

Serum Immunoglobulin A Response to Norwalk Virus Infection

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We describe the serum immunoglobulin A (IgA) antibody response to Norwalk virus infection in human volunteers and compare it with previously described IgM and total antibody responses. Whereas specific IgA and IgM peak within 2 weeks after onset of symptoms, titers of total blocking antibody continue to rise, implying mediation by IgG antibody.

Norwalk virus is recognized as the leading cause of outbreak-associated viral gastroenteritis in adults in the United States (7). Although total serum antibody (4) and specific immunoglobulin M (IgM) responses (2) to Norwalk virus have been described, dependence on conventional serum blocking immunoassays has limited study of other immunoglobulin classes. In this report, we introduce a newly developed IgA capture immunoassay and describe the serum IgA antibody response to Norwalk virus infection.

Sera from human volunteers challenged with Norwalk virus (8FIIa) were obtained from a study conducted at the University of Texas General Clinical Research Center in Houston (8). A total of 52 prechallenge- and convalescent-phase serum samples collected over a period of several weeks from 11 volunteers were tested for specific IgA. Five sequential serum samples collected over a longer period from an individual with possible laboratory-acquired Norwalk virus infection were also tested. In addition, 40 serum samples without Norwalk blocking activity selected from 15 non-Norwalk gastroenteritis outbreaks were tested to estimate the cutoff for a positive IgA test.

Norwalk-specific IgM was measured with a capture immunoassay as previously reported (3). Specific IgA was measured in a similar fashion with the substitution of a goat affinity-purified anti-human IgA α -chain antibody (KPL Diagnostics, Kirkegaard and Perry Laboratories) for capture antibody. In brief, capture antibody was absorbed overnight into a 96-well microdilution plate and, sequentially, patient sera, Norwalk-positive and Norwalk-negative antigen, biotinylated anti-Norwalk IgG, and streptavidin-peroxidase were added and incubated. Color was then developed with 3,3',5,5'-tetramethylbenzidine, the reaction was stopped with H₂SO₄, and the absorbance was read. Nonspecific binding of IgG antibody in both IgM and IgA assays was negligible, as determined by probing the plates with a monoclonal anti-human IgG-peroxidase conjugate.

Test results were expressed as P/N and P-N values, where P and N were the average absorbance values of duplicate wells of the test serum to Norwalk-positive and Norwalk-negative antigen, respectively. A positive cutoff for specific IgA was estimated by testing 40 Norwalk-negative serum samples; the mean P/N value was 1.45 (standard deviation, 0.66), with a range of 0.837 to 3.6. Seven serum samples (five from two outbreaks) had significantly higher P/N values, yielding a skewed distribution of values. Exclusion of these values normalized the distribution with a mean P/N value of 1.17 (standard deviation, 0.146). A cutoff ratio was selected

at the mean plus 3 standard deviations (P/N = 1.6). A positive cutoff for specific IgM had been previously determined (P/N = 1.3).

The kinetics of the IgM and IgA antibody responses were described by using P-N or attributable absorbance values. A capture IgG antibody assay could not be developed because detector antibody was derived from human convalescent-phase sera. Consequently, specific Norwalk IgG levels were interpreted from titers of total blocking antibody previously determined by a biotin-avidin immunoassay (5); results were expressed as the last reciprocal serum dilution that reduced a standard unblocked signal by $\geq 50\%$.

A fourfold-or-higher rise in total Norwalk blocking antibody titer had been previously demonstrated in 19 volunteers (Fig. 1) (4). Prechallenge antibody ($\geq 1:50$) was present in 14 of 19 volunteers (74%), and antibody was detected in all but one volunteer by 5 days postonset. Blocking titers increased from 5 days to a plateau at 23 to 24 days.

Of the volunteers who seroconverted by blocking assay, 18 had been tested for specific IgM (2). Of these, 17 (94%) developed an IgM response (Fig. 1); specific IgM was not detected in two other volunteers who failed to develop a blocking antibody response. Based on our cutoff criteria, preexisting IgM was absent in all volunteers tested but was detected in 6 of 18 volunteers (33%) by day 5 postonset. Specific IgM values peaked at 10 to 13 days and then declined over 2 weeks; one volunteer monitored for an extended period became negative for IgM by 2 months (data not shown).

In this study, nine volunteers who seroconverted by blocking assay were tested for specific IgA. All developed an IgA response, including one individual who failed to develop detectable IgM (Fig. 1); specific IgA was not detected in two other volunteers who failed to develop a blocking antibody response. Based on our cutoff criteria, preexisting IgA was detected in 5 of 9 volunteers (56%) and was present in 8 of 9 volunteers (89%) by day 5 postonset. Specific IgA values peaked at 10 to 13 days and then gradually declined over 2 weeks; IgA values remained elevated in one volunteer monitored for 2 months (data not shown). Attributable absorbance values for specific IgA were significantly higher than those for IgM. The IgA response occurred earlier, and the average decline of IgA antibody (25% by days 28 to 30) was less precipitous than that for IgM antibody (47% by days 28 to 30). Volunteers with the strongest IgA response also had the strongest IgM response but not necessarily the highest peak blocking titers. The continued rise in blocking titer beyond 10 to 13 days in the presence of declining IgM and IgA values probably represents a continued rise in specific IgG antibody.

