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Rotavirus

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ROTAVIRUS STRUCTURE AND ANTIGENS

Rotavirus Structure

Rotaviruses (RVs) form the genus *Rotavirus* of the Reoviridae family of viruses. The RV genome consists of 11 segments of double-stranded RNA and is enclosed within 3 concentric protein layers (capsids). Viral protein (VP) 2 represents the core or inner capsid, whereas VP6 constitutes the middle capsid, determines serogroup antigen specificity (A–G), and is the most immunogenic protein in RV infection. Group A, B, and C RVs all have been identified in humans, with group A RV responsible for the majority of disease (1,2). Group A RVs are differentiated by serotype, which is determined on the basis of the antigens expressed on the outer viral capsid, VP7 (a glycoprotein, which defines the G-type antigens) or VP4 (a protease-cleaved protein, which defines the P-type antigens) (1,3). Analogous to the classification of influenza viruses, RV classification is a binary system that includes both the VP4 and VP7 types (3,4). The serotype and genotype of a particular G-type antigen usually match, and therefore RVs generally are referred to by their serotype alone (eg, G1, G2, G3, etc) (1). On the other hand, because there are greater numbers of, and variation in, P-type genotypes than serotypes, concordance for P-type antigens is rare. Accordingly, P-type antigens are denoted with their genotype referenced in brackets (eg, P[4], P[8]) (1). G and P proteins induce neutralising antibodies involved in protective immunity.

The segmented nature of the RV genome allows for gene reassortment upon co-infection of a single cell with 2 different RV strains. This property has the potential to generate many combinations of G and P proteins (theoretically, 2^{11} different combinations), and thus creates serotype diversity. In practice, however, the number of G and P combinations is less than the theoretical number of possible reassortants (Table 1).

Distribution of Rotavirus Serotypes

The range of RV strains cocirculating in Europe is diverse, with predominant and emerging strains varying between regions and from year to year (2,5). At present in Europe, 5 common G/P combinations of RV predominate, namely G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] (2,5,6). There are at least 15 G types and 28 P types circulating (7–11). Analysis of strains collected between 1973 and 2003 indicates that G1P[8] was the most prevalent, being the strain responsible for 69.4% of infections in Europe (6). Surveillance for cocirculating RV strains has been initiated by the European Rotavirus Network in 11 European countries and data will be collected for the 3 years before introduction of RV vaccines and for 3 years afterward (12). The distribution

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Conflicts of interest of the working group members appear at the end of the article.

TABLE 1. *Diversity of rotavirus strains circulating in humans*

Rotavirus genotype	Possible derivation of human rotavirus strains
G1P[8] G2P[4] G3P[8] G4P[8]	Common human genotypes
G1P[4] G2P[8] G4P[4]	Reassortment among common human genotypes
G1P[9] G4P[6] G9P[8] G12P[8]	Reassortment between animal and human genotypes
G9P[6] G9P[11] G10P[11] G12P[6]	Possible zoonotic introduction

of RV G genotypes in Europe in the 2004–2005 RV season is shown in Figure 1; G1, G2, G3, G4, and G9 represented >98% of rotavirus gastroenteritis (RVGE) cases in children (5). The significance of these strains for vaccination recently was reviewed by Desselberger et al (2). All of the serotypes studied cause disease in humans, with no clear evidence of any consistent strain-specific differences in the severity of disease or the age of person affected (2,5), but several reports indicate that, on average, the duration of symptoms and severity of RVGE are greater than for non–RV-associated gastroenteritis in age-matched subjects (13,14).

DISEASE

Transmission and Pathology

RVs are most commonly transmitted via the faecal/oral route; there is also evidence to suggest that they can be transmitted in respiratory droplets (15,16). Transmission by the respiratory route does not, however, imply virus multiplication in the respiratory tract. Rather, RVs are swallowed with mucus, which also protects against gastric acidity. RVs and live oral RV vaccines are relatively acid labile, and thus need to be buffered to withstand gastric acidity (17).

