IMMUNODEFICIENCY IN HYPOPHYSECTOMIZED RATS

By

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ABSTRACT

The response of hypophysectomized, sham-operated and non-operated female Fischer 344 and Wistar-Furth rats was compared to various antigenic stimuli. Antibody production against sheep red blood cells, skin response to dinitrochlorobenzene and the development of adjuvant arthritis after treatment with Freund's complete adjuvant were all markedly suppressed in hypophysectomized animals. Sham-operated rats responded as well as did non-operated controls. Skin graft survival was also prolonged in hypophysectomized rats when compared to controls. These results indicate that the pituitary gland plays an important role in immune reactions.

There is much circumstantial evidence that hormonal mechanisms may influence immune reactions (Ahlqvist 1976). Thyroxine, growth hormone and insulin seem to be needed for the ontogenic maturation (Fabris et al. 1971a; Fabris 1973) and maintenance (Duquesnoy et al. 1969; Fabris et al. 1971a,b, 1972; Fabris 1973; Pierpaoli & Sorkin 1972; Pierpaoli et al. 1970) of the immune system. The immunosuppressive effect of adrenal steroids (White 1958) is well recognized, although small amounts of glucocorticoids are necessary for in vitro immune reactions (Ambrose 1964; Cohen et al. 1970; Sherman et al. 1973). Orchidectomy may protect against chemical carcinogenesis and against transplanted tumours (Castro 1974a,b,c) and cell-mediated immune reactions are depressed during pregnancy (Morton et al. 1974; Olding & Oldstone 1974; Purtilo et al. 1972; Thong et al. 1973). Thus it appears that endocrine factors, especially the hormones of the pituitary gland, have an important role in the maintenance of immunocompetence. We report here the suppressive effect of hypophysectomy on cell-mediated and humoral immune reactions.
**MATERIAL AND METHODS**

**Animals**

Female Fischer and Wistar-Furth rats, weighing 150–200 g, were obtained from Canadian Breeding Farm Laboratories, Ltd., Montreal, Canada, and from A.R.S. – Sprague Dawley, Madison, Wisconsin, USA, respectively. Hypophysectomy was performed by the parapharyngeal approach (Lostroh & Jordan 1955; Tarttelin & Gorski 1972). The completeness of hypophysectomy was determined for each experimental animal by autopsy at the end of the experiments. Sham-operated and non-operated animals were used as controls. Immunization was started two weeks after operation in order to avoid the possible detrimental effect of surgical trauma on the immune response. All the animals were maintained on a standard diet (Wayne’s Laboratory blocks with 6% fat content, Chicago, Illinois, USA) and on water supplied ad libitum.

**Induction of antibody response**

Sheep red blood cells (SRBC) were washed with phosphate buffered saline (PBS) at pH 7.2 three times and 10⁷ SRBC were injected ip to each animal in 1 ml of PBS. The rats were bled from the tail veins at the time of injection and on every third day afterwards up to day 15. The serum samples were individually stored at −20°C from each animal and titrated by haemagglutination (Takatsy 1955) at the end of the experiment. Some of the sera were treated with 0.1 M mercaptoethanol (ME) in order to destroy the haemagglutinating capacity of IgM antibodies (Froese 1971). The haemagglutinating capacity of such treated sera was then compared with the non-treated portion of the same samples.

**Induction of cell-mediated immunity**

Skin grafts were transplanted from Fischer donors to Wistar-Furth recipients and vice versa. Graft survival was established by daily macroscopic observation of grafted animals. In other experiments skin sensitization was carried out with dinitrochlorobenzene (DNCB). DNCB was dissolved in acetone (200 mg/ml) and 0.02 ml was applied onto the shaved skin behind the ears to an area of approximately 1 cm². Control groups were treated with 0.02 ml of acetone in an identical manner. In DNCB treated animals, the resulting inflammatory reaction was quantitated by measuring the diameter of the affected skin area by a caliper every day until day 6.

**Induction of adjuvant arthritis**

A single injection of 0.1 ml of Freund’s complete adjuvant (Difco) was given to each animal into one of the hind footpads (Waksman et al. 1960). Such treatment caused a severe inflammation of all four footpads which reached the maximum on day 12 after treatment. The diameter of swollen footpads was measured with a caliper every third day until day 15.

**Evaluation of experiments**

Each figure presented in this paper was constructed from two separate experiments using data generated by a total of 10 animals per group. Mean values ± SEM are represented in the figures. Data obtained in experimental and control groups on the same day of observation were compared using Student’s t-test in order to determine the significance of the differences.
RESULTS

The mean haemagglutination titers $\pm$ SEM for hypophysectomized, sham-operated and non-operated animals are plotted in Fig. 1. It is clear from the results that hypophysectomized animals were markedly deficient in antibody production while sham-operated rats responded as well as did non-operated controls. For this experiment sham-operation was carried out without drilling the skull. This experiment was repeated with sham-operated animals that were drilled similarly to those on which hypophysectomy was performed. Although the antibody response of such sham-operated animals was still significantly higher than that of the hypophysectomized group, non-operated animals responded somewhat better (Fig. 2). Titration of sera after treatment with ME revealed a partial loss of antibody activity, which indicates that both ME sensitive (IgM) and non-sensitive antibodies (IgG) were affected by hypophysectomy.

Fig. 3 illustrates that hypophysectomized rats were virtually non-responsive to DNCB while a vigorous response was observed in both control and sham-operated animals.

