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Local Antibody Response to Experimental Poliovirus Infection in the Central Nervous System of Rhesus Monkeys

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By employing the techniques of immunofluorescence and radioimmunodiffusion using ^{32}P-labeled poliovirus as the antigen, the immunoglobulin response to poliovirus in serum, nasopharynx, spinal fluid, and in different segments of the central nervous system (CNS) was studied after intramuscular, oral, intranasal, and intrathalamic administration of inactivated (Salk), live attenuated (Sabin), or live virulent (Mahoney) type I poliovirus. Spinal fluid γG antibody was detected after immunization with Sabin or Mahoney virus and intramuscular administration of Salk vaccine. The response in the CNS was characterized by the appearance of γG antibody after oral or intrathalamic administration of Mahoney virus and rarely after intrathalamic inoculation of Sabin vaccine. The antibody activity in CNS was limited to the areas of poliovirus replication. Intrathalamic immunization with Mahoney virus resulted in local γG antibody production in the CNS in the absence of any detectable response in serum. Discrete foci of γG-containing cells were observed in those areas of CNS which contained poliovirus antibody. No immunoglobulin-containing cells or poliovirus antibody was seen in the CNS of monkeys immunized with intramuscularly or orally administered Sabin or Salk vaccine and in sham-immunized control monkeys. It is suggested that the CNS, when stimulated locally with a potent replicating viral antigen, may manifest a specific local antibody response, which is independent of the response in serum.

Recent studies have demonstrated that many tissues and organs of the body are able to set up a specific local immune response which is largely independent of systemic responses. Local antibody responses have been demonstrated in the mucosa of respiratory and alimentary tracts, occular tissues, mammary glands, and genitourinary tract. These studies have been reviewed in several recent publications (6, 16, 23). Although polio- and echovirus-specific antibodies have been frequently demonstrated in the spinal fluid, it is not known whether such antibodies reflect local synthesis in meninges or central nervous system (CNS) tissue (7, 10, 15). However, studies of Webb et al. (25) with louping-ill and of Connolly et al. (1) with measles antibody in subacute sclerosing panencephalitis have suggested local antibody production in CNS, and such antibody activity may be associated with γM or γG classes of immunoglobulin.

The present investigation was undertaken to characterize the distribution and formation of antibody to type I poliovirus in serum, external mucosal surfaces, spinal fluid, and CNS tissues of rhesus monkeys after infection or immunization with poliovirus administered by different routes of inoculation. In addition, an attempt was made to correlate the appearance of poliovirus antibody in brain and spinal cord with the development of identifiable immunocompetent lymphoid tissue in the central nervous system.

MATERIALS AND METHODS

Rhesus monkeys. The population of monkeys selected for the study consisted of twelve 4- to 7-month-old male rhesus monekys, who were found to be seronegative for antibody against poliovirus types 1, 2, and 3. The monkeys were housed in individual cages and had no contact with one another until the study was terminated.

Immunization. Twelve monkeys were infected with live virulent type I poliovirus or immunized with live attenuated or inactivated poliovaccine by the following schedule. Two monkeys were immunized

with inactivated trivalent (Salk) poliovaccine. Of these, one animal was immunized intranasally, and the other animal received the vaccine by intrathalamic inoculation. Four monkeys were immunized with live attenuated type I poliovirus (Sabin) vaccine. The vaccine was administered orally in two animals and intrathalamically in the remaining two. Approximately log $10^{3.5}$ per 0.1 ml of vaccine virus was administered for oral or intrathalamic infection. Six monkeys were infected with live virulent (Mahoney) type I poliovirus. Three animals received the virus by oral feeding, and the remaining three animals were inoculated intrathalamically. The dose of virulent virus employed for infection was about log $10^{3.5}$ per ml.

For intrathalamic administration of the virus, two 0.5-inch (1.27-cm) diameter burr holes, approximately 0.25 inch (0.635 cm) caudal to the frontoparietal suture and 0.333 inch (0.847-cm) lateral to mid-line, were made over the shaved off skull of the anesthetized animals. A 24-gauge hypodermic needle was inserted 0.75 inch (1.905 cm) into the brain, and 0.5 ml of normal saline containing virulent or attenuated virus was injected in each side. Two animals died within 1 week of intrathalamic immunization. One of these monkeys had received virulent virus and the other had received live attenuated virus. However, the remaining animals survived and were sacrificed 2 months after immunization or infection.

