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Changes in Blood Hormone Levels during the Immune Response (39057)

H. BESEDOVSKY, E. SORKIN

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Schweizerisches Forschungsinstitut Medizinische Abteilung, 7270 Davos Platz, Switzerland

M. KELLER

F. Hoffmann-La Roche, Diagnostica, 4133 Schweizerhalle-Basel, Switzerland

AND

J. MÜLLER

Steroidlabor, Medizinische Universitätsklinik, Kantonsspital, 8006 Zürich, Switzerland

There is now a considerable body of evidence indicating that hormones can exert multiple influences on the immune system (1–5). In retrospect this is entirely logical in view of an established role of hormonal influence on such basic functions as protein synthesis, control of gene expression, cell replication and the allosteric arrangement of cell membranes, all of which play an essential role in immune functions. Furthermore, some hormones function through their capacity to influence the rate of formation of cyclic 3, 5 adenosine monophosphate (cyclic 3, 5 AMP) which is also known to affect strongly lymphoid cells (6–9).

Investigations on the involvement of hormones in the immune response have generally involved the parenteral administration of hormones to experimental animals and man or the ablation or blockade of endocrine glands. Numerous reports agree that hormone administration can lead to depressed or stimulated immune responses depending on the kind and dose of the hormones and the timing of administration. Thus, these experiments demonstrate that changes in the level of various hormones can considerably influence immune performance.

The aforementioned studies attest to modulation of the immune system by hormone passively administered. However, to the best of our knowledge the possibility that the immune response would *itself* bring about changes in hormone levels has not been previously considered. The latter possibility was explored in the study reported here, and it is shown that during the primary immune response of rats to a particulate antigen, sheep red cells (SRBC) or to soluble antigen Trinitrophenyl-haemocyanin (TNP-Hae), and of mice to TNP-horse red cells (TNP-HRBC) corticosterone levels increased several fold while there occurred a temporal decline in thyroxine levels in some systems (rats).

Materials and methods. Female Holtzmann rats, (Tierfarm Füllinsdorf) were used for SRBC or TNP-haemocyanin as antigens. Two months old rats were immunized by a single intraperitoneal injection of 5×10^9 sheep erythrocytes (SRBC). Nonimmunized rats and animals injected with 5×10^9 homologous rat erythrocytes (RRBC) served as controls. Groups of animals were sacrificed at various intervals after injection of antigen. The animals were between 9 AM and 11 AM killed by ether inhalation; they were invariably dead within 2 min. This procedure does not produce significant changes of serum corticosterone levels (10). Animals were kept singly each before and throughout the experiment. Blood was taken from the vena cava and the serum of three rats was pooled. Three different serum pools for corticosterone and thyroxine determinations for day 1, 3, 5, 6, 7, 8, and 10 after immunization were available.

In other experiments, female 6–8 months old rats were immunized with a single intraperitoneal injection of 224 μg TNP-haemocyanin. This antigen was prepared by coupling keyhole limpet haemocyanin (Calbio-

chem, San Diego USA) with TNP-sulfonic acid according to Rittenberg and Amkraut (11). Individual sera were assayed for hormones.

Female inbred mice (C3H/He) were injected ip with a single dose of TNP-horse erythrocytes. This antigen was prepared by coupling horse erythrocytes with TNP-sulfonic acid (12). Blood was taken from the retroorbital plexus at various times after immunization. Hormonal determinations were made on pools of sera from three or more animals.

The immune response to SRBC was evaluated by counting the direct plaque forming cells in the spleen as described by Jerne *et al.* (11). The immune response of rats to the injected TNP-haemocyanin was measured by counting the direct plaque forming cells in the spleen to TNP coupled to SRBC as described by Rittenberg and Pratt (12).

The response in C3H/He mice to TNP-horse red blood cells was evaluated by the same method with TNP coupled to SRBC (12).

Serum corticosterone levels in animals immunized with SRBC were measured by a modification (14) of the double isotope dilution derivative assay of Kliman and Peterson (15). A similar double isotope dilution technique as modified by Buus (16) was used for animals immunized with TNP-haemocyanin and TNP-horse red cells.

The competitive protein-binding assay of Murphy *et al.* (17) was used for thyroxin determination.

Results and discussion. The results shown in Fig. 1a, indicate that between days 5 and 7 after immunization with SRBC, the maximum number of PFC in the spleen were observed.

