Quantitative Study of the Splenomegaly Assay System of the Graft-versus-host Reaction in Chick Embryos[1]

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INTRODUCTION

When adult chicken blood or spleen cells are inoculated on the chorioallantoic membrane or injected into the embryo, a graft-versus-host (GVH) reaction ensues in which pock-like lesions appear on the membrane and splenomegaly, runting and mortality occur among the recipients (Simonsen, 1957; Boyer, 1960). The reaction is immunological, i.e., dependent on the antigenic disparity of donor and host (Jaffe and Payne,

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1961; Jaffe and McDermid, 1962), and only recently has the cellular basis become generally understood (Ebert, 1959; McBride, 1966; Jaffe and Fechheimer, 1966). The quantitative analysis of the chorioallantoic membrane reaction (Szenberg et al., 1962; Simons and Fowler, 1966; Coppleson and Michie, 1966) and of the GVH response of the host embryo (Terasaki, 1959; Seto and Albright, 1965; Seto, 1966) has resulted in further support for the clonal selection theory of immunity, has led to the identification of cell types involved, has demonstrated a contributory role of the host in the GVH reaction, and is useful in measuring variations in the homograft capacity.

The two basic assay systems metioned are approximately equivalent in their efficiency (Coppleson and Michie, 1965). Greater sensitivity in the splenomegaly assay can be achieved if biological and technical factors that influence the response are appropriately utilized. These conditions have been experimentally tested and are described in this paper.

Materials and Methods

Baby, juvenile and adult Game chickens and adult White Leghorn and Dominique chickens were tissue donors, and White Leghorn chick embryos were recipients. Citrated whole blood was obtained by cardiac puncture; and spleen, thymus and bursa of Fabricius were dissected from sacrificed birds. The organs were teased apart in sterile Tyrode's solution, dispersed further by gently forcing the tissue through a syringe, and clumps were strained out with stainless steel cloth (200 mesh/inch). The cell suspension was washed once with fresh Tyrode's solution, centrifuged, and the supernatant discarded. The pellet was resuspended in fresh Tyrode's solution and a cell count made with a hemocytometer. Approximately 4×10^6 cells in 0.1 ml or 0.1 ml equivalent of whole blood was intravenously injected into the chorioallantoic vessel of embryo recipients and 5-6 days later the body and organ weights were recorded.

Results

Experimental criteria of GVH capacity—Of several criteria available to assess the GVH reaction (Jaffe, 1966), the recipient mortality, weight loss (runting), and organ enlargement data are the most readily obtainable. Mortality is the least quantitative and informative criterion and was not used. A comparison was made of body weights, relative liver size *(liver index)*, and spleen weights of control and inoculated embryos (Table I). Decrease in body weight was not large enough for quantitative evaluations. Liver enlargement was a less sensitive index of GVH response than spleen enlargement. For example, among 53 experimental embryos with apparently normal liver indexes of 0.022 to 0.024 (Seto, 1966), there was a 66% incidence of splenomegaly. Splenomegaly was the main assay method used in this investigation.

TABLE I. MEAN BODY, LIVER AND SPLEEN WEIGHTS OF 19-DAY-OLD WHITE LEGHORN CHICK EMBRYOS INOCULATED AT 14 DAYS OF INCUBATION WITH 0.1 ML OF WHOLE BLOOD FROM JUVENILE GAME CHICKEN DONORS.

	Controls	Experimentals
No. of host embryos	31	54
Mean body weight (g)	28.4	24.6
Liver index*	.021	.035
Mean spleen weight (mg)	10.3	108.6

*Ratio of liver to body weights in g.

Donor tissue type and splenomegaly—Of the different tissues reported to be effective in eliciting homologous splenomegaly, blood, spleen and thymus tissues have been most commonly used. In addition to these, bursa cells were tested for their relative ability to promote spleen enlargement in recipient embryos (Table II). The data indicate that for equal numbers of mononuclear cells inoculated, the order of effectiveness was blood, spleen, thymus and bursa of Fabricius cells.

Blood was selected as the inciting donor tissue in the present investigations. When the results were compared with those obtained earlier with spleen cells (Seto and Albright, 1965), peak hepatosplenomegaly also occurred 5-6 days after injection and the pathological changes in spleen and liver were alike. Similarly, maximum response was elicited with 13- to 14-day-old hosts and within the blood dilutions tested, a fourfold dilution in donor cells resulted in twofold decrease in splenomegaly. The growth kinetics were alike with spleen cells and with blood as donor tissue.

