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Origin and Fate of a Thymocyte-Specific Antigen

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Summary. The incidence of a thymocyte-specific antigen in thymuses of rats at different ages was studied by immunofluorescence using an anti-thymus microsome serum. This serum, which could detect an antigen common to all lymphocytes, was made thymus-specific by absorption with non-thymic lymphoid organs. The thymic antigen was detected in the earliest embryo (32 mm) in which lymphoid cells are recognizable in the thymic anlage, where it appeared to be present in all thymic lymphocytes. With increasing age, both the proportion of lymphoid cells bearing the antigen and the amount of antigen present were decreased. In lethally irradiated marrow-injected animals, the thymus recovered its normal histology and lymphoid antigenicity within a month, but the thymocyte-specific antigen did not reappear. The findings suggest that the antigen is an exclusive component of native thymic lymphocytes.

INTRODUCTION

Two antigenically different populations of morphologically similar lymphoid cells have been demonstrated in the rat thymus (Potworowski and Nairn, 1967): the first could not be distinguished from non-thymic lymphocytes while the second had an additional antigen unique to the thymus. In this report, the origin and fate of the thymus-specific antigen have been investigated in an attempt to determine whether it is a normal attribute of native thymocytes or whether it is a transient feature of lymphoid cells passing through the thymus. We have found that in the embryonic rat thymus all the lymphocytes possess the thymic antigen and that the proportion of cells with antigen decreases as the animals grow older. In lethally irradiated rats injected with marrow, thymic antigen could not be detected in the lymphocytes repopulating the thymus. Thus the antigen appears to be present only in native thymocytes.

MATERIALS AND METHODS

Lister hooded rats of either sex maintained in closed colony since 1940 by the Commonwealth Serum Laboratories (C.S.L., Melbourne) were used. Antisera and general technical procedures were as reported previously (Potworowski and Nairn, 1967).

Thymus smears

The rats were killed with ether and thymuses were dissected out and teased apart in a solution containing 20 ml 20 per cent bovine serum albumin, 50 ml phosphate-buffered saline (0·01 M phosphate, pH 7·1), 8 ml 5 per cent aqueous EDTA, 100 units penicillin/ml,

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$NaHCO_3$ to pH 6·8 (van Furth, Schuit and Hijmans, 1966). The cells were drawn up and down a micropipette to ensure adequate separation and smears, made on chemically clean slides, were dried at 2° with a fan for a minimum of 3 hours. Immunofluorescent staining was by the sandwich technique as previously employed except that the final rinse was with a 1:5000 solution of Brilliant Cresyl Blue in phosphate-buffered saline. This counterstain gave the nuclei a reddish fluorescence which ensured that no cells without specific immunofluorescent staining were overlooked on microscopical examination. Differential fluorescent cell counts were made on 200 cells.

Irradiation

Twenty 6-weeks-old rats of both sexes were irradiated unanaesthetized, under the 25 cm cone of a Muller RT100 X-ray machine (70 kV, 10 mA, 1·25 mm Al filter) and received 1000 rads to the whole body over 10 minutes.

Marrow cell suspension

Marrow was obtained aseptically from the femurs of rats of the same strain and age as the irradiated animals by injecting Tyrode solution (CSL) through one end of the bone shaft with a syringe and the cells were dispersed by drawing the suspension in and out of a hypodermic needle. The nucleated cells were counted in an haemocytometer and their concentration was adjusted with Tyrode solution to 16×10^6/ml; 0·5 ml of this suspension was injected by the jugular vein in one group of rats within 4 hours of irradiation.

Antiserum

The anti-thymus serum used for immunofluorescent tracing of the thymus-specific antigen had been obtained previously from ascitic fluid of mice immunized with a microsome fraction of the rat thymus cells. When absorbed with rat liver homogenate the serum stained all lymphocytes, thymic and non-thymic alike, and is referred to as 'lymphoid-specific'. When further absorbed with rat non-thymic lymphoid organs homogenate, the serum stained only thymic lymphocytes and is referred to as 'thymus-specific'.*

Histology

Paraffin sections of Bouin-fixed lymphoid tissues were stained with Haematoxylin and Eosin.

Studies in other species

The thymus-specific antigen was sought in the thymuses of rats of different strains (Sprague–Dawley, Black CSL, White CSL), of mice (BALB/c, C57BL, NZB/BL, NZW, NZC, A/Jax, AKR), of chicken, of bullfrog (*Bufo marinus*), of fish (flathead) and of human.

