REVIEW ARTICLE

Human immunity to rotavirus

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Summary. Rotaviruses are the most important cause of severe gastro-enteritis in infants and young children. However, the determinants of protective immunity are poorly understood. Human immunity to rotavirus can be acquired passively or actively. It may be humoral or cell-mediated, protective or non-protective, homotypic or heterotypic and mucosal or systemic, or any combination of these. Mucosal immunity is protective against rotavirus illness, but not against infection, whereas systemic immunity reflects exposure, but probably has little if any role in protection. Both local and cell-mediated immunity are likely to be important in protection. However, there is no agreement as to a reliable surrogate marker of small intestinal protective immunity, and little is known about small intestinal cell-mediated immunity in man, especially infants. Passive mucosal immunity, but not systemic immunity, may contribute to protection in breast-fed infants, and in those at increased risk of serious illness who have been given oral immunoglobulin, either as prophylaxis or therapeutically. Animal and adult studies may have only limited relevance to those who are at greatest risk of serious illness. However, it is probably from such studies that hypotheses about small intestinal cell-mediated immunity in the protection of infants against rotavirus infection and illness will come. The determinants of susceptibility to serious rotavirus infection in man remain unclear, and this continues to hinder vaccine research.

Introduction

Acute diarrhoeal diseases are of public health importance in developed and developing countries, being the largest single cause of morbidity and mortality.¹ Rotaviruses are the most important cause of severe acute gastro-enteritis in young children and animals of many species, and of childhood mortality caused by diarrhoea and vomiting in developing countries.² Re-infections after childhood are common, but symptoms, if any, are usually mild.^{2,3} Therefore, protective immunity or resistance develops, but the mechanisms responsible for this are poorly understood.³

The main aim of this review is to consider what is known about the development of humoral immunity to rotavirus in man. Cell-mediated immunity will be mentioned only briefly, as much less is known about it. The determinants of protective immunity and the factors that result in resolution of rotavirus infections are of relevance to the development of an effective rotavirus vaccine. This is of current importance as a

rotavirus vaccine able to prevent severe illness in infancy and early childhood is much needed, and reference will be made to work which could be done to expand knowledge in this field.

Virus structure, classification and antigenic composition

Rotaviruses are double-stranded RNA viruses with a genome of 11 segments in the family Reoviridae. Complete virions consist of a triple-layered protein shell surrounding the genome.⁴ Different proteins are encoded by each of the 11 genome segments.^{2,4} The most abundant protein, VP6, the major structural component of virus particles, is highly antigenic and is located in the middle layer of the protein capsid. Group and sub-group classification is based on VP6. The outer capsid is composed of two proteins, VP4 and VP7, each of which, independently, induces neutralising antibodies. Classification into serotypes is based on identification of VP7, which is the major neutralising antigen (called G-serotypes because VP7 is a glycoprotein), and VP4 (called P-serotypes because VP4 is post-translationally cleaved by proteases).⁵ Therefore, the complete antigenic description of a rotavirus strain includes the group and sub-group

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(both based on VP6), and the serotypes (based on VP4 and VP7). There are seven serogroups, A–G.² Group A strains are further subdivided into sub-groups I, II, I+II and non-I non-II, 14 G serotypes,⁵ G1–G14, and at least 11 P serotypes, P1–P11.⁶

Epidemiology and pathogenesis

Rotaviruses are transmitted by faecal-oral spread, and possibly also by the respiratory route.² Following ingestion, the virus attaches to and then replicates in the differentiated villous columnar epithelial cells (enterocytes) lining the upper small intestine. Death and desquamation of infected enterocytes follows, and this impairs the normal digestive and absorptive processes so that an acute, temporary malabsorptive diarrhoea results. The lost enterocytes are replaced initially by undifferentiated cuboidal epithelium, and diarrhoea persists until sufficient differentiated enterocytes cover the villi to allow normal digestion and absorption. The infection is limited to the small intestinal mucosa and, as with other mucosal pathogens, immunity is not lifelong and re-infections occur. Worldwide, man is mainly infected by group A serotypes G1–G4, and less commonly by serogroups B and C, whereas animals can be infected by any serogroup.

Most children have been infected by the age of 3 years, with severe illness requiring hospitalisation commonest between 6 months and 2 years. Death is uncommon in developed countries, but it is estimated that over 800 000 children aged between 1 and 4 years die each year from rotavirus infections in developing countries.⁵

Infected neonates often have no symptoms,² and those strains causing endemic asymptomatic neonatal infection have been shown to have a different VP4 from strains associated with symptoms. Follow-up of infants for 3 years after neonatal rotavirus infection has shown that subsequent rotavirus infections are less frequent and less severe than in children not infected with rotavirus as neonates.³ Therefore, neonatal infection gives some protection against subsequent symptomatic illness, but does not prevent re-infection.

