Transplantation Antigens in Normal Mouse Organs.* (31379)

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Recent observations in several laboratories indicate that microsomal fractions obtained from lymphoid cells are rich in transplantation antigens(1,2,3). These fractions were found to possess the capacity of inducing in recipient animals strong homograft immunity as measured by the specific accelerated rejection of test skin grafts. In addition, tolerance across a weak histocompatibility barrier, the Y chromosome antigens, has been induced by inoculating microsomal preparations into newborn females(4,5,6).

In previous communications(2,7), we have reported observations on the antigenic properties of microsomal fractions obtained from liver and spleen cells. The current study was undertaken to determine: (a) the antigenic activity of the mitochondrial fraction and cell sap; (b) the effect of ultrasonic irradiation and RNAase treatment of spleen microsomes; and (c) the antigenic activity of microsomal fractions of kidney cells.

Materials and methods. The mice used in this study were obtained from Jackson Memorial Laboratry, Bar Harbor, Maine. Mice from strain A/Jax served as donors of skin grafts and antigenic materials while mice from strain CBA were the recipients. The skin grafts were fitted suprapannicular 11 mm in diameter applied to the lateral thoracic region. The grafts were covered with non-adhering dressing, held in position by latex bandage, and then the animals were wrapped with a strip of Scotch Tape.

Isolation of subcellular fractions. The technique of cell fractionation has been described previously(7). Briefly, the tissues were homogenized in cold 0.25 M sucrose and extracted twice at 900 $\times$ *g* for 10 minutes. The sediment was discarded and the supernatant was centrifuged in spinco rotor #40 at 3200 $\times$ *g* for 15 minutes to sediment the mitochondrial fraction. The mitochondrial pellet was washed 3 times with 0.25 M sucrose and sedimented at the same speed. The first supernatant following the 3200 $\times$ *g* spin was centrifuged at 10,000 $\times$ *g* in the same rotor for 10 minutes, the sediment discarded and the microsomal fraction was sedimented at 105,000 $\times$ *g* for 1 hour in the same rotor. For obtaining the cell sap, the 3200 $\times$ *g* supernatant was centrifuged at 105,000 $\times$ *g* for 2 hours to sediment the microsomal particles.

For convenience, the dose of microsomes, mitochondria and cell sap is expressed in units, one unit being the equivalent of 1 mg of original wet weight of the tissue used.

Ultrasound wave treatment. The microsomal pellet was suspended in sucrose and subjected to 5 or 10 minutes of ultrasonic waves using a 50/MSE unit. The suspension was either injected into recipient mice or centrifuged in spinco rotor #40 at 105,000 $\times$ *g* for one hour. The pellet and supernatant were separated and injected into different groups of recipient mice.

RNAase treatment. Tris buffered (pH 7.5) microsomal suspension, 300 units/ml, was incubated with RNAase (Worthington Biochemical Corp.) 50 μg/ml for 30 minutes at 37°C.

Assay system. The antigenic preparation was suspended in sucrose and injected into recipient mice by the i.p. route, approximately 0.5-1 ml volume/mouse. The skin grafts were applied 3-5 days later. The grafts were inspected 6 days following their application, the grafted animals were then sacrificed and representative histologic sections of these grafts were obtained. Skin grafts showing 50% or more destruction of surface epithelium were scored rejected.

The sixth day was taken as an endpoint since in this donor recipient combination, first set skin grafts are viable 6 days following

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TABLE I. Antigenic Activity of the Mitochondrial Fraction.

Recipient	Donor	Tissue	Dose*	Reject/ total†
CBA	A/Jax	Spleen	200u i.p.	19/20
		"	100u "	8/10
		"	50u "	15/18
		"	25u "	5/ 8
		Kidney	200u "	0/ 8
		Liver	100u "	1/10

* One unit being the equivalent of mitochondrial material elicited from 1 mg original wet weight of tissue used.

† No. of test grafts showing accelerated rejection/total No. grafted.

their application while second set grafts are completely destroyed.

