THE INFLUENCE OF CORTISONE ON EXPERIMENTAL VIRAL INFECTION

VIII. Suppression by Cortisone of Interferon Formation in Mice Injected with Newcastle Disease Virus*

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The suppressive effects of corticosteroids on interferon production have been well established in the chick embryo (1–3), and in cell culture systems (4, 5). Evidence that corticosteroids may influence interferon biosynthesis in vivo has been lacking. This has been due in part to difficulties in developing a suitable experimental animal model, and in part to the complex and multiple effects of cortisone in the intact animal.

Recently, Baron and Buckler reported that high levels of circulating interferon could be readily induced in mice by intravenous injection of Newcastle disease virus (NDV) and other viruses (6). Because NDV does not replicate in the mouse, when injected intravenously, the possible inhibitory effects of cortisone on virus multiplication (7) do not have to be considered. Also, circulating antibody to NDV does not appear in this experimental model during the phase of interferon increase and decline, which takes place within 24 hr. For these reasons, the NDV mouse system seemed particularly suitable for the study of the influence of cortisone on interferon formation, divorced from its potential ancillary effects on virus multiplication or antibody response. Earlier studies have defined a cortisone dose for the murine host sufficient to modify host response without suppression of antibody formation (8).

The present report will present evidence that a single injection of cortisone is associated with significantly reduced concentrations of interferon in the serum and tissue of mice injected with NDV.

Materials and Methods

Viruses.—Newcastle disease virus (NDV) (Hickman strain), grown in chick allantoic cavity, was employed for induction of interferon. Concentration of the seed virus was determined in terms of plaque-forming units (PFU) by titration in a human conjunctival cell var-

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iant (clone 1-5C-4) (9). Mice were injected in the tail vein with from $10^{7.7}$ to 10^8 PFU of NDV in 0.1 to 0.2 ml volumes of allantoic fluid diluted in saline.

Vesicular stomatitis virus (VSV) (Indiana strain) served as the indicator virus in interferon titrations. In order to obtain plaques of uniform size, which would facilitate assessment of plaque number, the seed virus was isolated by plaque purification and propagated in L cells. Titers in the order of 10^7PFU/0.2 ml were uniformly obtained. In interferon titrations the seed virus was diluted in medium 199 with 0.1% bovine albumin to give approximately 50 plaque-forming units (PFU)/0.2 ml.

Animals.—Male mice of the MF-1 pathogen-free strain were used (Supplied by Manor Farms, Staatsburg, New York). These mice provide more uniform response to influenza virus (10) and Coxsackie virus infection (Kilbourne, E. D., unpublished data). In all experiments mice between 8 to 9 wk of age were employed, weighing on the average 34 to 38 g. Five mice were used in each experimental group, unless stated otherwise. Animals were individually housed at the beginning of every experiment, to prevent fighting, the resulting "stress" of which could possibly elevate endogenous corticosteroid levels (11).

Processing of Specimens.—At different time intervals following injection of NDV, mice were bled by cardiac puncture under nembutal anesthesia, blood from each group was pooled, and allowed to clot at room temperature for 1 hr. Serum was then separated by centrifugation, and stored at 4°C. Organ extracts were prepared by homogenizing pooled organs as 10% (by wet weight) suspensions in medium 199 with 0.1% bovine albumin, and clarified by centrifugation at 12,800 *g* in a Lourdes centrifuge. Sera and organ extracts were not treated further prior to interferon titration, since in preliminary experiments no NDV could be detected in sera and organ extracts collected at 6 and 16 hr following its administration. This was determined by direct hemagglutination of specimens, (at 4°C) which in the case of organ extracrt were pretreated with receptor-destroying enzyme (RDE) (Behringwerke, Marburg-Lahn, Germany), to ensure release of receptor-bound hemagglutinin, and by inoculation of chick embryos for detection of infective virus. The latter procedure was done utilizing both untreated 11-day-old embryonated eggs, and eggs pretreated with cortisone (100 μg/egg) 1 hr prior to inoculation of the specimen, this to facilitate the detection of infective virus in the presence of possible autointerference engendered by NDV, should the virus be present in the inoculum in high concentration (12).

Cortisone.—Cortogen (cortisone acetate) (Schering Corporation, Bloomfield, New Jersey) was administered subcutaneously in doses varying from 0.5 to 10.0 mg per mouse, 24 hr prior to NDV inoculation, unless otherwise indicated.