After the neonatal period, most primary rotavirus infections cause symptoms, with subclinical or mild re-infections common thereafter. Asymptomatic infection in infants after the neonatal period has also been reported to be as protective as symptomatic infection, with protection lasting for at least 2 years.⁷ After early childhood, significant symptomatic rotavirus infection is uncommon except in immunocompromised patients or the elderly.⁸

Animal studies

The situation in human neonates contrasts with new-born animals, where peak susceptibility to illness

is in the first few days of life and is followed by the rapid development of resistance. VP4 and VP7 evoke antibodies that neutralise rotavirus *in vitro* and protect experimental animals *in vivo*, but their relative importance in protective immunity in man is not known. In several animal studies, the importance of antibody within the gut lumen has been shown.

In ungulates, unlike man, the structure of the placenta does not permit the transfer of immunity *in utero*.⁹ A study of gnotobiotic newborn lambs showed that feeding colostrum or serum containing antibody to rotavirus at the time of experimental virus challenge protected lambs from infection.¹⁰ Protection does not correlate with the level of serum antibodies alone in lambs,¹⁰ mice¹¹ or cows.¹²

Of animal models, only rabbits, suckling mice and gnotobiotic piglets mimic rotavirus infection in children. The homotypic and heterotypic serological and mucosal responses in the rabbit model have been studied in detail.¹³ It has been shown in a rabbit model that protective immunity against rotavirus can be induced by giving a parenteral vaccine.¹⁴ In this study, anti-rotavirus IgG, but not IgA, was detected in the intestinal lumen after immunisation, but whether the IgG originated from circulating IgG or was produced locally in the gut is not known. However, its presence was associated with protection against live virulent virus challenge and it was only after this challenge that intestinal anti-rotavirus IgA was detected.

It is not yet certain how relevant results from animal model studies are to infection with human rotavirus and disease, particularly as disease in man is not caused at a similar age. Intestinal cell-mediated immune responses are much easier to study in animal models than in man. Studies of mice have shown that, after oral or parenteral rotavirus inoculation, rotavirus-specific cytotoxic T lymphocytes (CTL) are present acutely in the intestinal mucosa, and it is possible that these CTL have an important role in surveillance and lysis of virus-infected epithelial cells.¹⁵ This is supported by the demonstration that suckling mice are protected from rotavirus gastro-enteritis after adoptive transfer of splenic lymphocytes from immunised animals,¹⁶ and that rotavirus-infected severe combined immunodeficient (SCID) mice reconstituted with immune CD8 T lymphocytes can clear chronic rotavirus infection in the absence of rotavirus-specific antibodies.¹⁷ This clearance is independent of serotype and can be mediated by CD8 T lymphocytes harvested after systemic immunisation with recombinant VP4, VP6 or VP7, the three major structural proteins, or with VP1, a putative polymerase protein.¹⁸ Again, in mice, cross-reactive CTL recognise target cells expressing VP7 better than those expressing VP4 or VP6,¹⁹ which may partly explain heterotypic protection after rotavirus immunisation.

There is also evidence in mice that active immunity following oral immunisation with rotavirus does not depend on the production of serum or intestinal neutralising antibody.²⁰ After heterologous infection

of mice with simian rotaviruses, anti-rotavirus antibodies detected by IgG and IgA ELISA and neutralisation assays in serum and intestinal contents do not correlate with rotavirus-specific responses by antibody-secreting cells in the small intestinal lamina propria.²¹ The number of rotavirus-specific plasma cells in the small intestine of the mouse varies, being highest in the duodenum and lowest in the ileum.²² Therefore, it is probable that cell-mediated responses made locally in the intestinal mucosa contribute to protection against rotavirus in animals.

Immunity in man

Overview

Local immunity in the gut lumen is also important in man, although the protective mechanisms involved are poorly understood. There have been many reports of human antibodies to rotavirus in different specimens. Most have reported serum or faecal antibodies, with fewer reporting intestinal, salivary or colostrum antibody levels. However, it is the levels in these latter fluids that are more likely to be important in protection against disease. Since natural immunity does not prevent asymptomatic or mild re-infection, in order to develop an effective immunisation strategy it is necessary to know what immunological markers indicate protection against disease in infants and young children.