Results. 1. Antigenic activity of the mitochondrial fraction. Mitochondrial fractions from kidney, liver, or spleen were injected into separate groups of recipient mice in varying dosages followed by the application of test skin allografts. It can be seen from Table I that spleen mitochondria induced a high degree of homograft immunity as indicated by the accelerated rejection of almost all the test skin allografts. Mitochondrial material obtained from 25 mg original wet weight of spleen tissue (25 units) represented the lower limit of antigenic activity. There was no antigenic activity in the mitochondrial fraction from kidney and liver cells.

2. Antigenic activity of the cell sap. Cell sap derived from 200 mg wet weight (200 u) of spleen, kidney or liver tissue was injected into separate groups of recipient mice. All the test skin allografts on these recipients were viable by the sixth day indicating lack of antigenic activity in this cytoplasmic component.

3. Exposure of spleen microsomal antigens to RNAase or ultrasound waves. Incubation of spleen microsomes with RNAase did not abolish their antigenic activity as measured by accelerated rejection of test skin allografts. Each of 10 CBA recipients was injected with treated microsomal suspension from 300 mg original wet weight of spleen tissue (300 units). All of the test skin grafts on these recipients were rejected.

Spleen microsomes retained their antigenic potency following exposure to 5 minutes of ultrasound waves. Centrifugation of the sonicated microsomal suspension at 105,000 $\times$ g for one hour resulted in a supernatant and a pellet. When separated and injected into different groups of mice, equal activity was found in the supernatants and pellet fractions. However, when exposure time to ultrasound was increased to 10 minutes, minimal antigenic activity was retained in the pellet following centrifugation at 105,000 $\times$ g while the supernatant lacked any antigenic activity (Table II). It is of interest that exposure to ultrasound caused some clearance in the opalescent appearance of the microsomal suspension.

4. Kidney microsome. Each of 8 CBA mice were injected with kidney microsomes from 200 mg original wet weight kidney tissue (200 units). None of the test grafts on these animals exhibited accelerated rejection. This amount represents an 8-fold increase over the amount of spleen microsomes which will cause rejection of a related skin homograft.

Discussion. Evidence is presented in this study to show that mitochondrial fractions obtained from spleen cells are potent transplantation antigens. The antigenic activity of this fraction parallels that which we have observed when microsomal fractions of spleen cells were employed(7).

Davies(8) and Basch *et al*(9) using hemagglutination inhibition and cytotoxicity re-

TABLE II. Effect of Ultrasound Waves on Antigenic Activity of Spleen Microsomes.

Sonication time	Dose	Centrifugation after sonication	Material injected	Reject/total
5 min	300u i.p.	None	Whole	9/9
5 "	300u "	105,000 $\times$ g for 1 hr	Supernatant	8/15
			pellet	7/14
10 "	200u "	*Idem*	*Idem*	0/10
				5/9

spectively as endpoints, also observed the association of transplantation antigens with mitochondrial fractions. In our experiments we have selected the biologic endpoint of accelerated rejection of test skin grafts as an index of antigenic activity. We believe this method to be more discriminating and a more reliable index of homograft immunity. The endpoint recorded excludes the difficulties in interpretation inherent in the *in vitro* tests (10). Monaco *et al*(11) using accelerated rejection of test skin grafts as an assay system also observed high antigenic activity in subcellular fractions from spleen cells sedimented at 5,000 $\times$ *g* which is the range for mitochondrial sedimentation.

The finding that spleen cell sap preparations free of particulate materials have no demonstrable antigenic activity, in contradistinction to the mitochondrial and microsomal fractions, indicates that transplantation antigen activity is associated with the particulate component of cytoplasmic fractions. This conclusion is supported by recent observations on the presence of transplantation antigens in the particulate fractions of cell-free culture medium from tissue cultures of rabbit spleen cells and human leucocytes. Mannick *et al*(12) using accelerated rejection of test skin allografts as an assay system, demonstrated the presence of transplantation antigens in cell-free medium from cultures of rabbit spleens. In these experiments, the antigenic material was recovered by centrifugation of the culture medium at 30,000 $\times$ *g* leaving an inactive supernatant fraction. Recently, Kasakura *et al*(13) showed that cell-free medium from cultured human leucocytes stimulated blastogenesis when added to cultures of homologous but not autologous leucocytes. Ultracentrifugation of the culture medium resulted in a precipitate with a higher blastogenic stimulating capacity than the supernatant. It may be, as Kasakura pointed out, that the stimulating factors are transplantation antigens released into the culture medium.