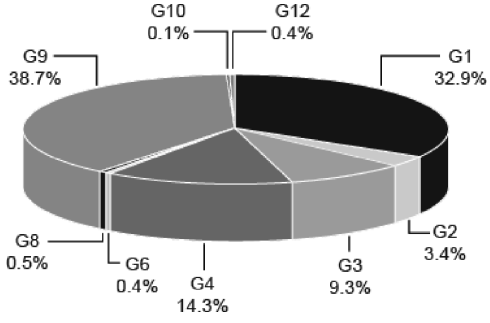

FIG. 1. The distribution of circulating rotavirus G genotypes (corrected for participation rates and sampling fraction) in 2004–2005 among children with rotavirus gastroenteritis in 7 European countries (Belgium, France, Germany, Italy, Spain, Sweden, and the United Kingdom) (5).

After ingestion, RVs infect the mature enterocytes at the tip of the villi in the small intestine. Virus particles replicate in the cell cytoplasm and are then shed, permanently damaging the cell and preventing effective uptake of fluids and nutrients. Secretory crypt cells proliferate extensively to compensate, leading to substantial fluid and electrolyte secretion into the gut lumen. Cellular dysfunction also can prevent the expression of some digestive enzymes, and owing to the increased osmotic potential resulting from the accumulation of sugars within the small intestine, may exacerbate fluid loss (18). The excess volume of fluid excreted in this manner results in the clinical manifestation of diarrhoea. The pathophysiology of RV diarrhoea is therefore a combination of osmotic and secretory mechanisms (17). The mechanism by which RV causes vomiting is not known.

It is now recognised that RV can spread from the intestine into the circulation, with RV antigen and RNA detected in blood (19). There is, however, no evidence that RV multiplies in extraintestinal sites (19). Although the clinical significance of systemic spread is largely unknown, it should be noted that RV has been detected in cerebrospinal fluid and also has been associated with central nervous system disease symptoms in case reports (20).

Other disease associations include a possible role of RV infection in the induction of 2 autoimmune diseases, type 1 diabetes, and coeliac disease. For type 1 diabetes, an Australian group first reported molecular mimicry between RV surface protein VP7 and immunodominant epitopes of 2 autoantibodies that are markers for T cell–mediated destruction of pancreatic islet cells, namely tyrosine phosphatase IA-2 and GAD65 (21). Subsequently Australian investigators found an association between RV seroconversion and increased antibody levels to IA-2, insulin, and GAD65 in children at risk for diabetes who were followed up from birth (22). However, a Finnish study failed to confirm this finding (23,24). Likewise, in coeliac disease, molecular mimicry has been reported between RV VP7 and transglutaminase. A subset of anti-transglutaminase antibodies from sera of patients with acute coeliac disease recognise RV VP7, suggesting a possible involvement of RV infection in the pathogenesis of the disease (25). Furthermore, a prospective study of US children with coeliac disease–risk human leucocyte antigen alleles followed up from infancy to 24 months of age identified multiple RV infections as a risk factor for coeliac disease autoimmunity (26).

The role of RV vaccination in relation to these newly identified, yet hypothetical, risk associations

is totally unknown. On the one hand, RV vaccination (especially repeated dosing) could be a similar risk factor to wild-type RV infection. On the other hand, RV vaccination with attenuated strains—including a mild and subclinical RV infection—will protect against severe RVGE, and therefore against the development of autoimmunity.

Symptoms and Diagnosis

A sudden onset of symptoms typically manifests in children 1 to 2 days after infection with RV. The clinical picture of RVGE is characterised by 4 to 7 days of acute febrile illness, vomiting, and watery, nonbloody diarrhoea. This combination can lead to rapid dehydration without appropriate intervention. Secondary infections with RV are clinically milder or asymptomatic (27,28).

Adults more frequently experience asymptomatic RV infection than children; however, when symptoms are reported in adults, the most common are diarrhoea, fever, headache, malaise, nausea, or cramping (27). Despite adults having milder symptoms of RV, they are still infectious, and thus can transmit the infection to susceptible children (27,29).