Attempts have been made to relate local intestinal anti-rotavirus antibodies to serum, faecal or salivary immune responses so as to use one of these antibodies as a marker of intestinal anti-rotavirus immunity, but none has proved ideal. When studying protection against re-infection, homotypic protection is probably of longer duration than heterotypic, but the incidence of symptomatic re-infection may be influenced by the variety of rotavirus strains circulating.

Serum anti-rotavirus IgG antibodies against VP6, as determined for instance by indirect ELISA, indicate previous exposure but not protective immunity,²³ whereas IgA antibodies against VP6 in secretions reflect ability to neutralise virus and, therefore, mucosal immunity and resistance to re-infection.^{24,25} Antibodies against VP4 and VP7, as determined either by neutralising antibodies in a plaque reduction assay or by VP4- or VP7-specific blocking ELISA, also indicate protective immunity.²³ VP7 is highly immunogenic and mostly induces serotype-specific but also cross-reactive antibodies.²³ As indirect ELISA tests are technically easier to perform than neutralisation tests or epitope-blocking assays, it is not surprising that much more is known about antibodies directed against VP6 than against VP4 or VP7.

Examples of research in man are given below, but comparisons of results would be easier if standardised results were obtained, as has been suggested for anti-rotavirus IgG ELISA by worldwide distribution of an international standard serum.²⁶ A clear and concise

summary of the many studies in man is difficult because of the many variables involved. These include:

- ages studied: infants, older children, adults;
- different control populations;
- natural infection or vaccine study;
- primary infection or re-infection with the same or with a different serotype;
- symptomatic or asymptomatic infection;
- how infection is defined in non-excretors;
- specimens collected: serum, saliva, intestinal secretions, faeces;
- frequency and timing of specimens;
- method of specimen collection, storage and processing before testing;
- tests performed: IgG, IgA, IgM, secretory immunoglobulin (SIg), neutralising antibodies;
- different laboratory methods, controls and cut-offs;
- clinical outcomes sought:
 - protection from significant symptomatic primary infection;
 - protection from significant symptomatic re-infection with the same serotype;
 - protection from significant symptomatic re-infection with any serotype.

Breast milk and breast milk antibodies

If the presence of antibody locally in the small intestine at the time of exposure to rotavirus is important in preventing infection or disease, or in preventing both infection and disease, the presence of anti-rotavirus IgA and neutralising antibodies in colostrum and breast milk should also be important. However, breast-feeding does not provide total protection against rotavirus infection, and its role in protecting against disease is uncertain.²⁷⁻³⁰ Human neonates are, therefore, different from new-born animals, where the value of colostrum is known. However, colostrum from cows hyperimmunised with human rotavirus has been found to shorten rotavirus excretion in infants.³¹ Confounding factors which are not considered in all breast-feeding studies include social class, smoking and parity. Breast-feeding perhaps postpones rather than prevents life-threatening rotavirus diarrhoea. In a study of infants in rural Bangladesh, breast-feeding gave no overall protection during the first 2 years of life, although exclusive breast-feeding in the first year did protect against severe rotavirus diarrhoea.³²

The differences reported may not wholly result from specific immunity acquired passively *via* colostrum or breast milk, but from the presence of trypsin inhibitors in breast milk,³³ or a qualitative or quantitative lack of the intestinal enzymes required to activate rotavirus infectivity,³⁴ or the different VP4 of neonatal rotavirus strains.³⁵ Gastric acidity and digestive enzymes have been shown to reduce rotavirus neutralising activity of bovine colostrum immunoglobulin.³⁶ Mucin prevents experimental disease in mice,³⁷ and its presence in

human milk may inhibit rotavirus replication. IgA anti-rotavirus antibodies have been reported variously as present in all milk specimens up to 1 week post-partum except in women with selective IgA deficiency,^{38,39} in milk samples in 24 of 25 women for up to 9 (mean 3.9) months,⁴⁰ to vary in breast milk,⁴¹ and as not always being present to give any protection.⁴² Neutralising antibodies varying with time have also been reported,⁴¹ and the ranges detected to seven serotypes were all wide.⁴³ Antibodies to the infecting serotype of rotavirus may be absent or only present at low levels when breast milk appears not to be protective.⁴⁴

Mucosal antibodies following natural infection

At all mucosal surfaces, IgA is the predominant immunoglobulin isotype.²⁵ Passive antibodies from breast milk may be a source of error in these specimens, especially saliva. Anti-rotavirus IgA or IgM responses, or both, have been detected in 47–73% of salivary samples after natural infection,^{45–47} whereas copro IgA detection in convalescence is more common,^{47–58} with higher levels reported in prolonged disease or in those excreting virus.⁵⁰ However, four infections (i.e., three re-infections) may need to occur before 100% copro IgA conversion.⁵⁵ Copro anti-rotavirus IgG was generally present in a minority^{49–51} or none.⁴⁷ In uninfected, breast-fed infants whose mothers had IgA and ScIg in their milk, there was a good correlation with the presence of these antibodies in the infants' faeces, even though these antibodies were found in only a few samples of the infants' duodenal fluid;⁴⁰ the explanation for this finding is uncertain.