Specimens. Specimens of serum, nasopharyngeal washing, and spinal fluid were obtained before and after immunization until the monkeys were sacrificed. At this time, brain and spinal cord from each animal were carefully removed. Parts of cerebral hemisphere, medulla, thalamus, cerebellar hemisphere, and segments of spinal cord were quick frozen for immunofluorescent studies, and the remaining portions of CNS tissue were weighed and processed for virus isolation and antibody determination.

Nasopharyngeal washing and spinal fluid were collected and processed for antibody determination as described previously (15). Specimens contaminated with blood were excluded from the study.

Immunoglobulin determination. Total concentrations of γG, γA, and γM immunoglobulin in the serum, spinal fluid, and other secretions were determined by radial gel diffusion and electroimmunodiffusion as reported previously (15).

Antisera to immunoglobulins. Rabbit antisera against human γG, γA, and γM immunoglobulin were employed in this investigation. The procedure for their preparation has been described previously (24). Antisera to human immunoglobulins were used in this study since they are known to cross-react freely with monkey immunoglobulin, although they are slightly less sensitive than antisera to monkey immunoglobulin, particularly for the detection of γG and γM monkey immunoglobulins (26). Monospecificity of antisera to human immunoglobulins was tested by Ouchterlony gel-diffusion and immunoelectrophoresis by employing purified human and monkey γG, γA, and γM immunoglobulin. (Monkey immunoglobulins and their antisera were provided by Konrad Wicher, Department of Microbiology, State University of New York at Buffalo.) Only antisera found to be free of cross-contamination were employed in the study.

Immunohistological studies. The presence of immunoglobulins and the appearance of immunocompetent lymphoid tissue in the CNS tissues were studied by the direct fluorescent-antibody staining technique. Tissue specimens of cervical cord, cerebellar vermis, thalamus, occipital cortex, and other parts of the brain were prepared for cryostat sectioning by the method of Eidelman and Davis (2) and stained with fluorescein-isothiocyanate (FITC)-labeled antisera to human γG, γA, and γM as described previously (18). Consecutive sections of each tissue of CNS were stained with the three antisera. Fluorescein blocking experiments included absorption of the antisera with purified preparation of different classes of human and monkey immunoglobulins before they were reacted with the tissue sections. All sections were examined immediately with a Leitz ortholux microscope equipped with an Osram 4BG 200-W high-pressure mercury vapor lamp, UGI exitation filter, and BG-38 barrier filter. Photographs were taken on Kodak high-speed film.

The FITC-labeled antisera to human immunoglobulins used in the studies were prepared from the antisera described above. The antisera were made specific for their respective heavy chains by appropriate absorption to remove light chain proteins (22). The specificity of each antiserum was tested by immunoelectrophoresis and Ouchterlony gel-diffusion. All antisera labeled with FITC had a molar F/P ratio of 3 to 4 and were employed at a dilution which provided 0.25 to 0.5 units of activity. All FITC-labeled antisera were absorbed with rabbit liver powder and human ABO red cells before use.

Poliovirus antibody determination. Portions of cervical cord, cerebellar vermis, thalamus, and occipital cortex were weighed, and 10 g of each tissue were homogenized in 15 to 20 ml of Hanks balanced salt solution. The homogenates were centrifuged at 1,000 rpm for 45 min. The supernatant fluid was concentrated approximately 10-fold by lyophilization. The CNS tissue extracts, nasopharyngeal washings, spinal fluid, and serum were examined for the presence of poliovirus antibody activity by the tissue culture neutralization technique (12). Poliovirus-specific antibody activity in γG, γA, and γM classes of immunoglobulin in the CNS tissue, serum, and secretions was determined by radioimmunodiffusion and autoradiography employing ^{32}P-labeled type I poliovirus as the antigen (12).

Recovery of poliovirus. All tissue specimens, sera, and secretions were tested for the presence of type I poliovirus by inoculation in primary rhesus monkey kidney cell cultures. Final identification of the isolation as poliovirus was made by specific neutralization with bovine antisera to type I poliovirus (12).

