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Mechanism of Mucosal Immunity to Viral Infections in γA Immunoglobulin-Deficiency Syndromes[1] (37900)

PEARAY L. OGRA,[2] PETER R. COPPOLA, MARGARET H. MACGILLIVRAY, AND JUDITH L. DZIERBA

Departments of Pediatrics and Microbiology, School of Medicine, State University of New York at Buffalo 14214

Considerable evidence has accumulated to suggest that viral specific secretory antibody present at external mucosal surfaces determines the outcome of a reinfection challenge with a virulent or attenuated virus (1–3). After naturally acquired or vaccine-induced viral infections, specific antibody activity has been shown to appear in the mucosal surfaces, and such activity is predominantly associated with locally produced γA class of immunoglobulin (4, 5). Although the presence of secretory γA antibody in respiratory and alimentary tracts is generally related to protection, a small proportion of normal human population is either markedly deficient or totally lacking γA immunoglobulin in serum and external secretions (6). Such apparently healthy subjects, and other patients with selective γA deficiency do not seem to be unduly susceptible to recurrent viral infections of the respiratory or alimentary tract (6–8). The mechanisms of mucosal defense in these subjects have not been clearly defined.

The present investigation was designed to characterize the nature and distribution of viral specific antibody response in serum and secretions of γA-deficient subjects. An attempt was also made to determine the role of γG and γM immunoglobulin against viral infections in external mucosal surfaces in γA deficiency syndromes.

Materials and Methods. Study population. The subject population studied consisted of 12 children ranging in age from 2–14 years who were seronegative for rubella and type I poliovirus antibody. Of these, four children were found to have markedly low levels of serum γA immunoglobulin. These children did not manifest any overt signs of ill health, recurrent respiratory infection, or chronic intestinal disease. Of the remaining eight children, four had total absence of γA immunoglobulin without any associated clinical disease, and four other patients were documented to have ataxia telangiectasia with absence of γA.

All patients were immunized with intranasal inoculation of 1 ml of inactivated (Salk) trivalent poliovaccine administered on 3 consecutive days as described previously (4).

After the initiation of these studies, five subjects developed naturally acquired wild rubella virus infection. However, the clinical course of their infection was uneventful with prompt recovery and no sequelae. Two subjects with isolated deficiency of γA who had acquired natural rubella infection were subsequently rechallenged with intranasal inoculation of RA27/3 live attenuated rubella vaccine,[3] 6–7 months after natural infection.

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[2] Reprint request: Dr. Ogra, Division of Virology, Children's Hospital, 219 Bryant Street, Buffalo, New York 14222.

[3] Wellcome Research Foundation, Kent, England.

TABLE 1. Serum Immunoglobulin Levels in Patients with γA Deficiency. Age Adjusted Normal Values (± Standard Deviation) are Included for Comparison.

Patient	Age yr	Clinical diagnosis	Serum immunoglobulin concentration (mg/100 ml)					
			γG		γM		γA	
			Patient	Normal	Patient	Normal	Patient	Normal
K.R.	10	Ataxia telangiectasia	1600	946 ± 124	380	69 ± 25	0	142 ± 50
S.B.	11	γA deficiency	2000	1000 ± 175	220	89 ± 20	0	148 ± 63
D.C.	8	Ataxia telangiectasia	900	960 ± 200	120	70 ± 15	0	130 ± 40
C.C.	14	Ataxia telangiectasia	1050	1250 ± 240	215	100 ± 36	0	148 ± 60
D.G.	6	γA deficiency	1150	950 ± 260	62	68 ± 18	0	128 ± 60
J.G.	2	γA deficiency	520	800 ± 200	100	60 ± 20	16	71 ± 37
K.C.	3	Ataxia telangiectasia	1500	875 ± 210	120	50 ± 18	23	75 ± 18
S.R.	5	γA deficiency	1100	900 ± 256	75	65 ± 20	40	124 ± 45
E.M.	3	γA deficiency	1200	875 ± 210	70	50 ± 18	6	75 ± 18
J.B.	5	γA deficiency	620	900 ± 256	40	65 ± 20	11	124 ± 45
A.B.	12	γA deficiency	350	1028 ± 200	110	90 ± 30	0	142 ± 41
N.C.	8	γA deficiency	1280	960 ± 200	78	70 ± 15	0	130 ± 40