The Cell Culture System.—The cell culture system employed for interferon titration consisted of L-929 cells (obtained from the South Jersey Medical Research Foundation, Camden, New Jersey), and found to be PPLO-free when tested at intervals during the study. The cells were grown in medium 199 with 5% inactivated calf serum and antibiotics, in 250 ml plastic flasks and in 60 mm plastic petri dishes (Falcon Plastic Company, Los Angeles). The dishes used for interferon titration were seeded with 0.5×10^6 cells per dish, and incubated in a 5% CO_2 atmosphere at 37°C. Complete monolayers were achieved in 48 hr.

Interferon Titration.—Interferon titration was carried out according to a modification of Wagner's (13) plaque-reduction method. Specimens consisting of sera or organ extracts were serially diluted 2- to 4-fold in medium 199 containing 0.1% bovine albumin. Cell monolayers were then overlaid with 2 ml aliquots of the diluted samples and incubated for 6 hr. At that time these fluids were removed, and monolayers were washed with phosphate-buffered saline (PBS without Ca^{++} of Mg^{++}) (pH 7.4) and challenged with an estimated 50 PFU of VSV. following 30 min absorption at 37°C, 5 ml of 1% agar with medium 199 and 1% heat-inactivated calf serum was added, and dishes incubated for 48 hr. Dishes then were stained with neutral red (0.02%) in medium 199, for 3 hr, and the plaques counted. The interferon titer was

taken as the reciprocal of the highest dilution giving 50% reduction in the plaque number, and expressed in terms of units per 2 ml. When the desired degree of plaque reduction fell between two dilutions, the titer was determined by interpolation. In replicate titrations of a given sample, the titers did not differ by more than 2-fold, in most instances.

RESULTS

Pathologic Effects of Intravenous Inoculation of NDV in Mice.—When injected intranasally, NDV does not achieve a complete cycle of replication in mice and produces no lesions other than those thought to be due to "toxic" effects when large doses of the virus are administered (14–15). However, Khoobyarian et al. (16) have reported that pretreatment of mice with cortisone caused a significant increase in the mortality when mice were inoculated intravenously with NDV (Victorian strain). Because of this observation and in order to get more information on the effects of NDV administration, in the dose range employed for interferon induction, with and without cortisone pretreatment, the following experiments were carried out. Animals were divided into four groups of ten mice each: Two groups were pretreated with 5 mg of cortisone and were given either NDV ($10^{7.7}$ PFU) or a saline placebo intravenously 24 hr later; two other groups that received saline instead of cortisone were similarly injected either with NDV or saline. These mice were then observed for 7 days. Only 1 death occurred, and that in the saline-pretreated group that received NDV. Autopsies performed at the termination of the observation period revealed no gross organ lesions; the only abnormal finding was a marked atrophy of the spleens in the cortisone-pretreated mice. In a confirmatory experiment, histologic sections of the spleens, lungs, livers, and pancreases were taken at 3 and 6 days following NDV injection from 4 experimental groups which had been treated as in the previous experiment. The only pathological finding was again confined to the spleen and consisted of a marked atrophy, particularly of the lymphoid follicles, in the cortisone-treated groups. In both experiments, all animals that received cortisone lost weight, whether or not they also received NDV, whereas animals that received only NDV gained weight to approximately the same degree as the control animals. It was therefore concluded from these experiments, that NDV in the doses administered, and by the intravenous route, produced no detectable tissue injury or gross pathophysiology suggestive of virus-induced cytonecrosis.

Kinetics of Interferon's Appearance in the Serum.—In a preliminary experiment, the observation of Baron and Buckler was confirmed and it was determined that following NDV inoculation an inhibitor of plaque formation appeared in the serum (Fig. 1). After intravenous inoculation of 10^8 PFU of NDV, groups of mice were killed at 2, 8, 12, 16, 24, and 48 hr, and sera collected. Inhibitory activity was demonstrable in the serum at 2 hr and persisted for 24 hr. None was discernible at 48 hr. The highest concentration of inhibitor was found at 12 hr, whereas moderately high titers were present at 6 and 16 hr.

Studies were carried out to determine whether the serum inhibitor fulfilled the criteria established for the definition of interferon. The inhibitor present in mouse serum 16 hr following injection of NDV, was found to be nondialyzable, and stable on dialysis against pH 2 glycine buffer for 24 hr. It was nonsedimentable on centrifugation at 105,000 *g* for 2 hr. It was diminished by 50% on heating at 37°C for 30 min. It was not directly virucidal as shown by the fact that incubation of VSV with serum inhibitor for 1 hr at 37°C, followed by dilution to give approximately 50 PFU prior to addition to L cells, did not result in reduction in the number of plaques. Finally, pretreatment of cells of

