Cellular Heterogeneity in the Thymus: Graft-versus-Host Activity of Fractionated Thymus Cells in the Chicken[1] (37296)

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(Introduced by S. Weinhouse)

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Thymic lymphocytes have generally a low capacity to initiate graft-vs-host (GVH) reactions as compared with peripheral lymphocytes (1–6). In mice, the weak GVH activity was shown to be due to the heterogeneity of thymic lymphocytes; the GVH activity is provided by a small subpopulation of highly competent cells, while the majority of thymic lymphocytes appear to have little or no GVH activity (7). A similar situation was suggested for the chicken thymus (6), although direct evidence for a cellular heterogeneity was not presented.

Recently, two cellular subpopulations have been demonstrated in the thymus of young chickens by electrophoretic analysis, differential centrifugation (8) and cell traffic studies (13–15). We now report experimental data which provide further evidence for the cellular heterogeneity in the thymus of young chickens. The experiments indicate that the two subpopulations (8) differ also with respect to their GVH activity.

Materials and Methods. Animals. White Leghorn Line 96 eggs, homozygous for the major histocompatibility locus (B^2/B^2), were obtained from Hy-Line Poultry Industries, Johnston, IA. These eggs were incubated and hatched in an incubator-hatcher (Ehret, Emmendingen, West Germany) under standard conditions. The chickens were then used as thymus cell donors, when 3, 6, or 14 weeks old. Eggs of specific pathogen-free, random-bred White Leghorn chickens were purchased from SPAFAS Poultry Farm, Norwich, CT, and incubated. Such 14-day embryos were used as recipients in the GVH assay.

Isolation and differential centrifugation of cells. The animals were killed by exsanguination. A cell suspension was made of thymic tissue in RPMI 1640 (Gibco, Grand Island, NY) containing 15% fetal calf serum. The cell suspension, freed from debris, was centrifuged at 750*g*, and resuspended in chicken plasma. This suspension was then sequentially centrifuged at 25*g*, 100*g*, 300*g*, and 750*g* for 10 min each time. The preparations were washed twice in phosphate-buffered saline and the cells counted in a Newbauer hemocytometer. All preparations showed over 95% viability when tested by trypan blue exclusion (0.125% trypan blue).

Graft-vs-host assay. The assay was carried out according to Simonsen (9, 10). Three thymus cell fractions, obtained at 100*g* (fraction I), 300*g* (fraction II), and 750*g* (fraction III) were injected into different experimental groups of eggs. The number of injected cells per recipient varied from 6×10^5 to 8×10^7. The cells were injected into a chorioallantoic vein of 14-day-old SPAFAS chick embryos, usually 5–6 embryos per experimental group. Five days later, the embryos were killed, and their spleen weight and body weight determined. The spleen weight and body weight of control groups of 10–12 embryos, which received no cells,

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were also determined. The spleen index for each animal was calculated as the spleen weight:body weight ratio of the animal, divided by the mean spleen weight:body weight ratio of the untreated controls. The logarithms of the individual spleen indices were finally used in the statistical analysis. The p values were calculated using Student's t test.

Results. Thymus cells were isolated from Hy-Line chickens at different ages and fractionated by differential centrifugation. Three fractions, I, II, and III, were tested for GVH activity in 14-day-old embryos from SPAFAS chickens. Thymus cells from high-speed centrifugation fractions tended generally to have lower GVH reactivity than cells from the low-speed centrifugation fraction.

The cells in the low-speed fractions (fraction I) from 14-week-old chickens showed significantly higher GVH activity compared with the cells in the high-speed fractions (fraction III) (Fig. 1, Table 1). The cells