Specimens. Specimens of serum and nasopharyngeal secretion were obtained from all patients before and after immunization with poliovaccine and after natural infection with rubella virus. The secretions were collected and processed for antibody determination as described previously (2). All nasopharyngeal washings were concentrated by lyophilization and adjusted to a final γG immunoglobulin concentration of 60–70 mg per 100 ml before further testing (2, 4).

Antisera to immunoglobulin. Monospecific antisera to human immunoglobulin γG, γA, and γM were obtained commercially,[4] and were tested for specificity and purity by Ouchterlony gel diffusion and immunoelectrophoresis before use. Only antisera found to be monospecific for the heavy (H) chains, and free of cross contamination were employed in the study (2).

Immunoglobulin determination. Total concentration of γG, γA, and γM immunoglobulin in serum and secretions was determined by the techniques of radial gel diffusion and electroimmunodiffusion (9).

Polio and rubella virus antibody determinations. Antibody activity against type I poliovirus and rubella virus in serum and secretions was determined by tissue culture neutralization and by hemagglutination-inhibition techniques, respectively (10) (2). Rubella and type I poliovirus specific antibody activity in γG, γA, and γM classes of immunoglobulin in serum and secretion was determined by radioimmunodiffusion and autoradiography employing P32 labeled rubella virus or type I poliovirus as the antigen as described previously (2, 11).

Results. The levels of serum γM and γG immunoglobulins were in general, within the normal age-adjusted values in all patients. The levels of serum γA immunoglobulin concentration are presented in Table I. No significant increase was observed in the preexisting serum immunoglobulin levels after intranasal immunization with inactivated vaccine or after natural rubella infection. Low levels of γA immunoglobulin were detected in the serum of five subjects (Table I). No γA was detected in the nasopharynx, although appreciable amounts of γG and γM immunoglobulins were observed in all secretions tested. Because of the dilution which oc-

[4] Melpar Laboratories, Falls Church, VA.

curred during the collection of nasopharyngeal secretions, no attempt was made to quantitate precisely the levels of immunoglobulins in the nasopharynx.

Antibody response to inactivated poliovirus. The poliovirus antibody response in the nasopharynx after intranasal inoculation with inactivated poliovaccine in six

TABLE II. Poliovirus Type I Antibody Response in Serum and Nasopharyngeal Secretions in Patients with γA Deficiency After Intranasal Immunization with Inactivated (Salk) Poliovaccine.

Patient	Days after immunization	Poliovirus Type I antibody titer[a]			
		Nasopharynx			Serum
		γG	γA	γM	(γG, γA, γM)
K.R.	0 PKVT × 3 (I.N.)[b]	<1	<1	<1	<1[c]
	10	64	<1	<1	<1
	60	16	<1	<1	<1
S.B.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	30	16	<1	<1	<1
	100	4	<1	<1	<1
D.C.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	15	128	<1	<1	<1
	170	8	<1	<1	<1
C.C.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	10	64	<1	<1	<1
	50	8	<1	<1	<1
	120	<1	<1	<1	<1
D.G.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	12	128	<1	<1	<1
	80	16	<1	<1	<1
J.G.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	15	64	<1	<1	<1
	120	32	<1	<1	<1
K.C.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	20	32	8	<1	<1
	100	4	<1	<1	<1
S.R.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	12	128	8	<1	<1
	50	16	2	<1	<1
E.M.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	20	64	8	<1	<1
	80	16	4	<1	<1
	140	<1	<1	<1	<1
J.B.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	10	8	<1	32	<1
	70	<1	<1	8	<1
	100	<1	<1	<1	<1
A.B.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	15	4	<1	64	<1
	60	<1	<1	8	<1
N.C.	0 PKVT × 3 (I.N.)	<1	<1	<1	<1
	10	32	<1	32	<1
	60	4	<1	16	<1
	100	<1	<1	<1	<1

[a] Expressed as reciprocal of dilution.

