

ASYMPTOMATIC CARRIAGE OF *CLOSTRIDIUM DIFFICILE* AND SERUM LEVELS OF IgG ANTIBODY AGAINST TOXIN A

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**ABSTRACT**

**Background** *Clostridium difficile* infection can result in asymptomatic carriage, mild diarrhea, or fulminant pseudomembranous colitis. We studied whether antibody responses to *C. difficile* toxins affect the risks of colonization, diarrhea, and asymptomatic carriage.

**Methods** We prospectively studied *C. difficile* infections in hospitalized patients who were receiving antibiotics. Serial stool samples were tested for *C. difficile* colonization by cytotoxin assay and culture. Serum antibody (IgA, IgG, and IgM) levels and fecal antibody (IgA and IgG) levels against *C. difficile* toxin A, toxin B, and nontoxin antigens were measured by an enzyme-linked immunosorbent assay (ELISA).

**Results** Of 271 patients, 37 (14 percent) were colonized with *C. difficile* at the time of admission, 18 of whom were asymptomatic carriers. An additional 47 patients (17 percent) became infected in the hospital, 19 of whom remained asymptomatic. The baseline antibody levels were similar in the patients who later became colonized and those who did not. After colonization, those who became asymptomatic carriers had significantly greater increases in serum levels of IgG antibody against toxin A than did the patients in whom *C. difficile* diarrhea developed ( $P < 0.001$ ). The adjusted odds ratio for diarrhea was 48.0 (95 percent confidence interval, 3.4 to 678) among patients with colonization who had a serum level of IgG antibody against toxin A of 3.00 ELISA units or less, as compared with patients with colonization who had a level of more than 3.00 ELISA units.

**Conclusions** We find no evidence of immune protection against colonization by *C. difficile*. However, after colonization there is an association between a systemic anamnestic response to toxin A, as evidenced by increased serum levels of IgG antibody against toxin A, and asymptomatic carriage of *C. difficile*. (N Engl J Med 2000;342:390-7.)

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**C**LOSTRIDIUM DIFFICILE is the leading infectious cause of nosocomial diarrhea in developed countries.<sup>1-4</sup> In one study, 21 percent of patients admitted to a general medical ward became colonized with *C. difficile* and diarrhea developed in 8 percent.<sup>5</sup> *C. difficile* diarrhea and colitis result from the actions of protein exotoxins released by pathogenic strains of the organism.<sup>1,6</sup> Toxin A and toxin B are proinflammatory and cytotoxic. Toxin A is also enterotoxigenic to both animal and human intestine, whereas toxin B may have enterotoxigenic activity in the human colon.<sup>1,6,7</sup>

The clinical outcome of infection with toxigenic *C. difficile* ranges from asymptomatic carriage to mild diarrhea to fulminant pseudomembranous colitis.<sup>1,5,8,9</sup> Host, rather than bacterial, factors appear to determine these differences in clinical presentation.<sup>10,11</sup> Serum and colonic antibody responses to *C. difficile* toxins are evident in more than 60 percent of the general population.<sup>12,13</sup> There is some evidence, albeit inconclusive, that the immune response to *C. difficile* and its toxins contributes to the wide spectrum of disease presentation.<sup>14-19</sup> In one study, for example, serum levels of IgG antibody against toxin A and fecal levels of IgA antibody against toxin A were higher in patients with mild *C. difficile*-associated disease than in those with prolonged or severe diarrhea.<sup>19</sup>

We hypothesized that acquired antibody responses to *C. difficile* can protect against diarrhea induced by *C. difficile* toxins. To test this hypothesis, we conducted a prospective study of hospitalized patients who were receiving antibiotics to determine whether antibody responses to *C. difficile* and its toxins are associated with a lower risk of colonization with hospital-acquired *C. difficile* and a lower risk of diarrhea in patients with nosocomial *C. difficile* infection.

**METHODS****Study Protocol**

Consecutive patients who were admitted to either of two general medical wards at our institution between January 5, 1998, and May 22, 1998, were screened for enrollment. Patients were eligible for the study if they were receiving antimicrobial therapy and if their expected length of stay was more than two days. The study was approved by the institutional review board, and informed consent was obtained from all patients or their health care proxies.

