THYMUS-DEPENDENT DIFFERENTIATION OF LYMPHOCYTES IN DOGS ASSESSED BY TESTS FOR ALLOGENEIC LYMPHOCYTES AND PHYTOHEMAGGLUTININ

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A decrease in the number of lymphocytes capable of responding to phytohemagglutinin (PHA) and in mixed culture was found in thymus-deficient dogs (thymectomized followed by lethal irradiation and transplantation of autologous bone marrow). On the basis of these results and the increase in the time before rejection of skin allografts in these animals, established previously, it was concluded that dogs have a population of lymphocytes of thymic origin that participate in the responses of cellular immunity and blast transformation under the influence of PHA.

KEY WORDS: thymus-dependent lymphocytes; allografting; differentiation of lymphocytes.

The histogenetic and functional heterogeneity of the composition of lymphoid populations in birds and mammals has recently been demonstrated. Two main categories of lymphocytes are distinguished, one of which requires the presence of the thymus for its differentiation (thymus-dependent or T-lymphocytes), whereas the other category, the thymus-independent or B-lymphocytes, can mature in the absence of the thymus.

The composition of the lymphoid tissue and the properties of its various populations have been studied chiefly in birds and rodents. The question of whether these principles that have been discovered are universal for all warm-blooded animals is therefore an interesting one. The writers previously studied the presence of thymus-independent and thymus-dependent categories of lymphocytes in primates using animals with experimentally induced thymus deficiency [2].

In the investigation described below the presence of the above categories of lymphocytes was studied in dogs.

EXPERIMENTAL METHOD

The reactions of blast transformation of lymphocytes in mixed culture and in a culture stimulated by phytohemagglutinin (PHA) were used as markers of the T-lymphocytes, for in both these tests chiefly the T-lymphocytes undergo transformation [5].

Experiments were carried out on 14 mongrel dogs of both sexes weighing from 5 to 12 kg. The following groups of animals were studied: 1) intact; 2) thymectomized; 3) irradiated, followed by transplantation of autologous bone marrow; 4) thymectomized, then irradiated, followed by transplantation of autologous bone marrow. The methods of thymectomy, irradiation, and obtaining the bone marrow were described in detail previously [3]. The animals were thymectomized while young, mainly under 6 months of age, and 2 months later they were irradiated in a dose of 700 to 1000 rad with γ-rays from Co^{60} or Cs^{137}, respectively. The bone marrow was aspirated immediately before irradiation in a dose of between 1.6×10^9 and

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TABLE 1. Incorporation of Thymidine-H^3 into Leukocytes (in pulses/min) Stimulated by PHA (M ± m)

Group No.	No. of animals	No. of expts.	PHA	Control
1	5	9	4717±1245	8,5
3	3	3	3093±658	0,7
2	3	5	2218±664	14,0
4	3	4	1512±364	23,0

TABLE 2. Incorporation of Thymidine-H^3 (in pulses/min) into Unidirectional Mixed Culture of Leukocytes (M ± m)

Group No.	No. of animals	No. of expts.	Mixed culture	Control
1	5	11	1158±432	39
3	3	3	1020±258	49
2	3	5	1031±296	57
4	3	5	458±156	49

9.4×10^9 nucleated cells. The ability of the dogs' lymphocytes to react to PHA and allogeneic leukocytes began to be studied 3-30 months after irradiation, and the study continued for 2 months. The methods of culture and of assessing the reaction from the incorporation of thymidine-H^3, as described in full by the writers for monkeys' leukocytes, were made more precise in order to obtain optimal conditions for culture of dogs' leukocytes. Red cells were sedimented from defibrinated blood by gelatin. The leukocytes were washed once with medium No. 199 and suspended in nutrient medium of the following composition: medium No. 199 in Earle's salt base with 15% healthy dog serum (a mixture from three donors), 1% L-glutamine, and kanamycin. Cell suspensions containing 10^6 lymphocytes in 1 ml were cultivated in tubes in a volume of 0.5 ml (culture with PHA) or 0.5 ml from each of two partners (mixed culture). To obtain a unidirectional reaction, the cells of one partner in the mixed culture were irradiated in vitro in a dose of 2124 rad (531 rad/min). Each test was repeated three times. Cultures with PHA were grown for 3 days and mixed cultures for 7 days. Thymidine-H^3 (specific activity 320 μCi/mmole) was added to the cultures 3 h before photography. The incorporated radioactivity was counted after the cells had been washed on Millipore filters.

In some experiments in which the partner animals for the mixed culture differed in sex, karyologic analysis of metaphase plates made it possible to determine the contribution of each of them to the reaction. In this case 0.2 μg/ml colcemid was added 2-3 h before the end of cultivation [3].

EXPERIMENTAL RESULTS

As Table 1 shows, both irradiation and thymectomy reduced the action of the lymphocytes to PHA, but not significantly; a statistically significant decrease was observed in the dogs of group 4 ($P < 0.05$). In mixed culture (Table 2) the reactivity of the lymphocytes in the dogs of groups 3 and 2 was virtually not reduced.

Preservation of normal reactivity of the dogs' lymphocytes in mixed culture during the 2 years after thymectomy suggests that the population of these cells can be sustained for a very long time in the absence of the thymus. This does not contradict the observations of Kisken [4], who found a decrease in the reaction of the lymphocytes from adult thymectomized dogs in mixed culture, for in that case the time after the operation was 3 years.

The decrease in the reactivity of the lymphocytes to allogeneic cells observed in the animals of group 4 was not statistically significant; a significant decrease ($P < 0.001$) was found in experiments in which the participation of the partners in the reaction was recorded by means of the chromosome marker. In each of three such experiments 50 metaphases were analyzed; of this number, 12, 3 and 7 metaphases, respectively, belonged to a partner from group 4, i.e., among the cells of intact animals and animals subjected to thymectomy, irradiation, and transplantation of autologous bone marrow reacting in mixed culture, the fraction of the latter group of metaphases was only about 15%.

The decrease in reactivity of the lymphocytes of the dogs of group 4 in mixed culture and in culture with PHA is indirect evidence of the existence in dogs of a thymus-dependent population of lymphocytes that participates in these reactions. The same population of lymphocytes was evidently responsible also for the reaction of cellular immunity in vivo, for in the dogs undergoing thymectomy, irradiation, and transplantation of autologous bone marrow the time for rejection of the skin allografts was substantially lengthened [1]. The results described above thus agree fully with the concept of the structure of the lymphocyte population established for birds and rodents.

It is interesting to note that, as the writers showed previously, in Macaca rhesus, unlike all other species so far studied including dogs, a thymus-deficient state is not accompanied by any significant decrease in the reaction of the lymphocytes to PHA, whereas their reaction in mixed culture is substantially depressed.

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