Laboratory diagnosis of RV infection using enzyme immunoassay involves detection of RV VP6 group A–specific antigen in diarrhoeal stools (7,30–34). Alternatively, the immunological detection of RV VP6 antigen is through the capture of antigen from faeces by anti-VP6–specific antibodies immobilised onto latex (reverse passive latex agglutination test), nitrocellulose, or nylon (rapid chromatographic assays). For enzyme immunoassay, captured antigen is detected by a second labelled anti-VP6–specific antibody and chromatographic assays, or by visible agglutination of latex particles. It is also known that demonstration of seroconversion in rotavirus immunoglobulin (Ig) A or IgG antibodies can detect more cases of RV than antigen detection. Serological diagnosis is seldom applied in the clinical setting. Antigen enzyme immunoassay detection usually is used as the laboratory determinant for a case definition of RVGE in a clinical setting, and subsequently for determination of vaccine efficacy in clinical trials of RV vaccines. However, the insensitivity of antigen detection techniques leads to an underestimation of RV disease and asymptomatic RV infection (35). Using real-time polymerase chain reaction, more RV infections will be detected due to its superior sensitivity (30,33–35), but it can be difficult to differentiate a causal association with symptoms from prolonged shedding using this method.

Outcome of Infection

Although RVGE is regarded as a self-limiting disease, the combination of watery diarrhoea, vomiting, and fever often can lead to rapid dehydration. Rehydration therapy is used for the treatment of dehydration associated with RVGE. This can be given orally, or in serious cases intravenously. In the developing world, lack of access to rehydration therapy and other medical care leads to the considerable mortality rates associated with RVGE (36). In industrialised countries, a delay in treatment can result in death, although this is rare (3,37).

Upon resolution of gastroenteritis symptoms, RV antigen is typically shed in the stools for approximately 1 week (38). However, shedding may continue for many months in immunocompromised people (39).

IMMUNITY

Although not completely understood, protective RV immunity is considered to be multifactorial, achieved through the combined action of secretory antibodies and humoral and cell-mediated immunity (40). IgA and IgG antibodies specific for structural proteins VP7, VP4, and VP6, as well as an enterotoxin called nonstructural protein 4, are involved. Quantitatively, the strongest antibody response is against VP6 (41). Possible immune correlates of protection for naturally occurring RV disease include neutralising antibodies against VP7 (G-type antigen) and VP4 (P-type antigen), as well as high levels (geometric mean anti-RV serum titre $>1{:}800$) of serum IgA antibody (largely against VP6 antigen) (16,40,42,43). However, none of these correlates—neither alone nor in combination—provide complete assurance of clinical protection.

Immune protection can be homotypic or heterotypic. In the case of RV, homotypic protection prevents infection with the same virus G-serotype, whereas heterotypic protection is associated with prevention of infection by a virus that is of a different G-type. Many RVs of different G-types express other proteins that are serologically or genotypically identical. This results from the relative lack of diversity, when compared with G-types, among P-types, VP6 subgroups, and nonstructural protein 4 genotypes circulating in the human population (Fig. 2). The relative importance of homotypic versus heterotypic protection against rotavirus infection still is not fully elucidated. The best available evidence comes from empirical observations of natural RV infections and RV vaccine trials.

Immune Protection Arising From Natural Rotavirus Infection

Natural infection with RV reduces the frequency of subsequent RV episodes, and particularly protects against clinically significant RV disease (28,42,44,45). The first study to show that asymptomatic neonatal RV infection protects against clinically severe RV diarrhoea during the first 3 years of life was published in 1983 (46). A study in Mexican children also showed that RV symptoms are the most severe with the first RV infection, and decrease in severity with increasing number of RV infections (28,42).

Virus strain	Virus protein and antigenic components				
	VP6 Group	VP6 Subgroup	VP7	VP4	NSP4
G1P[8]	A	II	G1	P[8]	B
G2P[4]	A	I	G2	P[4]	A
G3P[8]	A	II	G3	P[8]	B
G4P[8]	A	II	G4	P[8]	B
G9P[6]	A	I	G9	P[6]	A
G9P[8]	A	II	G9	P[8]	B
G10P[11]	A	I	G10	P[11]	A
G12P[6]	A	I	G12	P[6]	A
G12P[8]	A	II	G12	P[8]	B

FIG. 2. Antigens shared among common rotavirus strains. Shared antigens in columns are represented by identical shading or patterns. P[8] commonly is associated with viral protein (VP) 6 subgroup II and nonstructural protein 4 (NSP4) B, whereas P[4], P[6], and P[11] are associated with subgroup I and NSP4 A.

Two natural infections with RV provided complete protection against moderate-to-severe RV diarrhoea and 3 natural infections with RV protected against RV diarrhoea (28,42). Both symptomatic and asymptomatic infection provided comparable protection against future episodes of RVGE (28).