[b] Poliovaccine administered intranasally on 3 consecutive days.

[c] Undetectable in undiluted sample.

subjects with γA deficiency was characterized by the appearance of isolated γG poliovirus antibody in the nasopharynx within 8 days of immunization (Patients K.R.–J.G., Table II). Highest antibody titers were found initially, and subsequently the antibody level manifested a steady decline. Three patients manifested a detectable though minimal γA response in conjunction with γG poliovirus antibody response in the nasopharynx (patients K.C., S.R., and E.M., Table II). However, the γA response was transient and was undetectable after 30 days of immunization.

The nasopharyngeal antibody response in the remaining three patients with deficiency or total absence of γA immunoglobulin was characterized by the appearance of predominantly γM and to a lesser extent of γG poliovirus antibody after immunization with poliovaccine (Patients J.B., A.B., and N.C., Table II). The γM antibody activity appeared 8–10 days after immunization. Subsequently, the titers declined and no activity was observed after 90–100 days (Table II).

The serum γG, γA, or γM poliovirus antibody response after intranasal inoculation of inactivated vaccine was conspicuously absent in all patients studied.

Antibody response to rubella. Natural infection with rubella virus resulted in the appearance of significant γM and γG rubella antibody response in the serum. The antibody levels were quite similar to those observed in normal children after similar infection. The representative data of two children (J.B. and J.G.) are shown in Table III. The nasopharyngeal response was characterized by the appearance of γG rubella antibody 3–4 weeks after natural infection. It is interesting to note that natural rubella infection resulted in the development of persistent nasopharyngeal antibody which was detectable in stable titers for as long as 6–7 months after infec-

TABLE III. Serum and Nasopharyngeal Antibody Response After Naturally Acquired Rubella Virus Infection and Its Effect on Subsequent Intranasal Immunization with Live Attenuated RA27/3 Rubella Vaccine in Two Patients with γA Deficiency. The Data on a Rubella-Susceptible Normal (Control) Child Are Included for Comparison.

Subject	Month after infection	Rubella antibody titer[a]						Rubella virus excreted nasopharynx[b]
		Serum			Nasopharynx			
		γG	γA	γM	γG	γA	γM	
J.B.	0 N.R.[c]	<1	<1	<1	<1	<1	<1[d]	<1
	2	256	<1	4	64	<1	<1	<1
	6 RA27 (I.N.)[e]	128	<1	<1	32	<1	<1	<1
	7	64	<1	<1	32	<1	<1	<1
	10	64	<1	<1	32	<1	<1	<1
J.G.	0 N.R.	<1	<1	8	<1	<1	<1	<1
	3	128	<1	<1	4	<1	<1	<1
	6 RA27 (I.N.)	64	<1	<1	8	<1	<1	<1
	7	256	<1	4	4	<1	<1	<1
	9	128	<1	<1	32	<1	<1	<1
C.C.	0 RA27 (I.N.)	<1	<1	<1	<1	<1	<1	<1
(control)	1/2	16	<1	16	<1	<1	<1	2.5
	3	64	4	2	<1	8	<1	<1
	6	64	16	<1	<1	16	<1	<1

[a] Expressed as reciprocal of dilution.

[b] Expressed as $Log_{10/0.1}$ ml.

[c] Naturally acquired wild rubella virus infection.

[d] Undetectable in undiluted sample.

[e] Live attentuated RA27/3 rubella vaccine administered intranasally in a single dose.

tion (Table III). No γA antibody activity was detected in the serum or nasopharynx of children with deficiency or absence of γA immunoglobulin.