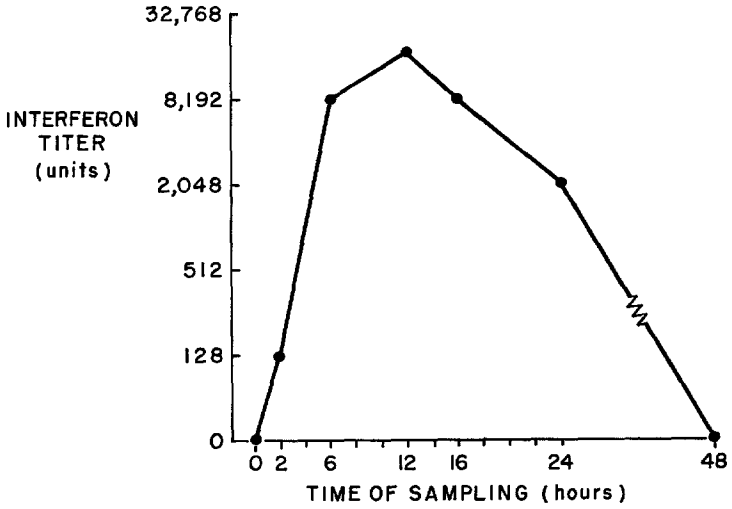

FIG. 1. Kinetics of interferon response as determined in the sera of mice injected with NDV at time 0.

another species, i.e., human conjunctival cells (clone 1-5C-4) (9), with mouse serum inhibitor did not result in reduction in the number of plaques, thus indicating a species specificity. It was concluded therefore, that the serum inhibitor fulfilled the major biological and physicochemical characteristics for the definition of mouse interferon.

Reduction in Interferon Titer with Cortisone.—In a preliminary experiment, it was determined that when 5 mg of cortisone was administered 1 hr prior to attempted induction of interferon with NDV there was no suppression of the interferon response as measured in the serum collected 16 hr later. Therefore cortisone in a dose of 5 mg per mouse was administered at 6 and 24 hr prior to NDV injection. The results of a representative experiment are presented in Table I. A 16-fold reduction in the interferon titer was obtained in mice which

were pretreated at 24 hr, and an approximately 5-fold reduction in mice pretreated at 6 hr.

Effect of Cortisone Dose.—In order to determine the range of effective dosage of cortisone, as well as the maximum degree of suppression attainable with a single injection, cortisone was administered in 0.5, 2.5, 5, and 10 mg doses 24 hr prior to interferon induction with NDV. As seen in Table II, in sera collected 16 hr after induction, an 8-fold inhibition was attained both with the 5.0 and the 10.0 mg doses. Thus, the 5 mg doses provided the maximal observed effect.

TABLE I

Interferon Concentrations in Sera of Mice Pretreated with Cortisone, Compared to Untreated Mice

Time of cortisone pretreatment	Interferon concentration
hr	*units per 2 ml*
No cortisone	16,000
−6	2,700
−24	1,000

TABLE II

Effect of Different Doses of Cortisone on Suppression of Interferon in Mice

Dose of cortisone	Interferon concentration
mg	*units per 2 ml*
0	4000
0.5	1500
2.5	1000
5.0	500
10.0	500

The 2.5 mg doses resulted in a 4-fold reduction; some suppression was also evident in the 0.5 mg dose.

An estimation of the duration of the suppressive effect of cortisone on interferon production was sought in the following experiment. Cortisone (5 mg) was administered 24 hr prior to NDV injection and sera were collected at 6 and 16 hr afterwards. In the cortisone-pretreated group the interferon levels were 750 and 1000 units at 6 and 16 hr, respectively, whereas in the untreated group the corresponding sampling yielded titers of 8000 and 5140 (Fig. 2). It would appear therefore that suppression is demonstrable both at 6 and 16 hr after induction of interferon, but that the relative reduction is less at the later time period.

Suppression of Interferon in Tissue.—When tissue homogenates from mice

killed 16 hr following administration of NDV were analyzed for interferon, approximately equal concentrations were found in the lungs and spleens (Table III), while no interferon was detectable in the 10% liver extracts at a dilution of ¼. In the animals pretreated with 5 mg of cortisone (24 hr earlier) an 8-fold reduction in the interferon titer was obtained in the spleen, and about a 2-fold reduction in the lung. The interferon concentration in the sera of the corresponding groups of mice was similarly suppressed by approximately 8-fold in the cortisone-treated animals. In a confirmatory experiment, when 10 mg of