RESULTS

Response to immunization with inactivated poliovirus. (i) Intramuscular immunization. The antibody response after intramuscular immunization is presented in Table 1.

Nasopharyngeal antibody activity was notably absent after such immunization. The antibody in the serum was characterized by the initial appearance of γM antibody, which was subsequently replaced by γG and to a small extent by γA antibody. Small amounts of γG antibody were detected in the spinal fluid, and its appearance seemed to correlate with the increase in γG titers in the serum. No poliovirus antibody activity was observed in CNS tissues studied (Table 1).

(ii) Intrathalamic immunization. Inoculation of inactivated poliovaccine via the intrathalamic route resulted in no detectable appearance of poliovirus antibody in the nasopharynx, serum, spinal fluid, or any of the CNS tissue tested (Table 1).

Response to immunization with live attenuated type I poliovaccine. (i) Oral immunization. The representative data obtained in one monkey after oral immunization with live attenuated vaccine are shown in Table 2. The nasopharyngeal antibody response was characterized by the appearance of γA poliovirus antibody 15 to 20 days after immunization. Such antibody activity could still be detected at 2 months when the animals were sacrificed. No γG or γM antibody activity was detected in the nasopharynx. The serum response was similar to the response obtained after intramuscular immunization with inactivated vaccine, although the γG antibody titers in the serum were somewhat higher after immunization with live attenuated vaccine. Small amount of γG antibody appeared in the spinal fluid concurrent to the increase of serum γG titer. No γM or γA antibody was detected in spinal fluid. No antibody activity was detected in the CNS tissues (Table 2). Infectious poliovirus was recovered in the nasopharyngeal washings collected 10 to 14 days after immunization. However, poliovirus was not recovered from spinal fluid or CNS tissues when the animals were sacrificed.

(ii) Intrathalamic immunization. No antibody or virus activity was observed in the nasopharynx and serum after intrathalamic immunization. Minimal though detectable antibody activity was found in the spinal fluid, cervical cord, cerebellar vermis, and thalamus 2 months after immunization when the animals were sacrificed. The antibody was found only in the γG class of immunoglobulin. No γA or γM immunoglobulin or poliovirus antibody was demonstrated in the CNS tissues (Table 2). In particular, no antibody activity was observed in the homogenates of occipital cortex.

Despite the presence of antibody in the CNS tissue, infectious poliovirus was not recovered from any of the secretions or CNS tissue homogenates studied (Table 2).

TABLE 1. *Poliovirus antibody response to parenteral or intrathalamic administration of inactivated poliovaccine in rhesus monkeys after 2 months of immunization*

Specimen	Intramuscular immunization				Intrathalamic immunization	
	Poliovirus antibody titer[a]			Recovery of poliovirus	Poliovirus antibody titer[a] (γG, γA, γM)	Recovery of polio virus
	γG	γA	γM			
Nasopharynx	<1	<1	<1	NT[b]	<1	NT
Serum	256	8	<1	NT	<1	NT
Spinal fluid	8	<1	<1	NT	<1	NT
Cervical cord						
Vermis						
Thalamus	<1	<1	<1	—[c]	<1	—
Occipital cortex						

[a] Expressed as the reciprocal of dilution.
[b] NT, Not tested.
[c] —, Undetectable in undiluted sample.

Response to infection with live virulent (Mahoney) type I poliovirus. (i) Oral infection. The antibody response in the nasopharynx and serum was similar to the response observed after oral immunization with live attenuated vaccine. However, certain quantitative differences were apparent. After infection with virulent virus, high titers of γA antibody and small amounts of γG antibody were regularly detected in the nasopharynx. The serum γG antibody response was two- to fourfold higher, and a low level of γM antibody was detectable 2 months after infection when the animals were sacrificed. The response in the spinal fluid was characterized by the appearance of high levels of γG antibody 5 to 10 days after infection. Two months after infection, the spinal fluid antibody levels were 8- to 16-fold higher, and the ratios of spinal fluid to serum γG antibody were 6- to 8-fold higher than those observed after oral immunization with live attenuated virus (Table 3). The antibody response in CNS tissues was characterized by the appearance of high titers of γG poliovirus antibody in the cervical cord, cerebellar vermis, and thalamus. After hematoxylin-eosin staining, these areas of CNS manifested histological evidence of poliomyelitis infection, with characteristic accumulation of mononuclear cells. On the other hand, no antibody activity was found in occipital cortex which conspicuously lacked histological evidence of infection (Table 3).