RESULTS

AGE DISTRIBUTION OF THE THYMIC ANTIGEN

Smears from the thymus anlagen of 20- and 32-mm rat embryos and from thymuses of rats at birth and 1, 2, 3, 4, 8, 12 and 14 weeks were stained with the lymphoid-specific and thymus-specific sera, at least two thymuses being examined from each age group. The thymus anlagen of the 20-mm embryos did not stain specifically with either of the

* The specificity of the serum has also subsequently been demonstrated by lymphocyte agglutination experiments.

antisera but at that stage lymphoid cells could not be recognized either in the smears or in conventional histological sections: pale staining epithelial cells with elongated nuclei were detected and these did not take up the antibody. The first recognizable lymphocytes were found in the thymuses of 32-mm embryos, at which stage it is not yet possible to distinguish histologically between medulla and cortex though lymphoid cells of all sizes are present. In smears stained by immunofluorescence, all the morphologically recognizable lymphocytes reacted both with the lymphoid-specific and the thymus-specific sera: staining was of similar intensity with either serum and was restricted to the cytoplasmic rim of the cells. In the neonatal animals, all the lymphoid cells of thymus smears reacted with the lymphoid-specific serum but only 93 per cent with the thymus-specific serum.

With increasing age the intensity of the staining with the thymus-specific serum became progressively less bright and the proportion of thymus-specific cells decreased logarithmically so that by 6 months only about 9 per cent of thymic lymphocytes could be stained. There was no obvious correlation between the loss of the antigen and the involution of the thymus (Table 1). The specific antigen gradually lost from the thymus could not be identified in any other organ.

TABLE 1

LOSS OF THYMUS-SPECIFIC ANTIGENICITY WITH AGE

Age (weeks)	Thymus cells staining with thymus-specific serum (per cent)	Ratio of thymus weight (mg) to body weight (g)
0	93	1·0
1	70	2·0
2	68	2·0
3	54	2·5
4	45	2·3
8	35	1·8
12	21	0·7
24	9	0·4

The lymphoid antigen could be detected in all lymphoid cells throughout life by means of the lymphoid-specific serum which gave no appreciable reduction of staining with advancing age.

Bone marrow smears of young and old animals have been examined but no specific staining was ever observed in any lymphoid cell with either the lymphoid-specific or the thymus-specific serum. Non-specific staining was very intense particularly in some large undifferentiated cells and in megakaryocytes, which made unambiguous interpretation difficult.

IRRADIATION EXPERIMENT

Rats were grouped as follows: (1) five rats lethally irradiated; (2) fifteen rats lethally irradiated and injected with bone marrow; and (3) five rats left untouched to provide normal controls. Irradiated rats within the first 9 days lost weight and hair, and all of Group 1 and two-thirds of Group 2 had died (Table 2). Post-mortem examinations, conducted whenever possible, revealed gross atrophy of thymus, lymph nodes and spleen,

with variable macrophage and fibroblastic reaction. Recognizable lymphoid cells were scanty and many of those present were distorted and had vacuolated nuclei; such degenerate cells did not react with either of the antisera. The thymuses of rats of Group 1 had occasional intact lymphocytes which gave specific staining with both antisera. The same was also true for thymuses from Group 2, but they had more intact non-staining lymphocytes presumably derived from the injected marrow.

Subsequent changes in the thymus of the irradiated marrow-injected rats killed singly at intervals were as follows. On the 14th day the thymus was very small but contained small lymphocytes and many blast cells scattered diffusely with some focal accumulations and there was a moderate patchy fibroblastic reaction. Of the lymphoid cells 24 per cent reacted with the lymphoid-specific antiserum but none with the thymus-specific antiserum. On the 20th day, the thymus was small, with signs of fibrosis and lymphoid cells were numerous. The small lymphocytes were mainly centrally located with the blast cells distributed more peripherally. The proportion of the lymphocytes reacting with the

TABLE 2
SURVIVAL OF IRRADIATED RATS

Days after irradiation	Group 1: irradiated (five rats)	Group 2: irradiated and marrow-injected (fifteen rats)	Group 3: normal controls (five rats)
3	5	15	5
4	3	15	5
5	2	8	5
6	1	8	5
7	1	7	5
8	0	6	5
9	0	4	5

No animals died after the 9th day.

lymphoid-specific serum was 41 per cent and again none reacted with the thymus-specific serum. On the 28th day, the thymus was larger, but below normal size; there was a clearly demarcated cortex of small lymphocytes with medullary zones largely populated with epithelial and blast cells and showing some fibrosis. Lymphoid antigen was detected in 45 per cent of the lymphocytes but none had the thymic antigen. On the 35th day, the thymus was practically normal in size and histology and 89 per cent of the lymphocytes had the lymphoid antigen, but the thymic antigen could not be demonstrated.