Figure 1b demonstrates a two- to three-fold increase in serum corticosterone levels above normal, at days 5, 6, 7 or 8 after immunization with 5×10^9 SRBC. The maximum level occurred on day 5. Rats injected with the same dose of rat red cells showed no changes in corticosterone levels at any time. On day 5, the serum corticosterone concentration was 44.5 ± 7.3 (means $\pm$ SD) μg/100 ml serum in rats treated with SRBC, 12.5 ± 0.9 μg/100 ml in untreated rats, and 8.6 ± 2.3 μg/100 ml in rats treated with RRBC.

In fact, that no significant changes in the serum corticosterone level occurred on day 1 and 3 after immunization indicates that the animals were not stressed by the manipulation or the injected cells. In SRBC-treated rats, the difference between the serum corticosterone values of days 5, 6, 7, or 8 and those of days 0, 1 and 3 were statistically significant ($P < 0.001$ according to t tests). In additional experiments, not recorded in Fig. 1, rats were treated with a lower dose (5×10^8) of SRBC; their serum corticosterone levels rose to 35.9 ± 1.4 and to 20.3 ± 2.9 μg/100 ml on days 5 and 7, respectively.

A biphasic change in the serum thyroxine concentration was observed in animals treated with the higher dose of SRBC (Fig. 1c). After an initial increase on day 3, the serum thyroxine decreased by approximately 30% below normal on day 5–8. The difference between the values of days 0 and 1 and those of days 5, 6, 7, and 8 was statistically significant ($P < 0.001$). No significant change was seen on day 1. Treatment of rats with the lower dose (5×10^8) of SRBC or with RRBC did not result in any significant alterations in the serum thyroxine levels.

The above observed hormonal changes in blood after injection of SRBC led us to test also a soluble antigen, TNP-haemocyanin, in rats. The results are summarized in Table I.

Again, the blood corticosterone levels were increased during the immune response, but in this system, the increase was earlier than in the SRBC injected rats. Thyroxin blood levels showed a decrease and then an increase in a way similar to the experiments with SRBC.

In order to verify whether such results can be reproduced in another animal species, C3H/He mice were immunized with TNP coupled with horse erythrocytes. The results, which are summarized in Table II show again a significant increase in blood corticosterone levels over the uninjected controls and mice injected with the same quantity of homologous red blood cells. This increase in corticosterone levels lasted throughout the experiment. No clear picture emerged as regard changes in thyroxine levels.

