AD 449730

449730

TECHNICAL MANUSCRIPT 157

EFFECT OF CORTISONE ON PROTECTION AGAINST RELATED ARBOVIRUSES IN VACCINATED MICE

AUGUST 1964

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 157

EFFECT OF CORTISONE ON PROTECTION AGAINST RELATED ARBOVIRUSES IN VACCINATED MICE

William P. Allen

Virus and Rickettsia Division
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1A012501B02801

August 1964

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this publication directly from DDC.

Foreign announcement and dissemination of this publication by DDC is limited.

ABSTRACT

Protection of mice vaccinated with Group A arboviruses against challenges with heterotypic viruses was investigated to determine whether resistance is fully manifested prior to challenge, or if such resistance develops as a result of the challenge stimulus. Cortisone acetate was administered intramuscularly in four daily doses of five mg each commencing the day before inoculation of the challenge virus to suppress the immune response to the challenge stimulus. This treatment inhibited protection against Venezuelan equine encephalitis virus (VEE) in mice previously vaccinated with Sindbis virus. Protection against Semliki Forest virus (SF) was partially inhibited by cortisone in mice vaccinated with VEE or Sindbis viruses. Homologous protection in immune mice was essentially unaltered by cortisone treatment. The data suggest that for certain cross-protection systems resistance to heterotypic viruses is manifested in the vaccinated animal as a result of the challenge stimulus and is not fully developed prior to the challenge.

Neutralizing antibodies to viruses against which the mice were vaccinated were present prior to challenge and probably accounted for the homotypic resistance, but neutralizing antibodies to heterotypic viruses were not detectable at that time. Normally, antibodies to the challenge virus appear in the serum within four days after the challenge is administered. Cortisone delayed the appearance of these antibodies by at least one day.

EFFECT OF CORTISONE ON PROTECTION AGAINST RELATED ARBOVIRUSES IN VACCINATED MICE

Adult mice vaccinated with Sindbis virus by the intracerebral route readily overcome the ensuing infection and develop resistance to peripheral challenges with Venezuelan equine encephalitis virus or Semliki Forest virus.* These and various other systems of cross-protection among related arboviruses have not been explained by the virus neutralizing capacity of the serum of the resistant host.[1] In view of this, studies were initiated to investigate the mechanism by which vaccinated mice resist challenge with heterotypic viruses.

One of the fundamental questions that needed answering was whether the vaccinated host possesses a fully developed immunity at the time that the heterotypic challenge virus is administered, or if this immunity arises as a result of the challenge stimulus. To resolve this question cortisone was administered to mice concurrently with the heterotypic challenge virus in order to suppress any immune response that otherwise might develop from the challenge stimulus.

The arboviruses used in these studies were all members of Casals' Group A[2] and included the following: (a) strain AR 339 of Sindbis virus; (b) the prototype strain of Semliki Forest virus (SF virus); (c) the virulent Trinidad strain of Venezeulan equine encephalitis virus (VEE virus); (d) an attenuated strain of VEE virus developed by Dr. Hearn of our laboratories;[3] and (e) the Oklahoma strain of Western equine encephalitis virus.

Young adult, Swiss albino mice from the Fort Detrick mouse colony served as hosts for all cross-protection systems studied.

In initial experiments two groups of mice were vaccinated. One group was inoculated with 70,000 suckling mouse LD_{50} of Sindbis virus by the intracerebral route. The second group received 3000 suckling mouse LD_{50} of attenuated VEE virus by the intraperitoneal route. A third group of mice remained unvaccinated. Four weeks later these groups were divided; half of each group was treated with cortisone while the other half was not. Cortisone acetate, U.S.P., was administered intramuscularly to mice in four daily doses of five mg each commencing the day before the mice were inoculated with the challenge virus. The challenge consisted of 2500 LD_{50} of VEE virus administered by the intraperitoneal route.

The summation of the results of three similar experiments are presented in Table I.

---

* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

TABLE I. HOMOTYPIC AND HETEROTYPIC RESISTANCE TO VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN MICE

Group	Cortisone[a] Treatment	Results of Challenge[b] With VEE Virus, Survivors/Total
Sindbis Immune	Yes	0/24
Sindbis Immune	No	21/24
VEE Immune	Yes	8/10
VEE Immune	No	10/10
Nonimmune	Yes	0/27
Nonimmune	No	0/28
Nonimmune-Unchallenged	Yes	17/20

a. 5.0 mg cortisone acetate per mouse for four days beginning one day before challenge.
b. $10^{3.4}$ IP-LD_{50} of VEE virus (Trinidad strain) inoculated by intraperitoneal route.

