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Immunosuppressive Effect of Male Mouse Submandibular Gland Extracts on Plaque-Forming Cells in Mice: Abolition by Orchiectomy

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Summary. The plaque-forming response to sheep red blood cells is suppressed in mice treated with crude saline extracts of normal male mouse submandibular glands. Extracts from adult male mice orchiectomized at 2 weeks of age are ineffective, suggesting that the presence of this suppressive substance in the male mouse submandibular gland is testosterone-dependent.

INTRODUCTION

Following observations that the male mouse submandibular gland contains a substance which has a suppressive effect on lymphoid tissues (Takeda, Yamasaki, Yamabe, Suzuki, Haebara, Irino and Grollman, 1967; Takeda and Grollman, 1968), we demonstrated the effectiveness of crude saline extracts of male mouse submandibular glands in prolonging the survival of H-2 incompatible skin allografts in mice (Kongshavn and Bliss, 1970a). We now report further investigations on the immunosuppressive action of male mouse submandibular gland extracts on the plaque-forming response to sheep red blood cells in mice, and the effect of orchiectomy on the presence of this suppressive activity in the male mouse submandibular gland.

MATERIALS AND METHODS

Experimental animals

Mice were from the inbred A strain maintained in this laboratory. Male or female mice 2–6 months of age were used for the plaque assay. Mouse submandibular gland extracts were prepared from male or female donors 2–12 months of age.

Preparation of mouse submandibular gland extract (MSGE)

MSGE was prepared from donor mice as described previously (Kongshavn and Bliss, 1970a). For control studies MSGE was prepared from female mice, instead of from kidney and liver tissues as before.

To prepare gonadectomized donors used to make MSGE, one half of the male and one half of the female mice in each litter were orchiectomized or oöphorectomized by normal surgical procedures at 2 weeks of age. Of the remaining litter mates, one half were sham-

operated and the other half left untreated. After killing, the presence or absence of testes or ovaries was confirmed.

Plaque assay

The method used for assaying IgM PFC was essentially the one described by Cunningham and Szenberg (1968) with certain modifications made in this laboratory. Spleen cell suspensions were prepared by gently tamping the spleen through a 50-mesh stainless steel screen, and collecting the cells in balanced salt solution (BSS) supplemented with 15% heat-inactivated calf serum. The spleen cells were washed and made up to 15 ml with BSS. SRBC were washed twice and made up to a 20% concentration. Complement (GIBCO) was diluted 1/10 with BSS. All stock solutions were kept on ice water until used. The test consisted of mixing 0·05 ml of spleen cells, 0·15 ml of SRBC and 0·75 ml of the complement solution in a test tube at 37°C. The whole mixture was immediately withdrawn and put into chambers prepared by glueing two 75 × 25 mm slides together with double-sided tape. Four to five slide chambers were necessary for each sample, filling up the last chamber if necessary with a blank solution of SRBC and complement in the same concentrations as for the test mixture. The slide chambers were sealed with warm paraffin wax, and incubated at 37°C for 45–60 minutes. (In earlier experiments, a 30 minute incubation period was used, but this proved to be insufficient time for full development of all plaques.) The number of PFC were counted by both macro- and microscopic examination. The total number of PFC per spleen was then estimated by multiplying the number of PFC in all chambers (0·05 ml spleen cells) by 300. Total spleen cell counts were also done in some experiments.

The method for assaying PFC as used in this laboratory appears to be sensitive and reproducible. For example, nine samples taken from one spleen gave the following results expressed as PFC/spleen; range 78,900–94,500; mean 84,967; standard error 1484; coefficient of variation 5·1%.

Experimental protocol

Each mouse received a total of four doses of MSGE, given intraperitoneally on days 1, 2, 3 and 5. Each dose was equivalent to 50 mg wet weight tissue. Mice were injected intravenously on day 2 with 5×10^8 sheep red blood cells (SRBC) per mouse, and on day 6 the mice were sacrificed and the spleens assayed for plaque forming cells (PFC) to SRBC.

RESULTS

Suppression of PFC response to SRBC with male MSGE

Administration of MSGE prepared from normal male mice resulted in suppression of the PFC response to SRBC (Table 1). The number of PFC/spleen obtained in the immunized groups receiving either normal female MSGE or no MSGE is approximately 10 times greater than the corresponding value obtained in the group receiving normal male MSGE in experiment 1, and 4–5 times greater in experiment 2. The number of PFC/10^6 spleen cells is also less in the group treated with male MSGE, in each experiment. Extract used in experiment 1 apparently had somewhat greater potency than that used in experiment 2. Presumably this difference is due to the rather crude method used to prepare extracts.

It is of note that, in mice receiving male MSGE, spleen counts fell approximately to half the normal value.

The shorter incubation time used in experiment 1 resulted in the overall formation of fewer PFC as compared to the number of PFC produced in experiment 2 in which a 45–60 minute incubation time was used.

In these experiments donor mice were 2–6 months old. Host mice used for the plaque assay were male.