At study entry we recorded age, sex, and information on known risk factors for *C. difficile* diarrhea and on potential confounding factors, including transfer from another health care facility; history of *C. difficile* diarrhea within the preceding year; antibiotic agents received; use of acid antisecretory drugs; type of feeding regimen (oral, tube [nasogastric, gastrostomy, or jejunostomy], or total parenteral nutrition); immunosuppression, including that induced by corticosteroid or immunosuppressive therapy; presence of renal disease, diabetes, dementia, or metastatic disease; a history of stroke; serum albumin level; score on the Charlson comorbidity index; and severity of the condition that led to hospitalization.<sup>20</sup> The last factor was assessed with use of a modified Horn's index in which the severity of disease is rated as mild (1 point), moderate (2 points), severe (3 points), or extremely severe (4 points).<sup>5,21-23</sup>

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We obtained a stool specimen for *C. difficile* culture and cytotoxin assay (or in some cases, a rectal swab for *C. difficile* culture) and a serum sample for antibody measurements at the time of enrollment, every three days during hospitalization, and on the day of discharge. Patients were monitored daily for the development of diarrhea, and if diarrhea occurred, an additional serum sample was obtained on the first day that the patient had symptoms.

### Definitions of Outcomes

Colonization with *C. difficile* was considered to have occurred during hospitalization if the culture and cytotoxin tests of stool (or rectal swabs) obtained at the time of admission were negative for *C. difficile* and any subsequent stool culture or cytotoxin test was positive. Among patients with hospital-acquired *C. difficile* infection, asymptomatic carriage was defined as a positive stool culture or cytotoxin test and the absence of diarrhea during hospitalization and during a 30-day period after discharge. Diarrhea was defined as the passage of three or more unformed stools for at least two consecutive days.<sup>5</sup> *C. difficile* diarrhea was defined as diarrhea that was not attributed to any other cause and that was associated with a positive stool cytotoxin test.

### Laboratory Studies

All laboratory studies were performed on coded specimens by investigators who were unaware of the patients' base-line characteristics, colonization status, and clinical outcome. Stool specimens and rectal swabs were transported and then stored at  $-80^{\circ}\text{C}$  as previously described.<sup>24</sup> They were later thawed, heated briefly, and then cultured for 72 hours under anaerobic conditions in selective (cycloserine, cefoxitin, fructose) broth medium.<sup>25,26</sup> Presumptive identification of *C. difficile* was made by subculturing of specimens on solid medium, examination of colony morphology, Gram's staining, and the use of a system of rapid identification based on enzymatic activity (Rapid-Ana, Innovative Diagnostics, Atlanta).

We assessed cytotoxin activity in filter-sterilized fecal supernatants using the tissue-culture cytotoxin assay.<sup>27</sup> To confirm that a cytotoxin effect was caused by *C. difficile* toxins, we mixed the fecal filtrate with neutralizing antibodies against *C. difficile* toxins according to the manufacturer's instructions (Techlab, Blacksburg, Va.). An aliquot of the fecal filtrate was supplemented with protease inhibitors (complete protease inhibitors, Boehringer Mannheim, Mannheim, Germany) and used for antibody testing.<sup>28</sup>

Toxin A and toxin B were purified from the supernatant of a culture of strain VPI 10463 (American Type Culture Collection 43255) as previously described.<sup>29,30</sup> Nontoxin antigens were prepared from a sonicate of two nontoxigenic strains of *C. difficile* (American Type Culture Collection 43597 and 43593). Levels of antibody against *C. difficile* toxin A, toxin B, and the nontoxin antigen preparation were measured by an enzyme-linked immunosorbent assay (ELISA) as previously described.<sup>13,17,19</sup> Serum samples or filter-sterilized fecal samples from a different population of patients, with high levels of antitoxin immunoglobulin (IgG, IgA, or IgM) were pooled, assigned an arbitrary value that was expressed in ELISA units, and used in all assays as standards.

### Statistical Analysis

All cutoff points for categories of variables were determined before we examined the study data. We separated antibody levels in patients with colonization into four categories on the basis of the distribution of antibody levels in the group of patients without colonization, as follows: levels below the 25th percentile were defined as low, levels ranging from the 25th to 75th percentile were defined as medium, levels ranging from the 76th to 90th percentile were defined as high, and levels above the 90th percentile were defined as very high. Age was categorized into three groups, on the basis of our knowledge of the age distribution of patients admitted to the study wards: 65 years of age or younger, 66 to 84 years of age, and 85 years of age or older. The severity of dis-

ease was dichotomized as mild to moderate (a score of 1 or 2 on Horn's index) or severe to extremely severe (a score of 3 or 4).