Antibodies in intestinal secretions have been measured in few studies of natural infection. Anti-rotavirus IgM is normally present after 7 days, but its presence is only short-lived. ScIg is usually present after 1 month, and persists for several months after the onset of illness.^{47,51,59} Variable anti-rotavirus IgG responses in duodenal fluid at 1 month, ranging from none⁵⁹ to 63%⁴⁷ have been reported. These may reflect passive transfer from serum when the serum anti-rotavirus IgG level is high. The importance of local intestinal immunity is supported by prophylactic and by therapeutic studies. Giving immunoglobulin orally to children hospitalised because of rotavirus gastroenteritis has been associated with faster recovery,⁶⁰ and has protected low birth-weight infants from contracting rotavirus diarrhoea.⁶¹

Serum antibodies following naturally-acquired infections in children

In neonates and infants, anti-rotavirus IgG and neutralising antibodies may reflect passive immunity obtained *via* the placenta rather than a response to infection. After primary infection, few neonates develop IgM,⁴⁵ whereas most older infants do.^{47,50,59,62}

However, when re-infections were studied in some of the same children, few produced IgM.⁵³ The ELISA method used may be especially important if accurate IgM and IgA results are to be obtained.⁶³

Anti-rotavirus IgA detection in children has varied from none in neonates,⁴⁵ or few,^{62,64} to most or all.^{47,48,50–52,59,65} When definite re-infections were studied, the anti-rotavirus IgA response rate was variable, being lower in the Australian study that found a lower IgM response,⁵³ but higher after second or third infections in a Sri Lankan study.⁵⁵ Variable anti-rotavirus IgG levels have also been reported, with neonates making little response,⁴⁵ while older children's convalescent sera were generally positive,^{47,50–52,55,59,62} but not always;⁶⁴ the higher IgA detection rates may have been predominantly in re-infected children. Reduced anti-rotavirus IgG has been found in more severe or prolonged infection.⁵⁰ In studies of sequential natural infections in children, protection has correlated with the level of homotypic neutralising antibody, but the duration of protection may only be short.^{66,67}

Vaccine and volunteer studies

In adults, these all represent rotavirus re-infection and are, therefore, studying the anamnestic response; the results cannot necessarily be extrapolated to infants and young children. Correlation^{68,69} and no correlation⁷⁰ between pre-challenge serum antibody and protection against experimental rotavirus infection or diarrhoea in adult volunteers have both been reported. Repeated challenge with the same virus strain after 9–12 months caused no illness and few infections; protection from infection correlated with levels of serum IgG antibodies.⁷¹ Protection against rotavirus illness or excretion has been reported to correlate with pre-challenge antibody against an epitope of VP7.⁷²

The relationship of pre-existing local neutralising activity in intestinal fluid to protection is also unclear as its absence was not a pre-requisite for the development of diarrhoeal illness,⁶⁸ but low jejunal neutralising antibody has been related to the probability of illness.⁶⁹

No relationship was found between pre-existing faecal anti-rotavirus IgA and protection from infection or illness, but rising faecal anti-rotavirus IgA in the absence of seroconversion was suggested as a reliable indicator of rotavirus infection.⁷³ Faecal anti-rotavirus IgA may be detectable for at least 1 year but, if faecal rotavirus antibody is representative of local intestinal antibody, antibody-mediated protection from re-infection may be short-lived if only high titres protect.^{69,74} An anti-rotavirus IgA response was detected in the saliva of all infected volunteers, but in none of the uninfected.⁷⁵

Many approaches to rotavirus vaccine development for infants have been tried. The aim has not been to prevent infection or mild disease, but to protect against

severe dehydrating diarrhoea.⁸ Protection does not always correlate with the serological response. In neonates, serum antibodies alone underestimate vaccine "take" and, even when serum and salivary responses are combined, the response rate only reaches 80% for bottle-fed infants.⁷⁶ Although breast-fed infants are less likely to have a serological response to rotavirus vaccines, it is not thought that this will interfere significantly with immunisation by means of a live vaccine, given orally.⁷⁷