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**Fig. 1.**

Effect of hypophysectomy on the antibody response of rats to sheep red blood cells (SRBC).

Each curve was constructed from two separate experiments, using data generated by a total of 10 animals per group. The skull of sham-operated controls was not drilled in these experiments.
Effect of hypophysectomy on the antibody response of rats to SRBC. These are identical experiments with those of Fig. 1, except that the skull of sham-operated controls were drilled.

Skin graft rejection was influenced the least by hypophysectomy. The average rejection time of controls was 8 ± 0.73 days, in sham-operated animals 7.8 ± 1.07 days, while in the hypophysectomized group, 12 ± 1.18 days as plotted in Fig. 4.

Effect of hypophysectomy on dinitrochlorobenzene induced contact sensitivity in rats. The data were averaged from two separate experiments (a total of 10 animals per groups). The skull of sham-operated controls was not drilled.
Finally, the strength of adjuvant arthritis as estimated by the swelling of fore and hind paws was markedly attenuated in hypophysectomized animals when compared to both control groups as shown in Fig. 5.
DISCUSSION

Present results clearly indicate that the humoral-, cell-mediated- and auto-immune responses in rats are impaired by hypophysectomy. Skin graft rejection was moderately delayed, while all the other responses were suppressed markedly with only minimal detectable responses in operated animals. The partial immunosuppressive effect of skull drilling in sham-operated controls was probably due to disturbed pituitary function, which resulted from surgical trauma to the neighbouring tissues.

The various immune reactions used in these experiments are known to be mediated by different cell types of the lymphoid system, e.g. antibody response is the result of the cooperation of macrophages with helper thymus derived (T) cells and bone marrow derived (B) lymphocytes (Feldmann & Nossal 1972). The response to DNCB is mediated by another T lymphocyte subset which has Ly surface antigens identical with helper T cells (Huber et al. 1976), although this T cell group appears to be different functionally from helper T cells. The principal effector mechanism in graft rejection is believed to be mediated by killer T cells which can clearly be differentiated from helper T lymphocytes by their Ly antigenic markers (Cantor & Boyse 1975a,b). Although the immunopathology of adjuvant arthritis is not elucidated as yet in full detail, it is known that non-T lymphoid cells are the major participants (Van Arman 1976) which are probably triggered by antigen-antibody reactions.

The mechanism underlying the immunoregulatory role of the pituitary gland is obscure. Hypophysectomized animals are known to have a disturbed metabolism which leads to loss of body weight (Powley & Morton 1976). In our experiments such animals weighed approximately 25% less than controls at the time of termination. Although this loss in weight may have influenced the results, it is unlikely that the observed differences in immune reactivity are the sole consequence of this metabolic effect. This assumption is supported by the fact that skin graft rejection was much less affected in hypophysectomized animals which militates against a general immunosuppressive factor, such as malnutrition. In order to solve this problem, presently pair-fed controls are fed the amount of food consumed by hypophysectomized animals. Severe protein-calorie malnutrition in animals has been shown to result in depressed cell-mediated and humoral immunity (Aschkenasy 1973). Animals with chronic protein deficiency which caused up to 60% weight loss, exhibited an enhanced resistance to grafts, to viruses and to tumours, but antibody production was impaired (Cooper et al. 1974; Jose & Good 1971). Malnourished children were found to have normal IgM and IgG serum levels with normal lymphocytic response to phytohaemagglutinin and with a normal delayed hypersensitivity to BCG vaccination (Rafii et al. 1977).
The direct effect of various hormones on the immune system (Ambrose 1964; Cohen et al. 1970; Duquesnoy et al. 1969; Fabris et al. 1971a,b, 1972; Fabris 1973; Ahlqvist 1976; Pierpaoli & Sorkin 1972; Pierpaoli et al. 1970; Sherman et al. 1973) suggests that the pituitary gland influences immune reactions specifically via hormonal mechanisms, rather than through the non-specific effect of malnutrition. In fact it is quite conceivable that the immunoregulatory function of hormones hinges on T lymphocytes which are known to play a key role in most, if not all, the immune reactions (Miller 1975). Thymic epithelioid cells were shown recently to produce at least one hormone (Komuro & Boyse 1973; Twoney et al. 1977) (thymopoietin) which seems to be necessary for the functional maturation of T lymphocytes and for the maintenance of normal immune reactivity. It may well be that the endocrine activity of the thymus is controlled by the pituitary gland similarly to that of other endocrine target organs.

Hypophysectomized mice and rats were shown by some investigators to exhibit a decreased antibody response (Enerback et al. 1961; Gisler & Schenkel-Hullinger 1971; Lundin 1960), while others found no change in antibody production (Kalden et al. 1970; Nagareda 1954; Tyrey & Nalbandon 1972) to various antigens. Similarly, it was reported that hypophysectomy had no effect on allogeneic skin graft survival (Enerback et al. 1961), whereas in another study (Comsa et al. 1975) graft survival was greatly prolonged in hypophysectomized rats. Finally, lymph node weights and the proliferation of lymph node cells after cutaneous application of DNCB were shown to be decreased in hypophysectomized rats (Prentice et al. 1976). The reason for these contradictory results is not clear at present. Differences in the methods used and in experimental conditions may have accounted for the divergent results. Our data tend to reinforce those findings which showed significantly decreased immune responses after hypophysectomy. Whatever is the underlying mechanism, the immunosuppressive effect of hypophysectomy is significant and deserves further investigation.

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