The lymph nodes at 14 days were very small without clear follicles and contained numerous degenerate lymphocytes and some plasma cells, but repopulation was evident from the many mitotic figures and aggregates of blast cells particularly at the periphery of the nodes: 19 per cent of the cells had the lymphoid-specific antigen. On the 20th day, the picture was essentially the same. On the 28th day, the size of the lymph nodes was normal, but they still contained no obvious follicles; several large aggregates of blast cells, and many plasma cells were present. Much the same proportion of cells gave fluorescent staining with the lymphoid-specific serum. On the 35th day, follicles were evident, some with germinal centres, and only 17 per cent of the cells had the lymphoid antigen. The recovery of the spleen resembled that of the lymph nodes, except that mitotic activity was inconspicuous and restoration of the follicular pattern occurred at the 28th day.

From the 20th day, the health of the animals deteriorated: they were losing hair and

weight which suggested the onset of secondary disease. This view was corroborated by the appearance of a large number of plasma cells in the cervical lymph nodes, though not in the thymus. It indicates that the animals used were not completely inbred, that the injected cells were not strictly syngeneic.

SPECIES SPECIFICITY

Apart from the other strains of rats examined, which had the thymus-specific antigen in approximately the same amount as the Lister hooded rats, this antigen was not detected in any of the other vertebrate species studied.

DISCUSSION

Studies on mouse (Auerbach, 1964) and hamster (Ackerman and Knouff, 1965) embryos suggest that thymic lymphocytes differentiate from the local epithelial cells and that the embryonic thymus is not, at first, colonized by cells from elsewhere. If we accept the view that the embryonic thymus is originally a sequestered organ, the appearance in it of the lymphoid antigen, as demonstrated by our study, would suggest that the cells which arise locally in the thymus are truly lymphoid, not only morphologically but antigenically as well. They possess, however, an additional, thymus-specific antigen from the very moment that they assume lymphoid morphology.

The reason for the subsequent loss of this thymus-specific antigen is not known. Possible explanations include 'maturation' with loss of antigen and/or simple dilution of antigen through cell division of the original thymocytes, perhaps accompanied by dilution of thymocytes by the immigration of non-thymus-specific lymphoid cells.

Whether cells other than original 'epithelial-derived' thymocytes have this antigen is at present not clear. Ford and Micklem (1963) have shown that in lethally irradiated animals, intravenously injected marrow cells migrated to the thymus. The present irradiation experiment, in which the thymus was depleted and re-populated by cells, originating presumably from the injected marrow, and which failed to develop the antigen, suggests that immigrant thymus cells are not conferred with specific thymic antigenicity, though they develop the lymphoid antigen. However, this experiment is not by itself conclusive, because any thymic factor responsible for expressing thymus-specific antigenicity in bone-marrow-derived cells could have been already lost in rats of the age group used or could have been destroyed by irradiation. Confirmation would require a strictly syngeneic system with neonatal thymic grafting of irradiated hosts.

We have no information about the nature or role of the thymus-specific antigen, which appears to be an exclusive component of native thymic lymphocytes. It is apparently rat-specific and not a mouse isoantigen such as the TL antigen (Old, Boyse and Stockert, 1963) as it could not be demonstrated in any of the mice including the A/Jax strain which is known to possess the TL antigen. No relationship has been established with the thymus-specific antigen demonstrated by Tallberg and Kosunen (1966) by gel diffusion: the present antigen could not be detected in this way.

It is not clear why the recovery of the lymphoid antigen is better in the thymus than in the lymph nodes. Among the possible explanations would be enhanced multiplication in the thymus of cells possessing the antigen or the preferential expression in that organ of lymphoid antigenicity in immigrant precursor cells, but there is no evidence in favour of either of these views.

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