Immune Protection Arising From Vaccination

Early studies with candidate animal (bovine and rhesus) RV vaccines demonstrated that heterologous RVs infect the human intestine and induce similar immune protection to that observed after natural RV infection (47–52). Protective efficacy against moderate-to-severe disease was between 80% and 90% (47–52). These early candidate vaccines demonstrated proof of principle for vaccination with a live heterologous virus. Animal–human reassortant RV vaccines, as well as vaccines derived from human RV strains, may perform better than early animal vaccines because they express human RV VP7 and/or VP4 antigens. Evidence from efficacy trials of the new rotavirus vaccines indicates that protective efficacy after 2 or 3 doses of live oral RV vaccines is largely similar to that induced by 2 natural infections with RV (53).

EPIDEMIOLOGY

In developing countries, RVGE is responsible for >500,000 deaths each year, likely related to limited access to medical provisions (36,54). However, in Europe and other industrialised regions, death from RVGE is rare (3,37). In these regions, the greatest health care burden of RVGE is the large number of medical visits and hospitalisations (3,37). RV disease therefore puts considerable pressure on medical resources and the economy, as well as reducing the quality of life of infected children and their families.

Age of Infection

Primary infection with RV peaks between 9 and 23 months of age (36,55–57), with most children infected, with or without evidence of symptoms, before 5 years of age (36).

Disease Incidence in Europe

In Europe and other regions with a temperate climate, RV has a seasonal distribution, with disease peaks occurring in winter and early spring. A south to north pattern of disease incidence has been reported in Europe. The RV season reportedly begins earlier in Spain, and progressively spreads northward to France, the United Kingdom, and eventually the Netherlands and Finland over the following months (1).

Medical Burden of Rotavirus Disease

Community-acquired Infection

In European Union (EU) countries, there are approximately 25 million children younger than 5 years, and 3.6 million births per year (37). The primary burden of RV disease is on child health. It is estimated that annually in Europe almost 700,000 children younger than 5 years will visit a medical practitioner as outpatients because of RV-associated disease (37).

In day care centre attendees, the median incidence of RVGE is 1.21/1000 child-days in children younger than 4 years (Table 2) (58–62). This number is higher than the median incidence of RVGE in the general population of children under 5 years in the EU (0.43/1000 child-days) (37), or calculated prospectively for placebo recipients younger than 2 years in RV vaccine trials in industrialised countries (0.37/1000 child-days; Table 3) (47,49–51,63–74), suggesting that children who attend day care centres may be at an increased risk of being infected with RV and developing RVGE.

In Europe, children who are hospitalised with severe RVGE represent the greatest medical burden of RV infection. A recent review using methodology based on the Centers for Disease Control and Prevention model estimates that almost 90,000 children younger than 5 years will be admitted to hospital with RVGE each year in the EU. This translates to a cumulative incidence rate of 1 in 54 children experiencing a severe RV infection requiring hospitalisation by the age of 5 years (37). Infants at ages 6 to 23 months have the greatest risk for being hospitalised with RVGE (75). Using the same Centers for Disease Control and Prevention model, it is estimated that

TABLE 2. *Incidence of rotavirus gastroenteritis in day care centres in industrialised countries (58–62)*

Reference	Country	Years	Incidence of RVGE	Incidence/1000 child-days	Incidence/1000 children/y
Rosenfeldt et al (58)	Denmark	1998–1999	0.032 child-month	1.07	384
Ford-Jones et al (59)	Canada	1997–1998	1.33 child-month	44.33	15,960
Ferson et al (60)	Australia	1994	0.021 child-week	3.05	1152.51
Bartlett et al (61)	United States	1986–1987	0.22 child-year	0.60	220
Hjelt et al (62)	Denmark	1981–1982	0.440 child-year	1.21	440.37
Median				1.21	440.37

RVGE = rotavirus gastroenteritis.

200 to 250 deaths are caused each year by severe RV in children ages 5 years or younger in the EU (37). However, limited data are available to demonstrate direct evidence for the actual number of deaths occurring as a result of RVGE in the EU.

Adults exposed to infected children have a high risk for contracting RV. Although infection in adults is more frequently asymptomatic, RV is still shed in their stools and can therefore be transmitted to children (27,29). Elderly adults and people with compromised immune systems are at greater risk of developing severe symptoms of RV infection and experiencing prolonged viral dissemination (27). As a result, these individuals more often require medical attention (27).