It was evident that in this heterotypic system of Sindbis immunization and VEE-virus challenge, resistance was completely suppressed by cortisone. It was also apparent that the homotypic resistance of VEE-immune mice was only slightly altered by the cortisone treatment. All nonimmune mice succumbed to VEE-virus challenge regardless of cortisone treatment, and only 3 of 20 nonimmune mice that received cortisone but were not challenged succumbed to nonspecific causes during the 28-day observation period. Pooled serum was obtained from each group of mice prior to challenge and assayed for neutralizing antibodies. Only serum from the VEE-immunized mice was capable of neutralizing the challenge virus when assayed by the constant serum-virus dilution technique and intraperitoneal inoculation into young adult mice.

Additional systems of cross-protection were studied in a similar way. Three separate groups of mice were vaccinated with Semliki Forest, Sindbis, or VEE viruses as follows: Group 1 was given three inoculations five days apart of Semliki Forest virus that was inactivated with beta-propriolactone.[1] Groups 2 and 3 were vaccinated with Sindbis or attenuated VEE virus respectively, as previously described. A fourth group included unimmunized mice.

Four weeks later half of each group was treated with cortisone as described above, and on the second day of cortisone treatment the mice were challenged with 3000 LD_{50} of SF virus by the intraperitoneal route.

Again, cortisone had little effect on homologous resistance to SF virus as indicated from the results presented in Table II. This was to be expected, because neutralizing antibodies for SF virus were already present in the mice at the time they were challenged.

TABLE II. HOMOTYPIC AND HETEROTYPIC RESISTANCE TO SEMLIKI FOREST VIRUS IN MICE

Group	Cortisone[a] Treatment	Results of Challenge[b] With SF Virus	
		Survivors/ Total	Mean Time to Death, days
SF Immune	Yes	8/10	7.0
SF Immune	No	10/10	-
Sindbis Immune	Yes	5/10	5.6
Sindbis Immune	No	9/10	9.0
VEE Immune	Yes	5/10	6.4
VEE Immune	No	9/9	-
Nonimmune	Yes	0/10	5.9
Nonimmune	No	1/10	8.2

a. 5.0 mg cortisone acetate per mouse for four days beginning one day before challenge.
b. $10^{3.6}$ IP-LD_{50} of SF virus inoculated intraperitoneally.

It was also observed that cortisone was not as effective in suppressing heterotypic resistance when SF virus rather than VEE virus was used as the challenge agent. Without cortisone treatment Sindbis-vaccinated mice were fairly resistant to SF virus in that nine of ten mice survived challenge. Cortisone reduced this resistance, but five of ten mice still survived. Similar results were observed among mice immunized with VEE virus. In both vaccinated and nonimmune groups of mice, cortisone substantially reduced

the mean time to death among those animals that succumbed to the challenge, as indicated in the column on the right. It should be mentioned here that neutralizing antibodies to SF virus were not detectable before challenge in either Sindbis-vaccinated or VEE-vaccinated mice.

Still another system of cross-protection was incorporated into these studies. In this system mice were vaccinated with Sindbis virus as in the previous experiments and challenged after four weeks with 700 LD_{50} of Western equine encephalitis virus by the intraperitoneal route. At the time of challenge antibodies were present in the sera of Sindbis-vaccinated mice that cross-neutralized Western equine virus. Cortisone, administered as previously described, had no effect on the heterotypic resistance of this system.

In summarizing the results of these studies it appears that a delicate balance exists between a challenge virus and the host with respect to some systems of cross-protection. In Sindbis-immune mice that were challenged by a peripheral route with VEE virus, the balance was weighted in favor of the host. Cortisone apparently negated this advantage. Heterotypic resistance was suppressed by cortisone but homotypic resistance was not. By inference from these results, it seems plausible to assume that resistance was incomplete at the time of challenge in the heterotypic system. Presumably, the challenge itself stimulated an immune response to the heterotypic virus and it was this response that was shown to be suppressed by cortisone. It has recently been determined that neutralizing antibodies to the heterotypic challenge virus appeared in the serum three to four days after the challenge and that cortisone delayed the appearance of these antibodies by at least one day. Possibly, the mode of action of cortisone in suppressing resistance was through its suppression of antibody formation.

The partial inhibition of cross-protection that cortisone affected in Sindbis-immune and VEE-immune mice challenged with SF virus is not presently understood. The difference between partial inhibition and complete inhibition by cortisone may be merely quantitative, but it may also be a reflection of the greater virulence that VEE virus possesses for mice than SF virus. It seems reasonable to assume that cortisone could tip the balance of resistance more easily when a highly virulent virus is used for challenge than when one of less virulence is used.

LITERATURE CITED

1. Allen W.P. "Cross-protection among group A arthropod-borne viruses," Bacteriol. Proc. pp 152, 1962.

2. Casals, J. "The arthropod-borne group of animal viruses," Trans. N.Y. Acad. Sci. Ser. II, 19:219-235, 1957.

3. Hearn, H.J., Jr. "Cross-protection between Venezuelan equine encephalomyelitis and Eastern equine encephalomyelitis virus," Proc. Soc. Exptl. Biol. Med. 107:607-610, 1961.