Antibody measurements were not distributed normally; therefore, we calculated the median levels and used Wilcoxon's rank-sum tests to assess the significance of any differences in median values between patients with colonization and patients without colonization and between patients with *C. difficile* diarrhea and asymptomatic carriers. We examined associations between other potentially predictive variables and the outcomes using chi-square tests or two-tailed Fisher's exact tests for categorical variables and Student's *t*-tests for continuous variables. We used Wilcoxon's rank-sum tests, the Kruskal-Wallis test, or Spearman correlation coefficients to assess associations between antibody levels and other variables.

Our main variables of interest were the *C. difficile* antibody levels at the time of admission and at the time of colonization. If these variables were significant in univariate analyses, we entered antibody levels (dichotomized as low, medium, or high vs. very high) into a multivariable logistic-regression model, along with age, sex, severity of disease, and the scores on the Charlson comorbidity index, since we hypothesized a priori that these might confound the immune response. Other variables associated with the outcome in univariate analyses ( $P < 0.10$ ) were also included in the multivariable model, and the adjusted odds ratios and 95 percent confidence intervals were calculated. Only confounders of antibody levels (variables that resulted in a change of more than 10 percent in the odds ratio for *C. difficile* diarrhea as compared with asymptomatic carriage) were included in the final model.

All analyses were performed with the SAS software system (version 6.12, SAS Institute, Cary, N.C.). The alpha level was set at 0.05. All *P* values were two-sided.

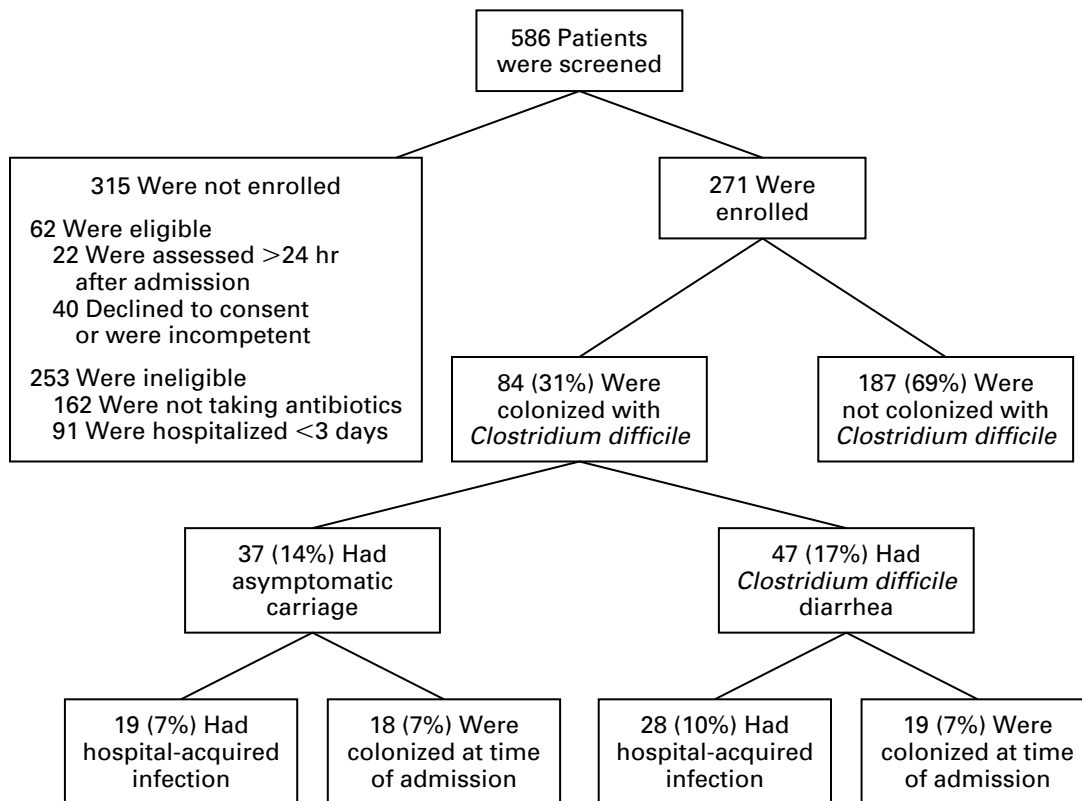
## RESULTS

### Characteristics of the Patients

The results of screening for enrollment, the reasons for nonenrollment, and the results of serial cultures of stool specimens and rectal swabs for *C. difficile* and stool cytotoxin assays are summarized in Figure 1. Of 586 consecutive patients who were hospitalized, 333 were eligible for the study and 271 of these patients were enrolled; all 271 completed the study.