TABLE 1

PFC RESPONSE TO SRBC IN SPLEENS OF MALE MICE TREATED WITH MOUSE SUBMANDIBULAR GLAND EXTRACT (MSGE)

Source of MSGE	SRBC	Experiment 1†		Experiment 2	
		$\frac{\text{PFC}}{\text{spleen}} \pm$ S.E.	$\frac{\text{PFC}}{10^6}$ cells $\pm$ S.E.	$\frac{\text{PFC}}{\text{spleen}} \pm$ S.E.	$\frac{\text{PFC}}{10^6}$ cells $\pm$ S.E.
Normal male	+	5160 ± 835	115·8 ± 26·5 (5)*	24188 ± 5954	339·8 ± 64·9 (8)*
Normal female	+	68775 ± 8811	590·2 ± 25·1 (4)	95820 ± 8028	636·5 ± 53·3 (5)
None	+	61000 ± 5381	474·1 ± 41·4 (3)	130100 ± 18397	707·7 ± 70·5 (3)
Normal male	—	900 ± 173	18·8 ± 4·2 (4)	200 ± 100	2·9 ± 1·5 (3)
None	—	900 ± 173	10·1 ± 3·3 (4)	200 ± 100	0·7 ± 0·7 (3)

* Number of animals in parentheses.
† Incubated 30 minutes only.

TABLE 2

PFC RESPONSE TO SRBC IN SPLEENS OF FEMALE MICE TREATED WITH MOUSE SUBMANDIBULAR GLAND EXTRACTS (MSGE) FROM NORMAL and GONADECTOMIZED DONORS

Source of MSGE	SRBC	PFC/spleen (Mean ± S.E.)
Normal or sham-operated male	+	14880 ± 5917 (5)*
Orchiectomized male	+	160140 ± 19948 (5)
Normal or sham-operated female	+	150900 ± 12480 (5)
Oophorectomized female	+	128900 ± 7398 (3)
None	+	140200 ± 5667 (3)
None	—	100 ± 100 (3)

* Number of animals in parentheses.

Effect of orchiectomy on suppression of PFC response by male MSGE

The marked (ten-fold) suppression of the PFC response to SRBC produced by administration of MSGE from normal or sham-operated male mice was not observed in mice receiving MSGE from orchiectomized male mice (Table 2).

Extract made from oophorectomized female mice was also tested but had no suppressive activity.

In this experiment donor mice were 1-year old. Host mice used for the plaque assay were female.

DISCUSSION

It is apparent that MSGE prepared from male mice has a suppressive effect not only on cellular immune responses, as observed previously (Kongshavn and Bliss, 1970a), but also on humoral immune responses as measured by the PFC response to SRBC. Reduction

in spleen size occurred and, since the number of PFC/10^6 spleen cells is reduced (Table 1), it appears that male MSGE has a relatively greater effect on lymphoid cells than on other cells in the spleen.

Mice used in the experimental results shown in Table 2 were 1-year old, demonstrating that this immunosuppressive substance persists in the submandibular gland for at least a year. Female mice were used for the plaque assay in this experiment, showing that the immunosuppressive substance in male MSGE is active in female hosts.

The results using MSGE from orchiectomized mice show that the presence of immunosuppressive activity in the male mouse submandibular gland depends on the presence of the testis, strongly suggesting that elaboration of this principle by the submandibular gland is testosterone-dependent. However, although the presence of the immunosuppressive substance is sex-dependent, it does not appear to be responsible for the sex difference in immunological responsiveness observed by us previously (Kongshavn & Bliss, 1970b). Experiments showing evidence for this will be the subject of a separate communication (unpublished results). Since the sex differences apparently cannot be attributed to the presence of immunosuppressive activity in the male mouse submandibular gland it would seem unlikely that this substance has a physiological role in controlling the immune system in the normal male mouse, but rather it would appear to be a pharmacological agent comparable to some of the other substances such as nerve growth factor (Cohen, 1960) which are found in this interesting gland.

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REFERENCES

COHEN, S. (1960). 'Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic anti-serum.' *Proc. nat. Acad. Sci. (Wash.)*, **46**, 302.

CUNNINGHAM, A. J. and SZENBERG, A. (1968). 'Further improvements in the plaque technique for detecting single antibody-forming cells.' *Immunology*, **14**, 599.

KONGSHAVN, P. A. L. and BLISS, J. Q. (1970a). 'Effect of mouse submandibular gland extracts on survival of H-2 incompatible skin allografts.' *Immunology*, **19**, 363.

KONGSHAVN, P. A. L. and BLISS, J. Q. (1970b). 'Sex differences in survival of H-2 incompatible skin grafts in mice treated with antithymocyte serum.' *Nature (Lond.)*, **226**, 451.

TAKEDA, T. and GROLLMAN, A. (1968). 'Inhibitory action of submaxillary gland on thymus and lymphoid tissues of the mouse.' *Amer. J. Physiol.*, **215**, 1337.

TAKEDA, T., YAMASAKI, Y., YAMABE, H., SUZUKI, Y., HAEBARA, H., IRINO, T. and GROLLMAN, A. (1967). 'Atrophy of the lymphoid tissues of mice induced by extracts of the submaxillary gland.' *Proc. Soc. exp. Biol. (N.Y.)*, **126**, 212.