The effect of nasopharyngeal reinfection with RA27/3 rubella virus vaccine in two patients is shown in Table III. Patient J.B., who had a nasopharyngeal rubella antibody titer of 1:32 prior to reimmunization, manifested no increase in preexisting serum antibody titers and no demonstrable virus shedding in the nasopharynx. Patient J.G., who had low levels (1:8) of nasopharyngeal antibody, developed a 4-fold rise in preexisting serum antibody levels, although no virus excretion could be demonstrated in the nasopharynx. On the other hand, a seronegative control (C.C.) developed a predictable serum and nasopharyngeal antibody response, and shed rubella virus transiently in the nasopharynx (Table III).

Discussion. Intranasal immunization with inactivated poliovaccine in γA-deficient patients resulted in a local nasopharyngeal antibody response to poliovirus, in the absence of any detectable antibody activity in the serum. The nasopharyngeal antibody response in these patients was largely associated with γG and occasionally with γM class of immunoglobulin. The antibody activity generally disappeared within 3–4 months of immunization. The nasopharyngeal antibody acquired as a result of natural rubella infection persisted for several months (10 months to date). Of particular interest is the observation that the levels of nasopharyngeal antibody appeared to determine the outcome of intranasal reinfection challenge with RA27/3 live rubella vaccine. As shown in Table III, patient J.B., who had high levels of nasopharyngeal γG antibody, resisted nasopharyngeal reinfection. However, in the presence of low levels of nasopharyngeal antibody, intranasal rechallenge resulted in successful infection as evidenced by the 4-fold rise in preexisting serum and nasopharyngeal antibody levels (patient J.G., Table III). It should be pointed out that at the time of intranasal reinfection, both patients had similar preexisting serum antibody levels.

These observations suggest that a deficiency or total absence of γA immunoglobulin system may be effectively compensated by local mucosal production of viral-specific γG and infrequently γM class of antibody in the nasopharynx and respiratory tract, particularly after local infection or immunization with a replicating or inactivated viral antigen.

Several recent investigations have clearly demonstrated that γA-deficient mucosal tissues in alimentary tract are quantitatively replaced by cells containing γM and to a lesser extent by γG cells (12–15). Although the immunohistologic characteristics of γA deficient respiratory mucosa have not been studied in detail, the content of γM in salivary secretions is frequently elevated (12). Savilahti (12) and Savilahti, Pelkonen, and Visakovpi (15) has reported that γG concentration in salivary secretions may often be higher than the concentration of γM immunoglobulin. However, studies of Brandtzaeg (16) have suggested that salivary γG in γA deficiency syndromes may be derived largely from the serum, rather than produced locally. Therefore, it has been suggested that γM and γG immunoglobulins in the secretions of γA-deficient patients may replace the biologic functions of secretory γA in the gastrointestinal and respiratory tracts, although their specific role in local immunity against bacterial or viral infections has not been examined.

The present report provides evidence for local induction of γG and occasionally γM viral specific antibody in the nasopharynx, with little or no detectable contribution from serum immunoglobulin components. These data suggest that the primary compensatory mechanism in the respiratory tract and nasopharyngeal mucosa in γA deficiency may be the replacement by γG-containing cells and local production of viral specific γG antibody. The relative proportion of γG, γA, and γM immunoglobulin containing cells in normal subjects is known to vary considerably between different portions of respiratory and alimentary tracts (2). In view of the available evidence, it is suggested that the replacement tissue in the gastrointestinal tract may consist of predominantly γM class of antibody-producing

cells, while the compensatory mechanism in the nasopharynx and respiratory tract may be primarily related to γG immunoglobulin. These observations suggest a mechanism of mucosal immunity to viral infections in patients with selective deficiency of γA immunoglobulin system.

Summary. Local production of nasopharyngeal γG and γM antibody to poliovirus was demonstrated in γA-deficient patients after intranasal immunization with inactivated Salk poliovacccine. Such local response appeared in the absence of a detectable antibody response in the serum. The level of nasopharyngeal γG antibody induced as a result of natural infection with rubella virus appeared to determine the success of intranasal reinfection with live attenuated rubella vaccine, regardless of the level of antibody in the serum. These data suggest that selective γA deficiency is effectively compensated by mucosal production of γG and γM viral antibodies.

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