Forty-seven patients (17 percent) acquired *C. difficile* infection while in the hospital. An additional 37 patients (14 percent) were found to be colonized with *C. difficile* at the time of admission. These 37 patients were excluded from all subsequent data analyses because our study was designed to examine prospectively the immune response to colonization. Cytotoxin activity was detected in the stools of all patients with *C. difficile* diarrhea and in 15 of the 19 asymptomatic carriers (79 percent). Three of the four asymptomatic carriers with negative stool cytotoxin assays were colonized with nontoxigenic strains of *C. difficile*. The *C. difficile* isolate of the fourth patient could not be recultured for the toxin assay.

The base-line characteristics of the patients are shown in Table 1. The majority were white and were older than 65 years of age; approximately one third had been admitted from nursing homes or other institutions. Most patients had serious coexisting conditions, and one third had severe or extremely severe disease (as indicated by a score of 3 or 4 on Horn's index) at the time of admission.



**Figure 1.** Results of Screening, Reasons for Nonenrollment, and Results of Serial Cultures of Stool Specimens and Rectal Swabs for *Clostridium difficile* and Stool Cytotoxin Assays.

The 18 patients who were asymptomatic carriers at the time of admission and the 19 patients with *C. difficile* diarrhea at the time of admission were excluded from all subsequent data analyses.

#### Serum Levels of Antibody against Toxin A, Toxin B, and Nontoxin Antigens

There were no statistically significant differences in median serum IgA, IgG, or IgM antibody levels against toxin A, toxin B, or nontoxin antigens at the time of admission between 47 patients who subsequently became colonized with *C. difficile* and 187 who did not become colonized.

Among the 47 patients who acquired *C. difficile* infection in the hospital, *C. difficile* diarrhea developed in 28 (60 percent) (Fig. 1). The 19 patients who were asymptomatic after infection remained so during the 30-day follow-up period after discharge.

At the time of colonization, the median serum level of IgG antibody against toxin A was significantly higher in asymptomatic carriers than in patients in whom *C. difficile* diarrhea developed ( $P < 0.001$ ) (Fig. 2). Median serum levels of IgG antibody against toxin B and against nontoxin antigens were also higher in the asymptomatic carriers than in the patients with diarrhea, but these differences were not statistically significant (Fig. 2). The median serum level of IgM

antibody against nontoxin antigens was also slightly higher in asymptomatic carriers than in the patients with diarrhea (0.29 vs. 0.21 ELISA unit,  $P = 0.03$ ). There were no significant differences between asymptomatic carriers and patients in whom *C. difficile* diarrhea developed in the median serum levels of IgA antibody against toxin A, toxin B, or nontoxin antigens; in the median serum levels of IgM antibody against toxin A or toxin B; or in the median fecal levels of IgA or IgG antibody against toxin A, toxin B, or nontoxin antigens.

Among asymptomatic carriers, but not among patients with diarrhea, serum levels of IgG antibody against toxin A were significantly correlated with serum levels of IgG antibody against toxin B ( $R = 0.49$ ,  $P = 0.03$ ) and with serum levels of IgG antibody against nontoxin antigens ( $R = 0.70$ ,  $P < 0.001$ ). Serum levels of IgG antibody against toxin A at the time of admission were also highly correlated with the levels at the time of colonization ( $R = 0.67$ ,  $P = 0.001$ ). There was no apparent association between serum levels of IgG antibody against toxin A and age,