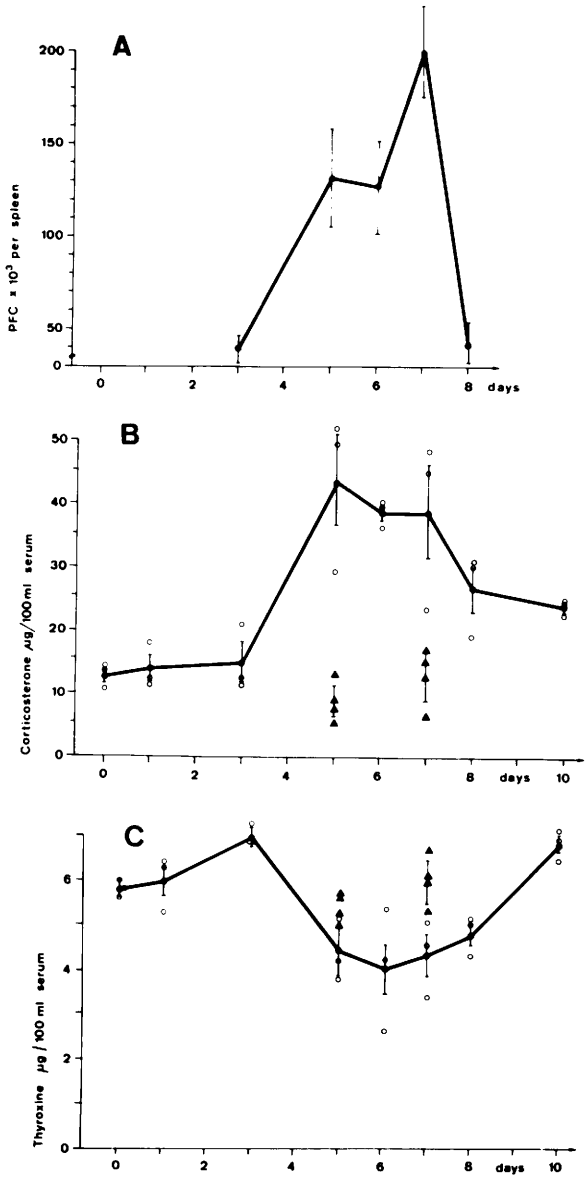

FIG. 1. Changes in serum corticosterone and thyroxine levels during the immune response to sheep red cells (SRBC) in rats. (A) Plaque forming cells (PFC) $\times$ 10^3 per spleen. (B) Corticosterone levels in serum: O—O animals immunized with SRBC; △—△ control injected with rat red blood cells (RRBC) (C) Thyroxine levels in serum: O—O animals immunized with SRBC; △—△ control immunized with RRBC.

This study has shown that in the course of the immune response to three different antigens in two animals species, major changes occurred in the blood levels of two hormones, i.e. corticosterone and thyroxine. It was already known that hormone administration, the ablation or blockade of endocrine glands influence the tempo and magnitude of the immune response. The present work has far greater implications in that it makes evident that the immune response *itself* affects hormonal levels in the blood. The corticosterone levels attained at the peak of the immune response to SRBC in rats were of the same magnitude as the concentrations observed in blood of stressed or

ACTH-treated mice which inhibited the capacity of spleen *in vitro* to respond to SRBC with plaque formation (18).

The present studies show that during the immune response hormonal changes occur that could regulate at least in part by a feedback mechanism the duration and possibly even the magnitude of the immune response. These findings may also be relevant to a different interpretation of antigenic competition, experiments where the antigens are administered sequentially. This report does not provide information about other possible endocrine changes and the manner in which the immune response may influence the endocrine glands or how the lymphoid cells change their hormone consumption. It is conceivable that chemical mediators are released by the transformed T-lymphocytes or by the antibody-producing B-cells during proliferation, and that these in turn may influence the target glands either directly or via hypothalamus → hypophysis → adrenals or thyroid. Also immune complexes, via activation of plasma constituents or cells could initiate a similar sequence of events.

The evidence, that a single injection of nontoxic and nonreplicating foreign cells or soluble antigen can produce important endocrine changes, whereas homologous red cells are ineffective and since, e.g. hormones affect the rate of tumour development it seems logical to suppose that endocrine changes during any immune response that may be aroused by cancer cells may determine at least in part their further fate (19–22).

TABLE I. SERUM CORTICOSTERONE AND THYROXINE LEVELS, PFC IN SPLEENS OF FEMALE RATS IMMUNIZED ip WITH 224 μg OF TNP-HAEMOCYANIN.

Day after immunization	PFC per spleen	Corticosterone (μg\100 ml)[a]	Thyroxine (*n*mole\liter)[b]
0		13.0 ± 3.9[c]	71.0 ± 2.4
1	5097	24.5 ± 11.3	51.66 ± 4.6
3	576260	51.0 ± 7.0	60.00 ± 7.4
4	529611	25.7 ± 9.1	66.5 ± 6.5
6	85119	36.06 ± 9.3	91.6 ± 7.4

[a] Corticosterone (Student's *t* test): Day 0 vs day 3, $P < 0.001$; Day 0 vs days 4 + 6, $P < 0.05$.

[b] Thyroxine (Student's *t* test): Day 0 vs day 1, $P < 0.001$; Day 0 vs days 3 + 4, $P < 0.05$; Day 0 vs day 6 $P < 0.05$.

[c] All results are expressed as mean ± standard error.

Summary. Injection of three different antigens into rats or mice led in the course of several days to about a threefold increase in serum corticosterone levels and concommitantly to a decrease in thyroxine (rats). In view of the known immuno-suppressive

TABLE II. SERUM CORTICOSTERONE AND THYROXINE LEVELS, PFC IN SPLEENS OF FEMALE C3H MICE IMMUNIZED ip WITH 4 × 10^8 TNP-HORSE RED CELLS.

