongation of the incubation time, antibodies capable of inducing the formation of pseudorosettes appeared in the culture fluid. If the rosette formation test was performed in the cold the formation of pseudorosettes in the residue after centrifugation was minimal.

KEY WORDS: rosette-forming cells; cytophilic antibodies.

One method of detecting the population of immunocompetent cells formed in response to injection of an antigen in vivo is the rosette-formation test. Existing methods of performing the test differ from one another in the time of incubation of the mixture of lymphocytes and red cells and also the incubation temperature. It has been suggested that at 4°C mainly cells carrying immunoglobulin receptors on their surface (antigen-recognizing cells) are revealed, whereas at 37°C antibody-forming cells (AFCs) can also be detected, for this temperature does not prevent antibody synthesis in vitro [1, 4, 6]. According to Wilson et al. [9], AFCs can also be detected if incubation is prolonged in the cold. However, the causes of formation of more rosettes at 37°C or on prolongation of the incubation time have not been finally elucidated. It may be that some of the antibodies synthesized during the reaction are cytophilic, i.e., they have the property of attachment to certain cells, as a result of which the cells become capable of specifically adsorbing antigen [3]. The pseudorosettes formed as the result of this process can be a serious obstacle to the use of the rosette-formation test.

The object of this investigation was to study whether cells binding antibodies synthesized in vitro can take part in rosette formation and to establish the optimal conditions for the reaction.

EXPERIMENTAL METHOD

Male CBA mice weighing 18-21 g, unimmunized or immunized 6 days before the experiments with sheep's red cells ($0.5 \cdot 10^9$ cells, intraperitoneally) were used. There were three series of experiments. In series I

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