**TABLE 1.** BASE-LINE CHARACTERISTICS OF THE PATIENTS AND THE RESULTS OF UNIVARIATE ANALYSES OF FACTORS ASSOCIATED WITH DIARRHEA IN 47 PATIENTS WITH HOSPITAL-ACQUIRED *CLOSTRIDIUM DIFFICILE* INFECTION.\*

| CHARACTERISTIC  | ALL PATIENTS<br>(N=234) | PATIENTS WITH HOSPITAL-ACQUIRED <i>C. DIFFICILE</i>        |                                    | P VALUE |
|---|-------------------------|--|------------------------------------|---------|
|   |                         | PATIENTS WITH<br><i>C. DIFFICILE</i><br>DIARRHEA<br>(N=28) | ASYMPTOMATIC<br>CARRIERS<br>(N=19) |         |
| Age   |                         |  |                                    |         |
| Mean — yr   | 74±16                   | 70±21  | 79±16                              | 0.07    |
| Range — yr  | 19–101                  |  |                                    |         |
| ≤65 yr — no. (%)  | 54 (23)                 | 11 (39)  | 2 (11)                             |         |
| 66 to 84 yr — no. (%)   | 122 (52)                | 10 (36)  | 12 (63)                            | 0.07    |
| ≥85 yr — no. (%)  | 58 (25)                 | 7 (25)   | 5 (26)                             |         |
| Male sex — no. (%)  | 94 (40)                 | 14 (50)  | 6 (32)                             | 0.21    |
| White race — no. (%)  | 198 (85)                | 24 (86)  | 17 (89)                            | 0.70    |
| Residence before admission — no. (%)†                             |                         |  |                                    |         |
| Nursing home  | 54 (23)                 | 8 (29)   | 9 (47)                             | 0.39    |
| Rehabilitation facility   | 11 (5)                  | 1 (4)  | 1 (5)                              | 1.00    |
| Other hospital  | 11 (5)                  | 5 (18)   | 0                                  | 0.14    |
| Home  | 158 (68)                | 14 (50)  | 9 (47)                             | 0.86    |
| Hospitalization within 30 days before current admission — no. (%) | 58 (25)                 | 12 (43)  | 4 (21)                             | 0.21    |
| Severity of disease (score on Horn's index) — no. (%)             |                         |  |                                    |         |
| Mild (1)  | 70 (30)                 | 3 (11)   | 4 (21)                             |         |
| Moderate (2)  | 86 (37)                 | 3 (11)   | 8 (42)                             | 0.01    |
| Severe (3)  | 50 (21)                 | 11 (39)  | 6 (32)                             |         |
| Extremely severe (4)  | 28 (12)                 | 11 (39)  | 1 (5)                              |         |
| Charlson comorbidity index — score‡                               |                         |  |                                    |         |
| Median  | 3                       | 3  | 2                                  | 0.36    |
| Range   | 0–13                    | 0–13   | 1–6                                |         |
| Time from admission to colonization — days                        | Not applicable          |  |                                    |         |
| Median  |                         | 6  | 3                                  | 0.08    |
| Range   |                         | 3–33   | 3–6                                |         |
| Admission to intensive care unit — no. (%)                        | 15 (6)                  | 8 (29)   | 0                                  | 0.01    |
| Acid antisecretory therapy — no. (%)                              | 107 (46)                | 18 (64)  | 7 (37)                             | 0.06    |
| Immunosuppression — no. (%)                                       | 59 (25)                 | 6 (21)   | 3 (16)                             | 0.72    |
| No. of antibiotics given  | 2.4±1.1                 | 2.5±1.3  | 2.3±1.0                            | 0.45    |

\*Plus-minus values are means ±SD.

†For the analysis of data on nursing homes, rehabilitation facilities, and other hospitals, the comparison is with data on “Home.” For the analysis of data on “Home,” the comparison is with the other groups.

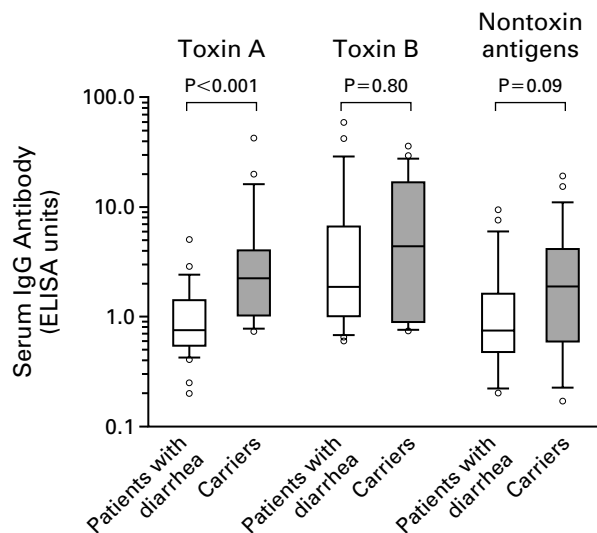
‡Higher scores indicate more serious coexisting conditions.

sex, score on the Charlson comorbidity index, severity of disease, presence or absence of immunosuppression, or serum albumin level.