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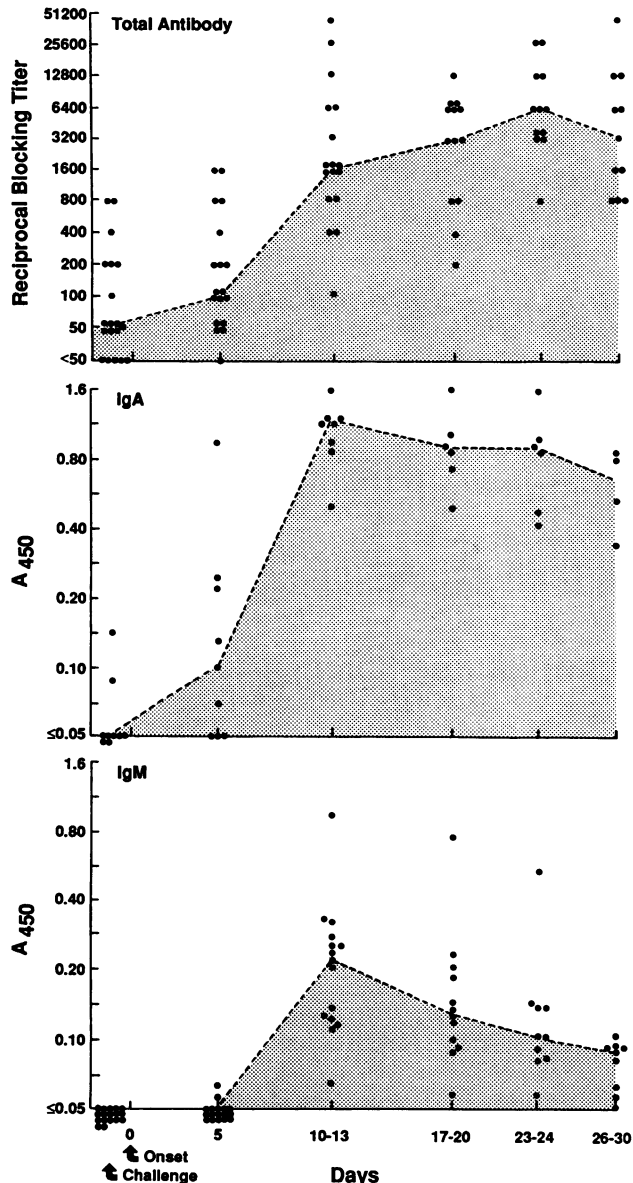


FIG. 1. Serum immunoglobulin responses of volunteers experimentally infected with Norwalk virus. Baseline serum specimens were drawn on the day of challenge, and approximately 1 day elapsed before onset of symptoms. The upper, middle, and lower panels show the subsequent total antibody (IgG), and IgA and IgM responses as measured by blocking and capture immunoassays, respectively. The kinetics of the IgA and IgM responses are described by using attributable absorbance (A_{450}) values plotted on a \log_2 scale.

Illness occurred within about 1 day of challenge in 15 of 19 volunteers (89%) who seroconverted by blocking assay, and symptoms ranged from mild diarrhea to severe diarrhea, nausea, and vomiting; four volunteers who seroconverted and two who did not were asymptomatic. Of five volunteers who seroconverted and had preexisting IgA, three (60%) developed mild-to-severe symptoms. Of four volunteers who seroconverted without preexisting IgA, two (50%) were symptomatic; two volunteers without preexisting IgA and who failed to seroconvert were asymptomatic.

Sequential serum samples from an individual with Norwalk virus infection acquired in a laboratory accident were tested for specific antibody. The first serum sample collected 2 days

after onset of illness had a total blocking antibody titer of 1:400 and specific IgA antibody but no specific IgM. At day 32, the blocking titer had risen (1:12,800), and both IgA and IgM values were elevated. Whereas blocking antibody remained stable at days 46 and 57, IgA and IgM values declined, the latter to borderline levels. At 15 months, both blocking titer (1:3,200) and specific IgA values remained elevated above initial levels, while specific IgM was no longer detectable.

A specific serum IgA response appears to be a consistent feature of Norwalk virus infection in human volunteers. The diagnostic utility of specific IgA in field specimens warrants further study; although the persistence of IgA may limit its usefulness as a diagnostic indicator with single serum specimens, it may serve as a sensitive measure of recent infection with paired serum specimens and a useful adjunct to established assays in the diagnosis of Norwalk virus outbreaks.

Earlier reports indicate an unusual pattern of immunity to infection with Norwalk virus (1, 9). When challenged, volunteers manifested either of two forms of resistance: (i) short-term immunity followed by renewed susceptibility to infection, or (ii) long-term immunity which appears related more to nonimmune factors than to a host immune response. In our study, the presence of preexisting serum IgA did not appear to be associated with resistance to infection or a lessening in severity of symptoms; this contrasts with rotavirus, in which preexisting serum IgA has been associated with milder symptoms (6). It is possible that the secretory fraction of total serum IgA would better correlate with secretory levels in the gut and with short-term immunity to rechallenge with Norwalk virus seen in some volunteers (9).

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