Nosocomial Infection

Nosocomial RV infection represents a particular problem for children in Europe. Results from observational studies in European and other industrialised countries indicate the rate of incidence of RVGE during hospitalisation is 7.0 to 15.8/1000 child-days of hospitalisation for infants younger than 2 years, and 0.7 to 8.1/1000 child-days of hospitalisation for children younger than 5 years (57,76). In a systematic review of observational studies of RV disease burden in Europe, it was estimated that for every 4 children <5 years of age admitted to hospital with community-acquired RVGE, there was 1 case of nosocomial RVGE (57).

Economic Burden of Rotavirus Disease

When evaluating the overall economic burden of disease, direct costs (medical and nonmedical), indirect costs, and other costs to individuals and society should be considered (77). Direct medical costs include visits to the doctor and emergency room, hospitalisation, and the cost of laboratory diagnosis; other direct (nonmedical) costs include additional nappies (diapers), over-the-counter medicines, transportation, and child care. Indirect costs

TABLE 3. *Prospectively determined incidence of rotavirus gastroenteritis in infants up to 2 years old enrolled in control groups (placebo recipients) of randomised clinical trials of historical RV vaccines in industrialised countries (47,49–51,63–74)*

Reference	Country	Years	No. of cases of RVGE	No. of children in study	Time of observation, mo	Incidence/ 1000 child-days	Incidence/ 1000 children/y	% Children with event/ outcome
Bernstein et al (63)	US	1988–1989	26	103	22	0.38	137.69	25.2
Bernstein et al (64)	US	1989–1991	65	330	22	0.30	107.43	19.7
Bernstein et al (65)	US	1997–1998	33	107	22	0.47	168.22	30.8
Gothefors et al (50)	Sweden	1985–1986	17	52	18	0.61	217.95	32.7
Joensuu et al (66)	Finland	1993–1995	188	1207	12	0.43	155.76	15.6
Madore et al (67)	US	1988–1990	21	73	12	0.80	287.67	28.8
Ruuska et al (47)	Finland	1984–1985	40	481	12	0.23	83.16	8.3
Treanor et al (68)	US	1993	26	118	12	0.61	220.34	22.0
Vesikari et al (49)	Finland	1983	32	163	18	0.63	130.88	19.6
Vesikari et al (69)	Finland	1985–1987	16	100	24	0.22	80.00	16.0
Vesikari et al (51)	Finland	1985–1987	24	217	24	0.15	55.30	11.1
Vesikari et al (70)	Finland	1987–1989	18	120	24	0.21	75.00	15.0
Wright et al (71)	US	1984–1986	9	32	24	0.39	140.63	28.1
Bernstein et al (65)	US	1997–1998	18	107	12	0.47	168.22	16.8
Bernstein et al (72)	US	1997–1999	15	93	12	0.45	161.29	16.1
Vesikari et al (73)	Various	1998–2001	315	2839	12	0.31	110.96	11.1
Vesikari et al (second season) (73)	Various	1998–2001	88	756	12	0.32	116.40	11.6
Vesikari et al (74)	Finland	2000–2002	23	123	18	0.35	124.66	18.7
Median						0.37	134.28	17.8

RVGE = rotavirus gastroenteritis.

include loss of earnings to attend to a sick child and work time lost, and other costs can include those incurred as a consequence of side effects of disease, infection-control measures, reduced quality of life, and family disruption and stress.

A study in Finland conducted in the mid-1990s indicated that direct medical costs accounted for 89% of the total cost of RV disease and, of those, 75% were related to hospitalisation (78). In other countries, the relative importance of direct medical costs compared with nonmedical costs (including loss of work productivity and travel-related costs) may be different. In England, according to 1994 figures, nonmedical costs due to loss of earnings for caregivers were 47% of the total cost of disease, whereas National Health Service costs for general practice accounted for 28% of the total cost of disease (79). For Germany, it was estimated that 51.2% of the country's RV economic burden was related to the cost of hospitalisation, 27.4% to outpatient visits, and 21.4% to productivity losses (80).