**Other Variables Associated with *C. difficile* Diarrhea**

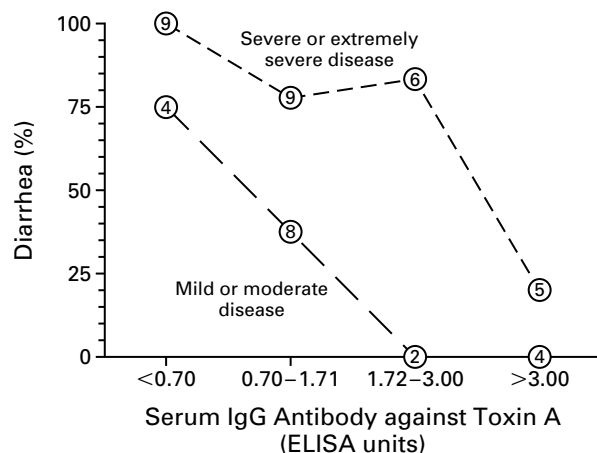
Among patients with colonization, other variables that were significantly associated with *C. difficile* diarrhea included increasing severity of disease and admission to the intensive care unit (Table 1). There was a trend for patients who were 65 years of age or younger and for those who had received acid antisecretory therapy to be more likely to have *C. difficile* diarrhea. There was no significant association between *C. difficile* diarrhea and the other variables examined.

For the multivariable logistic-regression analyses, we constructed a model to compare the risk of *C. difficile* diarrhea associated with a serum level of IgG antibody against toxin A of 3.00 ELISA units or less and a very high level (more than 3.00 ELISA units). Although admission to the intensive care unit was significantly associated with *C. difficile* diarrhea in the univariate analysis, we excluded this variable from the model because it was correlated with severe or extremely severe disease (P=0.001). In the final model, severe or extremely severe disease, as compared with mild or moderate disease, was found to be an independent predictor of *C. difficile* diarrhea (odds ratio, 7.7; 95 percent confidence interval, 1.5 to 39.9). Among patients with colonization and se-



**Figure 2.** Serum Levels of IgG Antibodies against Toxin A, Toxin B, and Nontoxin Antigens at the Time of Colonization in 28 Patients in Whom *Clostridium difficile* Diarrhea Developed and in 19 Asymptomatic Carriers.

The boxes indicate the 25th percentiles, median values, and 75th percentiles; the I bars indicate the 10th and 90th percentiles; and the circles indicate outlying values. Antibody levels are expressed in arbitrary ELISA units on a logarithmic scale. Median serum levels of IgG antibody against toxin A were significantly higher in carriers than in the patients in whom *C. difficile* diarrhea developed ( $P < 0.001$ ).



**Figure 3.** Relation between Serum Levels of IgG Antibody against Toxin A and *Clostridium difficile* Diarrhea in 47 Patients with Colonization, According to the Severity of Disease at Admission.

Serum levels of IgG antibody against toxin A were categorized as low (<0.70 ELISA unit), medium (0.70 to 1.71 ELISA units), high (1.72 to 3.00 ELISA units), or very high (>3.00 ELISA units) relative to the levels in the group of patients without colonization, as described in the Methods section. The total numbers of patients in each subgroup are shown in the circles.