Day after immunization	PFC per spleen	Corticosterone[a,c] μg/100 ml	Thyroxine[a] *n* mol/l
0		3.9 ± 0.4[d]	108.6 ± 4.3
1	550	6.4 ± 2.5	105.5 ± 5.3
3	3401	8.33 ± 1.0	104.25 ± 2.3
5	7435	6.17 ± 0.2	123.45 ± 9.7
6	3597	7.75 ± 1.6	125.0 ± 7.4
7	2443	11.25 ± 0.3	107.0 ± 1.3
8	1514	9.4 ± 1.4	102.75 ± 8.2
10	2817	6.1 ± 2.9	109.5 ± 4.1
5 + 7[b]		4.25 ± 0.3	105.0 ± 4.1

[a] Corticosterone and thyroxine determination were made in serum pools of at least three mice.

[b] Mice injected with 4 × 10^8 homologous mice red cells.

[c] Corticosterone (Student's *t* test): Day 0 vs day 1, $P < 0.05$; day 0 vs day 3, $P < 0.05$. Day 0 vs days 5 + 6, $P < 0.01$; day 0 vs day 7, $P < 0.001$; Day 0 vs day 8, $P < 0.01$. Homologous mice red cells on days 5 + 7 vs TNP-horse red cells on days 5 + 7 $P < 0.01$.

[d] All results are expressed as mean ± standard error.

effect of the glucocorticoids the possibility is considered that the endocrine changes induced during the immune response could significantly modulate the subsequent character of the immune response, e.i. magnitude, duration and lymphoid cell proliferation, however, a more complete pattern of hormonal variations and their cause needs to be established.

These findings while admittedly preliminary, suffice to provide an indication of a temporal pattern of hormonal change during the immune response which could be important in immunoregulation.

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1. Billingham, R. E., Krohn, P. L., and Medawar, P. B., Brit. Med. J. **1**, 1157 (1951).
2. Billingham, R. E., Krohn, P. L., and Medawar, P. B., Brit. Med. J. **2**, 1049 (1951).
3. Dougherty, T. F., Physiol. Rev. **32**, 379 (1952).
4. Dougherty, T. F., Berliner, M. L., Schneebeli, G. L., and Berliner, D. L., Ann. N. Y. Acad. Sci. **113**, 825 (1964).
5. Hormones and the Immune Response, Ciba Foundation Study Group Nr. 36 (Edit. by Wolstenholme, G.E.W., and Knight Julie) Churchill, J. and A., London 1970.
6. Ishizuka, M., Ganfni, M., and Brown, W., Proc. Soc. Exp. Biol. Med. **134**, 963 (1970).
7. Rixon, R. H., Whitfield, J. F., and McManus, J. P., Exp. Cell Res. **36**, 110 (1970).
8. Makman, M. H., Proc. Nat. Acad. Sci. **68**, 885 (1971).
9. Watson, J., Epstein, R., and Cohn, M., Nature (London) **246**, 405 (1973).
10. Cook, D. M., Kendall, J. W., Green, M. A., and Kramer, R. J., Endocrinology **93**, 1019 (1973).
11. Rittenberg, M. B., and Amkraut, A. A., J. Immun. **97**, 431 (1966).
12. Rittenberg, M. B., and Pratt, Kathrine, L., Proc. Soc. Exp. Biol. Med. **132**, 575 (1969).
13. Jerne, N. K., Nordin, A., and Henry, C., "Cell Bound Antibodies." (Amos, B. and Koprowski, H., eds.), The Wistar Institute Press, Philadelphia 1963.
14. Müller, J., Acta Endocrinol. (Kbh) **48**, 283 (1965).
15. Klimann, B., and Peterson, R. E., J. Biol. Chem. **235**, 1639 (1960).
16. Buus, O., Excerpta Medica, Int. Congress Series **157**, 107 (1968).
17. Murphy, B. E. P., Pattee, C. J., and Gold, A., J. Clin. Endocrinol. Metabol. **26**, 247 (1966).
18. Gisler, R. H., and Schenkel-Hulliger, Lotte, Cell. Immunol. **2**, 646 (1971).
19. Shigeru Shiba, Takeshi Tominaga, Tsuguo Shehara, Takeshi Tokuyuma, Yoshikazu Omukai, Keishi Matzumoto, and Tokuichiro Seki, Gann. **57**, 307 (1966).
20. Galton, V. A., and Ingbar, S. H., Cancer Res. **79**, 964 (1966).
21. Deshpande, N., Jensen, V., Carson, P., Bulbrook, R. B., and Lewis, A. A., J. Endocrinol. **45**, 571 (1969).
22. Marmorston, J., Ann. N. Y. Acad. Sci. **125**, 959 (1966).

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