Earlier reports showed that transplantation antigens in kidney and liver cells exhibit weak sensitizing activity(1,2,14). The current experiments show that mitochondrial and microsomal fractions prepared from kidney cells are devoid of antigenic activity as were liver mitochondrial fractions. It is not known why whole organ transplants of liver or kidney induce an effective homograft reaction which culminates in the rejection of the entire organ while cellular and subcellular extracts of liver or kidney function as weak transplantation antigens. It may be that some sort of enzymatic inactivation or degradation of the antigens takes place during the preparation of cells or antigenic extracts from these organs. Certainly, the observations of Mandel *et al*(15) that liver homogenates inactivate antigenic extracts from spleen cells is pertinent in this connection. Furthermore, these authors showed that the capacity of liver homogenates to inactivate transplanation antigens is heat labile. Thus, other than skin itself, cells of the leucocyte series furnish the most effective source of extractable antigens.

Physical treatment of spleen microsomes (ultrasound waves) can yield antigenic extracts which remain in solution after centrifugation at 105,000 $\times$ *g*. It is not clear whether this is due to elution of antigenic material from microsomes or to further fragmentation of the endoplasmic reticulum.

It is known that exposure of microsomes to RNAase degrades the ribonucleic acid (RNA) moiety. The observation that RNAase is without effect on the antigenic activity of spleen microsomes indicates, in an indirect manner, that homograft antigens are not associated with the RNAase sensitive RNA component of this fraction. Our earlier observation that desoxycholate inactivates spleen microsomes as transplantation antigens suggests, therefore, a direct effect of this detergent on the antigen itself rather than due to a dissociation of the RNA from the lipoprotein. Similar observations on the action of detergents on transplantation antigens have been made by Brent *et al*(16).

Summary. 1. Mitochondrial fractions obtained from spleen cells were found to be potent transplantation antigens. 2. No antigenic activity was detected in a) spleen, liver, or kidney cell sap; b) kidney mitochondria and microsomes. 3. Minimal antigenic activity was associated with liver mitochondria.

4. Exposure of spleen microsomes to RNAase did not affect their antigenic activity. 5. Brief exposure of spleen microsomes to ultrasound waves yielded a semisoluble antigenic preparation.

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Evidence for Thyrocalcitonin in Man. (31380)

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Studies with several species of experimental animals have demonstrated that there is a calcium-lowering principle whose secretion is stimulated by hypercalcemia(1,2,3). It was initially thought that this hormone was secreted by the parathyroids(1), but subsequent investigations have shown that it is secreted by the thyroid gland(4,5,6). It has therefore been designated thyrocalcitonin(4). Apparently this hypercalcemia-induced calcium-lowering thyrocalcitonin and the long known hypocalcemia-induced calcium-elevating parathyroid hormone are both important in calcium homeostasis: by their combined, but opposite, effects, the two hormones maintain the plasma calcium concentration within a very narrow physiologic range. Most studies of the homeostatic effects of thyrocalcitonin have been carried out on experimental animals(1-3,5,6). However, a substance has been extracted from the human thyroid gland which is hypocalcemic in rats(7), and injection of porcine thyrocalcitonin into human subjects causes lowering of plasma calcium (8). Therefore it seems probable that thyrocalcitonin is present and has important physiologic effects in the human species. Thyrocalcitonin is a polypeptide(9) and is therefore biochemically and physiologically distinct from thyroxine, but apparently both hormones are secreted by the same thyroid cells(10). Therefore patients with hypothyroidism (of excessive surgical removal, excessive I^{131} therapy, or idiopathic etiology) may well have a deficiency of thyrocalcitonin. If these patients do have thyrocalcitonin deficiency, they should have impaired calcium homeostasis, especially in their ability to prevent or promptly reduce hypercalcemia when subjected to an excessive calcium intake.

This study was undertaken to determine whether a thyroid hypocalcemic principle, thyrocalcitonin, can be demonstrated in man, and whether a deficiency of thyrocalcitonin