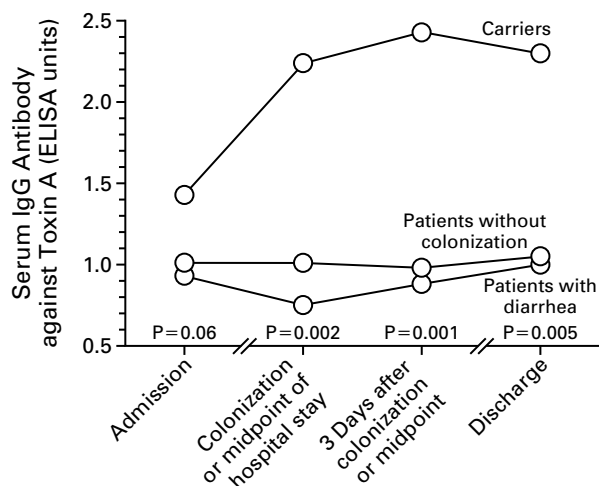
vere or extremely severe disease at admission, diarrhea occurred in 88 percent of those with a serum level of IgG antibody against toxin A of 3.00 ELISA units or less and in 20 percent of those with a level of more than 3.00 ELISA units ( $P = 0.007$ ) (Fig. 3). Among patients with mild or moderate disease at admission, diarrhea occurred in 43 percent of those with a serum level of IgG antibody against toxin A of 3.00 ELISA units or less and in none of those with a level of more than 3.00 ELISA units ( $P = 0.25$ ). After adjustment for the severity of disease, age, and sex, the odds of *C. difficile* diarrhea associated with a serum level of IgG antibody against toxin A of 3.00 ELISA units or less increased from 19.6 (95 percent confidence interval, 2.2 to 176) to 48.0 (95 percent confidence interval, 3.4 to 678; chi-square for the model, 23.3;  $P < 0.001$ ). We did not identify any other independent predictors for *C. difficile* diarrhea or potential confounding factors among patients with colonization.

**Serum Levels of IgG Antibody against Toxin A during Hospitalization**

Figure 4 shows the median serum levels of IgG antibody against toxin A during the course of hospitalization in patients with hospital-acquired *C. difficile* diarrhea, asymptomatic carriers, and the patients without colonization. At the time of admission, there was a trend ( $P = 0.06$ ) for patients who subsequently became asymptomatic carriers of *C. difficile* to have higher serum levels of IgG antibody against toxin A (median level, 1.43 ELISA units; range, 0.05 to 41.92) than patients in whom *C. difficile* diarrhea developed (0.93 ELISA unit; range, 0.45 to 7.96) or those who did not become infected with the organism (1.01 ELISA units; range, 0.00 to 25.47). At the time of colonization, the levels increased in patients who became asymptomatic carriers and dropped significantly in patients in whom *C. difficile* diarrhea subsequently developed (mean decrease, -0.6 ELISA unit;  $P = 0.02$ ). At this point the median serum level of IgG antibody against toxin A was three times as high in asymptomatic carriers (2.24 ELISA units; range, 0.04 to 42.50) as in patients in whom *C. difficile* diarrhea developed (0.75 ELISA unit; range, 0.20 to 5.07;  $P < 0.001$ ). Thereafter, there continued to be significant differences in the levels among the three groups. The serum levels of IgG antibody against toxin A did not change significantly in patients who were not colonized with *C. difficile* during hospitalization.

**DISCUSSION**

We found that asymptomatic carriage of *C. difficile* was strongly associated with an immune response to *C. difficile* toxins that was manifested by high serum levels of IgG antibody against toxin A. Conversely, patients who became colonized by *C. difficile* but



**Figure 4.** Median Levels of IgG Antibody against Toxin A during Hospitalization.

The results are shown for 28 patients in whom *Clostridium difficile* diarrhea developed and 19 asymptomatic carriers at the time of hospitalization, at the time of colonization by *C. difficile*, three days after colonization, and at discharge. The median interval between admission and colonization was 3 days (range, 3 to 33), and the median interval between the third day after colonization and discharge was 12 days (range, 2 to 56). The results are also shown for 187 patients without colonization at admission, at the midpoint of the hospital stay, three days after the midpoint, and at discharge. The P values refer to the comparison among the three groups (by the Kruskal–Wallis test).

who had low levels of serum IgG antibody against toxin A had a much greater risk of *C. difficile* diarrhea. The presence of severe disease on admission to the hospital was also independently associated with the risk of *C. difficile* diarrhea. The risk of diarrhea among patients with colonization who had severe or extremely severe disease was eight times that of patients who were less severely ill. All patients who were colonized with *C. difficile* but who had high serum levels of IgG antibody against toxin A and less severe disease were asymptomatic carriers, whereas all patients with colonization who had low antibody levels and severe disease had *C. difficile* diarrhea.

