IMMUNOLOGY

Microsomal Fractions as Transplantation Antigens

Recent emphasis has been placed on the lipid-protein composition of transplantation antigens derived from normal and neoplastic cells[1] and there is serological evidence for the occurrence of some of these antigens at cell surfaces[2,3]. The presence of transplantation antigens outside the nucleus of a cell implies the operation of a mechanism whereby the genetic individuality of nuclear components is expressed in cytoplasmic materials which are characteristic of the individual.

Such a mechanism, biochemical and structural, is available in the endoplasmic reticulum, which provides not only for much of the cytoplasmic protein synthesis but also for structural continuity between nucleus and cytoplasm[4]. The possibility therefore arose that cell fractions rich in endoplasmic reticulum might contain individual-specific materials capable of sensitizing a recipient to produce an accelerated rejection of a subsequent skin graft. For this purpose we have assayed the capacity of microsomes isolated from mouse spleen and liver to function as homograft antigens.

Washed microsomal fractions were obtained from freshly excised livers and spleens of inbred donor mice by differential centrifugation of tissue homogenates in cold M/4 sucrose. The donor strains used were *A/J* and *C57BL/6*; the microsome suspensions were tested for homograft antigenicity in mice of strains *CBA/J* and *DBA/2* respectively. The assay procedure was based on that of Billingham, Brent and Medawar[5]. The test skin grafts were applied on the third day following intraperitoneal or intradermal injection of donor microsomes, the grafts being removed for histological investigation after a further 6 days.

The results are presented in Tables 1 and 2. In the histological assessment of each graft, rejection was considered to have occurred only if more than 50 per cent of the graft epithelium was found to have been destroyed.

The results clearly show that microsomal suspensions from spleens of inbred donor mice are capable of sensitizing recipient mice to cause accelerated rejection of subsequent skin grafts from the donor strain (Table 1). The failure of spleen microsomes from *A/J* mice to cause accelerated rejection of *C57BL* skin, together with the failure of *C57BL* microsomes to cause accelerated rejection of *A/J* skin (Table 2), provides evidence for the specificity of the induced sensitization. Similarly, the injection of *CBA* spleen microsomes into *CBA* mice also failed to cause accelerated rejection of grafts of *A/J* skin (Table 2).

Furthermore, it was found that the antigenic effectiveness of the inoculum was related to the dose of spleen microsomes injected. Thus the injection of microsomes from 30 mg of whole *A/J* spleen represented the limit of antigenic activity; this was equivalent to approximately 600 μg of dry weight of microsomal material.

It is of interest that liver microsomes were also antigenic, though they were less active and sensitized with less regularity than did spleen microsomes. At least 60 per cent of the liver cell population is represented by parenchymal cells[6]; the lesser activity of liver microsomes may thus be due to their commitment to elaborate specialized products of cell differentiation[7]. On the other hand, the spleen may contain a majority of undifferentiated cells the synthetic machinery of which may not yet be diverted from the manufacture of individual-specific materials.

Tissue-specific antigens have been found in microsomal fractions of liver[8,9], kidney[10] and thyroid[11] and it has been suggested that they may be associated with membranes of the endoplasmic reticulum[8]. The findings reported in this communication extend present interest in the antigenic properties of microsomes and the possibility arises that microsomal preparations may represent useful starting-points for the isolation of more specifically active transplantation antigens. Further work is in progress to investigate the homograft antigenicity of microsomal sub-fractions.

Table 1. Homograft Antigenicity of Microsomes from Spleen and Liver

Accelerated rejection of *A/J* skin grafts in *CBA* mice that were sensitized with microsomes from liver and spleen of *A/J* mice

Spleen microsomes (wet wt. of spleen, mg)	No. of test grafts rejected	Liver microsomes (wet wt. of liver, mg)	No. of test grafts rejected
300	30/30	300	23/38
100	19/19	100	7/18
30	4/4	30	2/10
10	1/9		

Table 2. Specificity of Accelerated Homograft Rejection induced by Spleen Microsomes

Recipient mice each injected with microsomes from 300 mg wet wt. of spleen

	Strain combinations: Donor of microsomes → Recipient strain ← Donor of skin graft	No. of skin grafts rejected
Experimental group	*C57BL* → *DBA/2* ← *C57BL*	20/25
Control groups	*A/J* → *DBA/2* ← *C57BL*	0/10
	C57BL → *CBA* ← *A/J*	0/10
	CBA → *CBA* ← *A/J*	0/8

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[1] Davies, D. A. L., in *Ciba Found. Symp. Transplantation*, edit. by Wolstenholme, G. E. W., and Cameron, M. P., 45 (J. and A. Churchill, Ltd., 1962).
[2] Herzenberg, L. A., and Herzenberg, L. A., *Proc. U.S. Nat. Acad. Sci.*, **47**, 762 (1961).
[3] Moller, G., *J. Exp. Med.*, **114**, 415 (1961).
[4] Porter, K. R., in *Biological Structure and Function*, edit. by Goodwin, T. W., and Lindberg, O., 127 (Academic Press, 1961).
[5] Billingham, R. A., Brent, L., and Medawar, P. B., *Nature*, **178**, 514 (1956).
[6] Daoust, R., in *Liver Function*, edit. by Brauer, R. W., 3 (Amer. Inst. Biol. Sci., 1958).
[7] Campbell, P. N., *Biol. Revs.*, **35**, 413 (1960).
[8] Vogt, P., *Z. Naturforsch.*, **15**, b, 221 (1960).
[9] Asherson, G. L., and Dumonde, D. C., *Brit. J. Exp. Path.*, **43**, 12 (1962).
[10] Nairn, R. C., Ghose, T., Fothergill, J. E., and McEntegart, M. G., *Nature*, **196**, 385 (1962).
[11] Trotter, W. R., and Belyavin, G., in *Advances in Thyroid Research*, edit. by Pitt-Rivers, R., 138 (Pergamon Press, 1961).

Genetic Control of a Guinea Pig Serum Factor toward which Natural Delayed Iso-hypersensitivity occurs

Recent investigations[1–3] have revealed the natural existence, in certain guinea pigs, of a state of delayed iso-hypersensitivity toward a heritable factor present in the serum of other normal guinea pigs. The observation that this serum factor, which was first detected in animals of Rockefeller Institute stock, is present also in guinea pigs of the more inbred (nevertheless also heterozygous) Hartley strain but not in animals of the homozygous Wright strains II and XIII (ref. 2) suggested a means for investigating the mechanism of its heritability. Results presented here, obtained by interbreeding strain XIII and Hartley animals, indicate that the presence of serum factor in guinea is pigs controlled by an autosomal dominant gene.

Serum factor was detected by skin tests; 0·1 ml. of undiluted serum from animals at least two months old was injected intradermally into each of 2–4 Rockefeller stock animals lacking factor and known to be dermally reactive (naturally iso-hypersensitive). Sera were judged