For Europe, the mean cost per case hospitalised from RVGE was estimated to be €1216 (77,81). Multiplied by 87,313 (the estimated annual number of RVGE hospitalisations in children younger than 5 years of age in the EU 25 countries from 2000–2003) (37), the annual total cost of hospitalisation exceeds €106 million. The burden on health care resources is particularly prominent during the colder months, when the seasonal peak of RV cases coincides with a peak in incidence of other diseases such as influenza and respiratory syncytial virus bronchiolitis (77,79).

Nosocomial RV infection contributes a substantial economic burden to society owing to extended hospital stays and the cost of closing wards to new admissions to prevent further transmission of the virus (76,77,79). In France during 2001–2002, each case of nosocomial RV infection incurred an extra €1930 of direct medical costs, compared with hospital admissions that did not become infected with RV (82). The distribution of costs varies among individual countries, depending on the structure of health and social services.

CONCLUSIONS

In Europe, the estimated 700,000 annual cases of symptomatic RV disease requiring medical attention are responsible for considerable morbidity among infants and young children younger than 5 years of age. This figure translates to 1 symptomatic RV infection for every 7 children each year, and of these symptomatic cases 1 in 54 children (age $\leq$5 years) will require hospital treatment (37). With the development of vaccines against RV, RVGE has been promoted to the most common vaccine-preventable illness in infants and young children within the EU (37). This presents a new opportunity to prevent severe cases of RVGE in Europe.

Conflicts of Interest of Working Group Members

J.G. is the principal investigator and coordinator of a European rotavirus strain surveillance programme supported jointly by Sanofi Pasteur MSD and GlaxoSmithKline (GSK). He is also the principal investigator of a burden-of-disease study funded by Sanofi Pasteur MSD. For both of these activities, J.G. is funded entirely by the Health Protection Agency (HPA). He has also received travel grants and honoraria for consultancy services from Sanofi Pasteur MSD. T.V. has received honoraria for consultancy services and lectures from Chiron, Merck, GSK, MedImmune, and Wyeth. He has been the principal investigator in clinical trials for RotaShield (Wyeth-Lederle Vaccines), RotaTeq (Merck), and Rotarix (GlaxoSmithKline Biologicals). P.V.D. has been the principal investigator of vaccine studies for Merck, Sanofi Pasteur, Sanofi Pasteur MSD, GSK Biologicals, Wyeth, and Berna Biotech (a Crucell company), for which the University of Antwerp obtains unrestricted educational grants; the University of Antwerp received travel support grants and honoraria from Sanofi Pasteur MSD, Merck, and GSK Biologicals. C.G. has been the principal investigator in epidemiological studies supported by Sanofi Pasteur MSD and GSK Biologicals. He has also received honoraria for consultant services, and educational and research grants from Abbott, Bristol-Myers Squibb, Gilead, GSK, Sanofi Pasteur MSD, and Tibotec. J.M. has received honoraria for consultant services and lectures from GSK, MSD, Wyeth, Nutricia Poland, Nestlé Poland, Sanofi Pasteur Poland, and Pfizer, research grants from Nutricia and Wyeth, and financial support for scientific congresses from Nestlé Poland and GSK. A.G. is a member of the Italian Rotavirus Advocacy Committee; members of his group have received travel grants to attend meetings from companies active in the field of gastroenterology. He received research grants from Milupa, Dicofarm, and GSK. R.D. has been a scientific consultant to and a principal investigator for studies supported by Aventis Pasteur, Berna Biotech, GSK, MedImmune, Merck, Novartis, and Wyeth-Lederle Vaccines. He has also received lecture fees from GSK, Wyeth, Sanofi Pasteur, and Novartis. H.S. has received lecture fees and/or honoraria for consultant services from Nestlé, Nutricia Poland, Numico, Mead Johnson Nutritionals Poland, Mead Johnson International, Biocodex France, Danone, Crotex, Merck, Biomed Lublin, Biomed Kraków, and GSK. She has received research grants or donations from Dicofarm Italy, Nutricia Research Foundation, and Biomed Lublin, and sponsorship to attend meetings from Nestlé Poland, Danone, and GSK. V.U. has been the principal investigator of studies supported by GSK, Novartis, and Wyeth-Lederle Vaccines. He has also been a scientific consultant to Aventis Pasteur, Baxter, GSK, Merck, and Wyeth-Lederle Vaccines, and has received

sponsorship from these companies to attend scientific meetings.

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