Shim et al. reported that asymptomatic colonization with *C. difficile* was associated with a decreased risk of subsequent *C. difficile* diarrhea.<sup>31</sup> However, the mechanism of this protective effect was not identified. Our study shows that asymptomatic carriers mount an effective immune response to *C. difficile* toxins. The findings that asymptomatic carriage was associated with high levels of IgG antibody and that serum levels of IgG antibody against toxin A increased rapidly after colonization both indicate the occurrence of a systemic anamnestic response to *C. difficile* toxin A.

Our findings advance the understanding of the

pathophysiology of nosocomial *C. difficile* diarrhea.<sup>1,5,6,31-33</sup> Antibiotic therapy alters the normal colonic microflora, permitting opportunistic infection by *C. difficile*. If antibiotic therapy is administered in an environment where *C. difficile* and its spores are prevalent, such as a hospital or nursing home, colonization by toxigenic *C. difficile* is more likely to occur. We suspect that patients with colonization who have a serum IgG response to *C. difficile* enterotoxin usually become asymptomatic carriers; patients who lack protective immunity have diarrhea and colitis.

We studied the immune response to *C. difficile* in a cohort of elderly hospitalized patients, many of whom had severe coexisting disease. These are the patients at highest risk for *C. difficile* diarrhea.<sup>5,21,34-38</sup> We were able to determine the time of acquisition of *C. difficile* and to measure antibody levels at the time of colonization, before the onset of diarrhea. We examined a variety of relevant markers of immune response (serum IgA, IgM, and IgG and fecal IgA and IgG) to biologically important *C. difficile* antigens (toxin A, toxin B, and nontoxin antigens) and prospectively examined potential confounding variables. However, our study also had some limitations. The severity of disease was measured at base line but was not reassessed at the time of colonization. More important, the power of the study to detect differences in median antibody levels other than serum IgG antibody against toxin A was limited because of the relatively small number of patients with colonization. This lack of power may account for the absence of any statistically significant differences in fecal levels of antibody against *C. difficile*. The significant correlations between serum levels of IgG antibody against toxin A, toxin B, and nontoxin antigens indicate that asymptomatic carriers have humoral immune responses to toxin A and toxin B as well as to nontoxin antigens. However, whether immune responses to antigens other than toxin A are important with respect to protection against diarrhea is not clear.

Two previous studies examined antibody levels in asymptomatic carriers and patients with *C. difficile* diarrhea. Johnson et al. found that serum and fecal levels of IgA antibody against toxin A were significantly higher in 21 patients with *C. difficile* diarrhea than in 9 asymptomatic carriers.<sup>15</sup> In contrast, Mulligan et al. reported that serum levels of IgA, IgM, and polyvalent immunoglobulins against somatic-cell antigens of *C. difficile* were higher in 5 asymptomatic carriers than in 21 patients with *C. difficile* diarrhea.<sup>18</sup> However, in both studies, antibody levels, the putative marker of protection, were measured after the outcome (i.e., the onset of diarrhea or established asymptomatic carriage), the total number of patients studied was small, and potential confounding variables were not examined.

Approximately one third of the patients in our study (31 percent) were colonized with *C. difficile*, either

at the time of admission to the hospital or during hospitalization. This high rate of *C. difficile* infection is similar to that reported in previous prospective studies of similar populations of patients.<sup>5,35-38</sup> In this prospective study, we found no evidence that antibody responses to *C. difficile* or its toxins influence colonization.

Previous studies indicate that an inadequate immune response to *C. difficile* toxins predisposes patients to severe, prolonged, and recurrent *C. difficile* diarrhea.<sup>16,39</sup> We have reported previously that passive immunotherapy using pooled normal human immune globulin (which contains IgG antibody against toxin A) is effective in treating recurrent or refractory *C. difficile* diarrhea.<sup>17,40</sup> Clinical studies of the safety and immunogenicity of a candidate vaccine against *C. difficile* toxins are now under way.<sup>16,39</sup> Our findings support the appropriateness of the use of vaccines and hyperimmune globulin by providing evidence that the host immune response to *C. difficile* has a substantial role in determining the clinical outcome of infection. It also highlights a potential marker of immune protection (very high serum levels of IgG antibody against toxin A) that can be used as a surrogate marker of vaccine efficacy. Ultimately, passive or active immunization against *C. difficile* toxins may prove to be an effective way to prevent and control nosocomial *C. difficile* diarrhea.

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