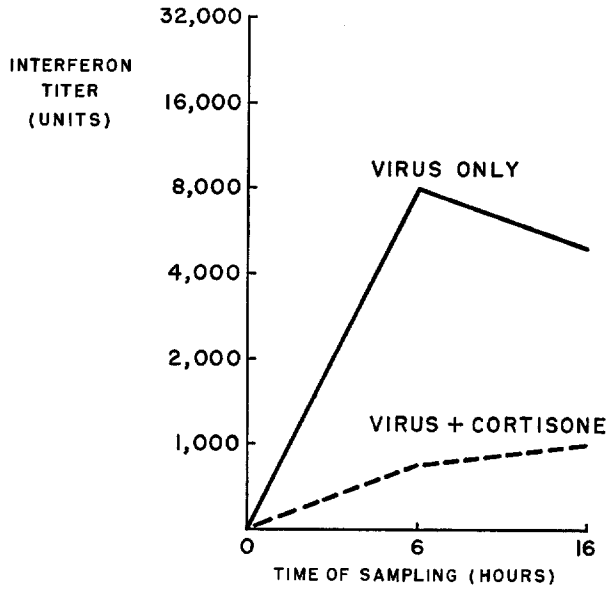

FIG. 2. The effect of pretreatment with cortisone on interferon induction in mice by NDV injected at time 0. Solid line, untreated mice. Dashed line, mice pretreated with 5 mg of cortisone 24 hr prior to interferon induction.

cortisone was administered 24 hr prior to injection of NDV and sera and spleen extracts obtained at 6 hr after induction were assayed, comparable but more pronounced differences were noted. In the untreated mice the serum titer was 8000 units and the spleen extract titer 1500 units, whereas in the cortisone pretreated mice the titers were 500 units and 100 units respectively.

The observation that no interferon was detectable at a ¼ dilution in the 10% liver extracts is not readily explainable. It is particularly surprising since the liver has been shown to clear most of the intravenously injected P_{32}-labeled NDV (up to 65% of injected radioactivity) whereas the spleen absorbs only minimal amounts (1.8%) (17). Others (18, 19) have reported detectable interferon in liver, but these titers were significantly lower than those found in the

other organs tested, particularly the spleen. A possible explanation may reside in the proteolytic enzyme activity of liver, which could cause inactivation of interferon *in situ*, or during preparation of the extracts for interferon assays.

TABLE III

Interferon Concentrations in Organ Extracts and Sera of Mice Pretreated with Cortisone, Compared to Untreated Mice

Specimens	Interferon concentration	
	Treatment	
	None	Cortisone
	units per 2 ml	*units per 2 ml*
Serum	7440	1000
Spleen*	768	100
Lung*	776	426

* 10% extracts (wet weight).

DISCUSSION

This study, prompted by lack of information on the effects of corticosteroids on interferon production in intact, mature animals, revealed that cortisone significantly inhibits interferon production in mice, as measured in the serum and organ extracts. Since initiation of the present study, 2 groups of investigators have reported similar results. Mendelson and Glasgow (20) found that hydrocortisone lowered Sindbis virus-induced interferon levels in sera of mice, and Tokumaru (21) noted similar effect of hydrocortisone on serum interferon induced in guinea pigs with herpes simplex virus. The importance of these observations lies in providing a possible explanation for the deleterious effect that corticosteroids are known to exert on resistance to viral infections.

For example, adult mice treated with cortisone develop fatal infections with Coxsackie B-1 virus, which is normally virulent only for newborn mice (22). Poliovirus type II infection in hamsters, normally a mild disease may become lethal (23) if cortisone is administered, and monkeys pretreated with cortisone may become more susceptible to minimal amounts of infective poliovirus (24). In man, vaccinia may become progressive (25), and varicella a serious, disseminated disease in patients on corticosteroid therapy (26). Soave has shown that administration of ACTH may reactivate latent rabies infection in guinea pigs (27). Finally, Jensen and Rasmussen have reported that "stress", possibly operating through increased endogenous corticosterone output (11), increases susceptibility of mice to VSV infection (28); and Chang and Rasmussen have recently demonstrated that stress actually results in suppression of interferon synthesis (29).

These adverse effects of corticosteroids on viral infections, could be mediated through several possible mechanisms; for example by suppressing inflammatory response (30) and antibody formation (8). The demonstration that cortisone significantly lowers interferon levels in vivo, in dosage inadequate to diminish antibody response (8) and the increasing evidence for interferon's role in resistance to viral infections (31), provides an additional mechanism to account for the lowering of resistance by corticosteroids.

SUMMARY

The administration of 5.0 mg of cortisone 6 to 24 hr prior to the intravenous injection of mice with NDV was associated with a marked reduction in the interferon response as measured in serum and spleen. Reduced concentrations of interferon following cortisone pretreatment were demonstrable from 6 to 16 hr following injection of virus.

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