TABLE II. RELATIVE EFFECTIVENESS OF CELLS FROM DIFFERENT TISSUE SOURCES* TO INDUCE SPLENOMEGALY IN EMBRYONIC CHICK RECIPIENTS.

Donor tissues	Mononuclear Cells Injected	Number Donor	Number Host	Mean spleen weight (mg)	Percent enlargement
Whole blood	$4 \times 10^{6**}$	7	25	179	100
Spleen	4×10^{6}	7	23	101	54
Thymus	4×10^{6}	7	27	95	50
Bursa	4×10^{6}	3	18	15	3

* 5-to 10-week-old Game chicken donors.

** The value for blood has been doubled to make it equivalent to other tissues.

GVH capacity of donors of different ages and chicken breeds—Game chicken donors of ages 3-8 days, 4, 8-9, and 12-13 weeks, and White Leghorn and Dominique donors of 8-9 weeks of age were tested for their ability to induce the GVH reaction in White Leghorn embryos. The data for individual donors, grouped together by age and breed, have been summarized in Table III. The data indicate that the GVH reactivity was present at a low level in the blood of baby chicks, increased in juvenile birds, and reached a mature level in 12- to 13-week-old donors. When the performances of different donor breeds were compared, game chickens as a group elicited the greatest splenomegaly consistently.

DISCUSSION

There is tremendous variability in the degree of splenomegaly observed in embryo recipients exposed to immunologically competent cells from adult allogenic donors. Some investigators reported only 3- to 5-fold splenic enlargement (Ebert, 1954; Solomon, 1961, Fennell, 1966), while others observed 6- to 12-fold increases (Biggs and Payne, 1961; Jaffe and McDermid, 1962; Seto and Albright, 1965). Since many factors can influence the severity of the GVH reaction elicited in the host (Russell and Monaco, 1965), the variability in the observations can be attributed to the different donor-host combinations, tissue sources, and

TABLE III. COMPARISON OF THE GVH REACTION CAPACITY OF BLOOD FROM DONORS OF DIFFERENT AGES AND CHICKEN BREEDS.

Donor Breed	Age	No.	Mean Spleen weights (mg) Individual	Group
Game	4- 8 days	7	15, 17, 25, 29, 35, 40, 70	35
	4 weeks	8	53, 70, 95, 103, 108, 139, 162, 197	113
	8- 9 weeks	9	91, 91, 94, 95, 95, 105, 154, 190, 207	127
	12-13 weeks	6	142, 199, 229, 245, 324, 341	247
White Leghorn	8- 9 weeks	6	38, 38, 45, 57, 65, 68	52
Dominique	8- 9 weeks	1	86	—

technical procedures used by the investigators.

The quantitative reinvestigation of homologous embryonic splenomegaly reported here show that:

(1) splenomegaly was the most sensitive index as compared with embryonic mortality, runting and liver enlargement;

(2) blood was more effective than either spleen or thymus cells when evaluated on the basis of equal numbers of mononuclear cells inoculated;

(3) certain chicken breeds—Game chickens in this study—elicited greater GVH response than others (cf. Jaffe and Payne, 1961);

(4) the GVH capacity of donors increased with age (cf. Solomon, 1961).

Furthermore, as reported earlier in experiments with donor spleen cells (Seto and Albright, 1965) and repeated here with donor blood tissue, consistently greater GVH responses were obtained under the following conditions:

(5) intravenous injection of blood rather than the grafting or inoculation of donor cells on the chorioallantoic membrane; and

(6) the choice of 13- to 14-day-old recipients (Isacson, 1959; Solomon and Tucker, 1962).

When factors conducive to maximum GVH responses are utilized, the efficiency of the spleen assay system can be significantly improved. Investigators aware of these factors have reported splenomegaly of 16 fold or greater and enlargements of 25-fold or more have been obtained in this study (Table II).

SUMMARY

A quantitative study was made of several factors known to influence the expression of the graft-versus-host (GVH) reaction in chick embryos. Of several criteria—embryonic mortality, decrease in body weight, liver index and splenomegaly—the last was the most sensitive measure of the

GVH reaction. The cell sources in their order of effectiveness in eliciting the GVH reaction were: blood, spleen, thymus and bursa of Fabricius. The severity of the GVH reaction is dependent on the amount of blood inoculated, mode of administration, and age of recipients at the time of inoculation. Of three breeds tested as donors, Game chickens consistently produced the greatest splenomegaly in White Leghorn hosts. The splenomegaly capacity was present in the blood of newly hatched Game chicks and increased to the mature level in 12- to 13-week-old birds.

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