TABLE 2. *Poliovirus antibody response to oral or intrathalamic administration of live attenuated type I poliovirus vaccine after 2 months of immunization*

Specimen	Oral immunization				Intrathalamic immunization			
	Poliovirus antibody titer[a]			Recovery of infectious poliovirus	Poliovirus antibody titer[a]			Recovery of infectious poliovirus
	γG	γA	γM		γG	γA	γM	
Nasopharynx	<1	32	<1	+[b]	<1	<1	<1	—[c]
Serum	512	8	<1	NT[d]	<1	<1	<1	—
Spinal fluid	16	<1	<1	—	2	<1	<1	—
Cervical cord	<1	<1	<1	—	4	<1	<1	—
Vermis	<1	<1	<1	—	8	<1	<1	—
Thalamus	<1	<1	<1	—	8	<1	<1	—
Occipital cortex	<1	<1	<1	—	<1	<1	<1	—

[a] Expressed as reciprocal of dilution.
[b] Virus recovered in the nasopharyngeal washing at 10 to 14 day after immunization.
[c] —, Undetectable in undiluted sample.
[d] NT, Not tested.

TABLE 3. *Poliovirus antibody response to oral or intrathalamic administration of live virulent (Mahoney) type I poliovirus in rhesus monkey after 2 months of infection*

Specimen	Oral infection				Intrathalamic infection			
	Poliovirus antibody titer[a]			Recovery of infectious poliovirus	Poliovirus antibody titer[a]			Recovery of infectious poliovirus
	γG	γA	γM		γG	γA	γM	
Nasopharynx	8	64	<1	+[b]	<1	8	<1	—[c]
Serum	1,024	16	4	+[b]	16	<1	<1	—
Spinal fluid	64	<1	<1	+[b]	32	<1	<1	+[b]
Cervical cord	128	<1	<1	+	64	<1	<1	+
Vermis	512	<1	<1	—	128	<1	<1	+
Thalamus	128	<1	<1	+	128	<1	<1	+
Occipital cortex	<1	<1	<1	—	<1	<1	<1	—

[a] Expressed as reciprocal of dilution.
[b] Virus recovered 10 days after infection.
[c] —, Undetectable in undiluted sample.

Poliovirus was recovered from the specimens of serum, nasopharyngeal washing, and spinal fluid collected approximately 10 days after oral infection. In addition, the virus was recovered from cervical cord and thalamus when the animals were sacrificed. The virus could not be recovered in the tissues of cerebellar vermis and occipital cortex (Table 3).

(ii) Intrathalamic infection. The response to intrathalamic infection with live virulent virus was characterized by the detection of low levels of γA antibody in the nasopharynx of one monkey 14 days after infection. However, such antibody activity was not demonstrated after 2 months. The remaining monkeys failed to elicit any nasopharyngeal antibody response. Little or no antibody activity was observed in the serum. Only minimal γG response was observed in the serum of one monkey, and the serum response was conspicuously absent in the remaining monkeys. The antibody response in spinal fluid and CNS tissue was characterized by the appearance of high levels of γG antibody. Highest titers of poliovirus antibody were seen in cerebellar vermis and thalamus, and no antibody activity was detected in the occipital cortex. No γA or γM activity was observed in the spinal fluid or CNS tissues tested.

Infectious poliovirus was regularly recovered in spinal fluid, cervical cord, cerebellar vermis, and thalamus. However, no virus was recovered from nasopharynx, serum, and occipital cortex (Table 3). The recovery of virus from CNS tissue was confined to the susceptible areas which manifested histological evidence of infection and the development of poliovirus antibody.