Trial results cannot necessarily be expected to be reproducible in another country, and reviews of many trials have shown that few infants receiving a monovalent vaccine make a heterotypic antibody response.^{78,79} This finding is different from that in adults, where most individuals make both heterotypic and homotypic responses.⁸⁰ Therefore, it is now thought that a monovalent vaccine is unlikely to be successful and, instead, multiple doses in the first year—before the rotavirus season—with a multivalent vaccine containing epitopes similar to the circulating rotavirus serotypes are favoured. There are trials, some of which have been completed and others that are still in progress, with multivalent vaccines.^{78,81,82}

A completely different approach would be to immunise pregnant women with an inactivated vaccine, aiming thereby to maximise the transfer of passive immunity across the placenta and in breast milk. This approach has been tried in cows.⁹

Discussion

Despite extensive research on rotavirus, it is still not known which immunological marker or markers correlate best with protection against dehydrating rotavirus diarrhoea, as distinct from protection against rotavirus infection. Such knowledge is needed to aid vaccine research.⁵ It is agreed that local immunity in the small intestine at the time of exposure to infection is important in the prevention of serious illness. In old age, symptomatic rotavirus infection may be more common than in earlier adult life because of changes in mucosal immunity.⁸³

It is difficult in human subjects to obtain the appropriate specimens to study local immunity in the small intestine, especially local cell-mediated immunity; therefore, it is tempting to extrapolate from animal studies or to use a surrogate marker. Non-human primates would, presumably, be close to the ideal choice for these studies, but they have not as yet been used. Unfortunately, there is no ideal non-primate animal model, but it seems probable that both local small intestinal humoral immunity (IgA and neutralising antibodies) and cell-mediated immunity have a role in protection in man as well as in animals. As yet, there is no agreement about a suitable surrogate marker for small intestinal IgA or neutralising antibodies. Following infection, IgM and IgA responses

occur in the small intestine and in saliva, as well as IgM, IgA and IgG responses in serum. Thus, faecal, serum and salivary antibodies have all been suggested as surrogate markers, and some workers maintain that they are reliable. For instance, faecal IgA,^{47,84} serum IgA^{51,85,86} and faecal neutralising antibody⁸⁷ have been reported to reflect, respectively, small intestinal IgA and neutralising antibody. However, there is extensive day-to-day variation in faecal antibody levels, probably resulting from proteolysis by intestinal and bacterial enzymes as well as variable amounts of dilution in the gut, and this variation argues against faecal antibody measurements providing a reliable surrogate marker of small intestinal immunity.^{51,86,88}

It is known that the systemic and mucosal humoral immune responses to other antigens are dissociated, for example in coeliac disease.⁸⁹ Therefore, it seems probable that they are also dissociated in rotavirus infection and that measurements of serum IgA, IgM and neutralising antibodies will not reflect their levels in the small intestine because of local synthesis of these antibodies in the gut mucosa. The correlation of serum antibody levels with protection against rotavirus illness is controversial for IgA^{85,90} and for IgG.^{85,91} Serum neutralising antibodies do correlate with protection against rotavirus disease, but not against infection, and the protection may either be only to the homotypic virus⁹² or there may also be heterotypic antibodies that protect.⁹¹ In heterotypic protection, the sequence of infecting G-serotypes may be important.

With serum IgG levels, although they may reflect small intestinal IgG, since most mucosal IgG is derived from serum and as it is usually only present in the small intestine at a low level, it is unlikely to have a role in protective immunity. Therefore, it is not considered appropriate for use as a surrogate marker.⁹³

The lack of importance of mucosal IgG is further supported by the detection of rotavirus and IgG anti-rotavirus antibodies, but not IgA anti-rotavirus antibodies, in the same faecal specimens obtained from children.⁹⁴ Thus, serum IgG reflects exposure to, but not protective immunity against, rotavirus. Because serum IgG may persist after infection, it is not surprising that some investigators have found that its presence correlates with protective immunity, but this association is probably casual rather than causal.

Measurement of salivary antibodies is a possible surrogate way of testing the intestinal antibody status. Immunocompetent cells primed at one mucosal site are known to migrate and secrete antibodies at distant mucosal sites.²⁵ Salivary antibodies are not exposed to intestinal enzymes so changes in salivary antibodies may reflect intestinal mucosal response, as has been shown in studies on cholera in adults.⁷⁸ In the breast-fed infant, passively acquired immunity may cause inaccuracies. This is unfortunate since it is especially in young children that a surrogate marker in a non-invasive sample would prove helpful in furthering understanding of protective immunity to rotavirus.

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