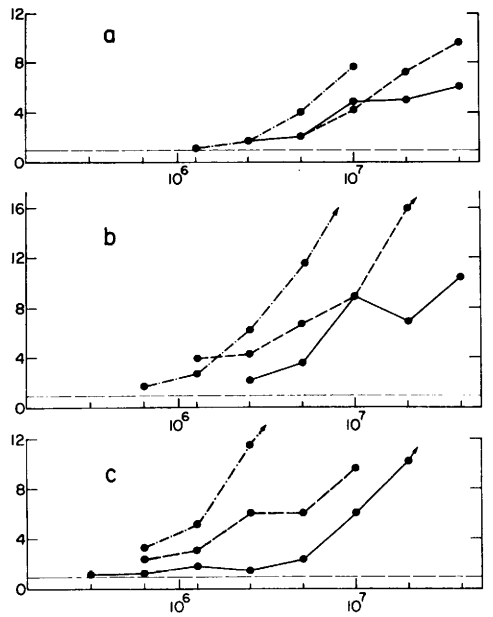

FIG. 1. Graft-vs-host activity of different thymus cell preparations. (a) Thymus cells from 3-week-old birds; (b) thymus cells from 6-week-old birds; (c) thymus cells from 14-week-old birds. -•-•-•-•- fraction I (25–100g); - - - - - fraction II (100–300g); ———— fraction III (300–750g). Abscissa: number of injected live thymus cells. Ordinate: splenomegaly index.

of the 3-week-old chicken thymus showed a comparably low GVH activity in all preparations.

Discussion. It has been demonstrated earlier (8) that the young chicken thymus contains two electrophoretically distinct main cell populations. After differential centrifugation the electrophoretically faster population is greatly diminished in the high-speed fraction (fraction III), while that population makes up a substantial part of the low-speed fractions (8).

The GVH activity provides an additional parameter to characterize the cellular subpopulations. The data presented here indicate that the electrophoretically slower cell population is the least active one in the GVH assay. This is supported by two findings: (a) The high-speed fractions (III), which have previously been shown to contain practically only the electrophoretically slower subpopulation (8), do not produce high spleen indices except when large numbers of cells are used and there is an imminent risk of minor contaminating cell populations being present in numbers great enough to cause an effect. (b) The early postnatal thymus, which contains practically only electrophoretically slow-moving cells even in the low-speed fraction (8), shows lower GVH reactivity in all fractions than that of older birds.

It has been previously reported that the young chicken thymus contains a quantitatively significant bursa-derived cell population (13–15) and that a bursa-dependent cell population is found highly enriched in the high-speed centrifugation fractions of thymus cell preparations (8, 16). The present data indicate that this bursa-dependent cell population has low GVH activity. The GVH competence of the preparations seems to parallel the proportions of electrophoretically faster cells in these preparations. The adult chicken thymus contains mainly the electrophoretically faster cell type (8) and has a higher GVH activity than does the young chicken thymus (6). This fact also suggests that the two electrophoretically defined populations do not correspond to the two types of GVH active T-cells in mice (T1 and T2), described recently (11, 12). However, a het-

TABLE I. GVH Reactions Obtained by Different Preparations of Thymic Lymphocytes.[a]

Age of donors (weeks)	No. of cells transferred	Thymus I (25–100g)	Thymus II (100–300g)	Thymus III (300–750g)	p value[b]
3	2.5×10^6	0.25 ± 0.02	0.20 ± 0.05	0.24 ± 0.04	NS
	1.0×10^7	0.79 ± 0.16	0.59 ± 0.09	0.62 ± 0.14	NS
6	2.5×10^6	0.75 ± 0.11	0.68 ± 0.06	0.34 ± 0.05	NS
	1.0×10^7	1.23 ± 0.14	0.96 ± 0.06	0.94 ± 0.07	NS
14	2.5×10^6	1.04 ± 0.10	0.76 ± 0.07	0.16 ± 0.05	<0.0005
	1.0×10^7	1.32 ± 0.04	0.97 ± 0.05	0.70 ± 0.17	<0.03

[a] Log of spleen indices, $\pm$ SE.

[b] The p-values for thymus I vs thymus III were calculated by using Student's t test.

erogeneity of this type may exist in the electrophoretically faster, nonbursa-dependent cell population.

Summary. Thymus lymphocytes from young chickens have been fractionated by differential centrifugation and tested for their graft-vs-host activity by the splenomegaly test. The experiments provide further evidence for the cellular heterogeneity in the young chicken thymus, previously demonstrated by electrophoretic analysis, differential centrifugation and cell traffic studies. The low-speed centrifugation fractions have been shown to consist of two populations with electrophoretically different mobilities, while the high-speed fraction is highly enriched in one electrophoretically slow-moving population. The data from the present study indicate that the thymus cell fraction obtained at high-speed centrifugation (750g), which is highly enriched in bursa-dependent cells, has little GVH activity. The low-speed fractions (100g and 300g) carry most of the GVH activity, presumably by a bursa-independent subpopulation.

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