Immunohistological localization of immunoglobulin-staining cells in CNS. Hematoxylin-eosin staining of CNS tissue sections and cellular sediments of spinal fluid demonstrated the presence of a large number of mononuclear

cells. Many of the cells morphologically resembled lymphoid or plasma cells. The tissue sections containing the cellular infiltrates were stained with FITC-conjugated antisera to human immunoglobulins. The distribution of immunologically reactive cells observed in CNS tissue after infection is shown in Table 4.

No immunoglobulin-staining cells were detected in the CNS tissue after intramuscular or intrathalamic immunization with inactivated poliovaccine or after oral immunization with live attenuated virus (Table 4). After intrathalamic immunization with live attenuated poliovaccine, 10 to 20 γG-staining mononuclear cells were observed per 10 high fields of cervical cord, cerebellar vermis, and thalamus. No immunoglobulin-staining cells were found in the cellular sediment of spinal fluid or in the sections of occipital cortex. Oral or intrathalamic infection with live virulent virus resulted in the appearance of approximately 45 to 160 γG immunoglobulin-staining cells per 10 high-power fields in the CNS tissues. Such cells were most frequently observed in the cervical cord, thalamus, and vermis (Fig. 1). Occasionally, γG-staining cells were observed in the cell sediments of spinal fluid. Approximately similar numbers of immunoglobulin-staining cells were observed after oral or intrathalamic infection with live virulent virus, although the highest number of such cells was seen in the thalamus of monkeys infected via the intrathalamic route (Table 4). Immunoglobulin-staining cells were rarely observed in the sections of occipital cortex. Staining for γA and γM immunoglobulin in the CNS tissue and spinal fluid was notably absent.

DISCUSSION

The serum and nasopharyngeal antibody response in rhesus monkeys after oral infection with live virulent or live attenuated poliovirus and after parenteral immunization with inactivated poliovirus reported here is strikingly similar to the response observed after natural or induced poliovirus infection or immunization in man (12, 15, 16). The regular appearance of nasopharyngeal γA antibody appears to be a function of oral immunization with live poliovirus, and such response was notably absent after parenteral or intrathalamic immunization with inactivated vaccine. Although a predictable antibody response in γM, γG, and frequently in γA classes of immunoglobulin was observed in the serum, poliovirus antibody response in the spinal fluid was essentially confined to γG immunoglobulin. The appearance of γG poliovirus antibody in the spinal fluid closely paralleled the development of increasing γG antibody titers in the serum after oral infection or parenteral immunization with inactivated virus. These data suggest that, under the conditions of infection or immunization listed above, the spinal fluid γG antibody may largely be derived from serum γG immunoglobulin as a result of direct transport.

The observations of particular importance are: the appearance of poliovirus-specific γG antibody in the CNS tissues and spinal fluid after intrathalamic infection with virulent or attenuated poliovirus and after oral infection with live virulent virus, and the demonstration of infectious virus in the spinal fluid and CNS tissues in the absence of a detectable serum antibody response or viremia after intrathalamic infection with live virulent virus, and infrequently after intrathalamic immunization with live attenuated vaccine.

The CNS tissue is considered to be an immunologically privileged site, which has been found to respond poorly when challenged with a locally introduced antigen (4, 5). This hypothe-

TABLE 4. *Distribution of cells staining for γG immunoglobulin in the central nervous system of monkeys 2 months after parenteral, oral, or intrathalamic administration of inactivated, live attenuated, or live virulent poliovirus*

Poliovirus type	Route of administration	Average no. of γG immunoglobulin-staining cells observed per 10 high-power fields				
		Spinal fluid	Cervical cord	Vermis	Thalamus	Occipital cortex
Inactivated	Intramuscular	0	0	0	0	0
	Intrathalamic	0	0	0	0	0
Live attenuated type I	Oral	0	0	0	0	0
	Intrathalamic	0	10	10	20	0
Live virulent type I	Oral	5	45	55	60	4
	Intrathalamic	10	50	120	160	6

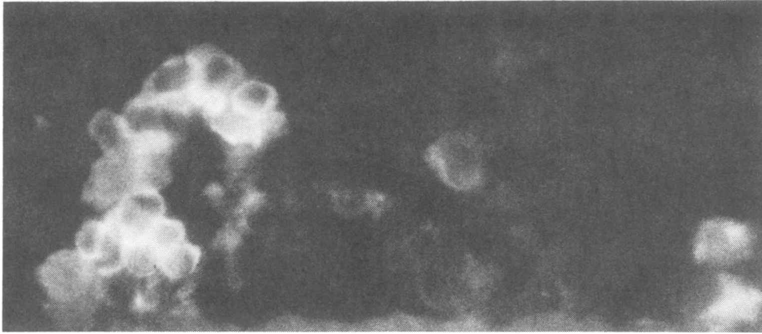

FIG. 1. *Immunofluorescent detection of γG immunoglobulin-staining cells in the cerebellar vermis, after intrathalamic inoculation of live virulent (Mahoney) type I poliovirus in rhesus monkey. A tissue section of vermis was reacted with fluorescein-conjugated rabbit antiserum to human γG immunoglobulin. Note specific γG immunoglobulin staining of a collection of cells. Reactions with fluorescein-conjugated antiserum to γA and γM immunoglobulin failed to demonstrate any cellular staining. Replicate sections were stained with fluorescein-conjugated antiserum to γG immunoglobulin absorped with purified γG. No immunoglobulin staining was observed after such blocking experiments.*

sis has been supported by earlier observations which have failed to demonstrate histological evidence of graft rejection response after implantation of homologous skin graft into the brain (3, 8). On the other hand, recent investigations have demonstrated rejection of primary and secondary sets of tumor grafts in nonisologous mouse strains (19–21). In addition, the classic experiments of Morgan about 30 years ago clearly demonstrated that monkeys injected intracerebrally with poliomyelitis virus have high titers of poliovirus-neutralizing substances in the CNS tissue, at a time when little or no such activity was found in the blood (9–11). Such antiviral activity must be presumed to be viral-specific antibody. However, the exact origin of such antibody and the distribution of specific immunoglobulin classes with which antiviral activity is associated could not be defined at that time.

The development of a viral-specific antibody response in the mucosal tissues of respiratory, gastrointestinal, and genitourinary tract, the mammary gland, and the conjunctiva appear to be determined by the local stimulation of mucosal immunocompetent tissue by the available antigen (13, 14, 17). The observations presented here suggest that select tissues of CNS which are susceptible to infection with poliovirus are able to produce specific poliovirus antibody in response to a local challenge with replicating poliovirus. A specific CNS antibody response and accumulation of immunoglobulin-staining cells were regularly observed after intrathalamic inoculation of live virulent virus, and less frequently after live attenuated virus. The absence of such a response after intramuscular immunization with inactivated virus or administration of attenuated vaccine, and the conspicuous lack of antibody response in occipital cortex and other nonsusceptible areas, regardless of the virus type and route of administration, strongly suggest that the production of poliovirus antibody in the CNS may be determined by the availability of sufficient immunologically active poliovirus antigen to the susceptible areas of CNS. However, although the predominant local antibody response in most external surfaces and mammary glands is associated with γA immunoglobulin, the local response in CNS was limited to γG immunoglobulin. No γM or γA antibody activity was detected in any CNS tissue studied.

It is well known that the CNS of most mammalian species including man are essentially devoid of any organized lymphoid structures or identifiable immunocompetent tissue (6). As a result, the mechanism underlying the local appearance of poliovirus-specific antibody and γG immunoglobulin-staining cells in CNS remains to be precisely defined. However, two possible explanations may merit special consideration. Mononuclear cells predominantly of lymphoid series are present diffusely in the brain tissue for 2 to 3 weeks after paralytic poliomyelitis infection. Subsequently, the cellular infiltrate may persist as conspicuous perivascular cuffing for several months (16). It is suggested that these cellular infiltrates mobilized from the blood stream to the areas of antigenic challenge may include a large number of circulating immunocompetent cells, which could be responsible for local antibody production. Alternatively, it is tempting to postulate that glial tissue of the central nervous system may contain precursor immunocytes which,

because of the relative inaccessibility of CNS to environmental antigens, may not manifest immunological activity under physiological conditions. Although no direct experimental evidence is available to support the hypothesis, it is conceivable that, under certain pathological conditions (e.g., stress of highly potent antigenic stimulus), these cells may exhibit varying degrees of immunological function.

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