

Antibody Response to *Cryptococcus neoformans* Capsular Polysaccharide Glucuronoxylomannan in Patients after Solid-Organ Transplantation

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Cryptococcosis is an important complication of solid-organ transplantation, but the risk factors for disease are poorly understood. The goal of this study was to investigate whether specific or nonspecific serum immunoglobulin levels determined in samples obtained before and after solid-organ transplantation differed in patients who did or did not develop cryptococcosis after transplantation. We analyzed pretransplantation sera from 25 subjects, 15 who subsequently developed cryptococcosis and 10 who did not, and posttransplantation sera from 24 subjects, 13 who developed cryptococcosis and 11 who did not. All subjects received a tacrolimus-based immunosuppressive regimen. Total immunoglobulin levels were measured by immunodiffusion, and *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan (GXM)-specific serum antibody levels were determined by enzyme-linked immunosorbent assays. The results showed that solid-organ transplantation had a significant effect on total immunoglobulin and GXM-reactive antibody levels. GXM-reactive antibody levels differed in subjects who did and did not develop cryptococcosis. In pretransplant serum samples, the levels of GXM-reactive immunoglobulin M (IgM) were significantly lower in subjects who developed cryptococcosis after transplantation than in those who did not. For posttransplant serum samples, the levels of GXM-reactive IgM and IgG were significantly higher among the subjects who developed cryptococcosis than among those who did not. These findings suggest that perturbations in the preexisting antibody or B-cell repertoire and/or related to treatment of rejection, transplantation, or immunosuppressive therapy could translate into an increased risk for transplant-associated cryptococcosis.

Cryptococcus neoformans is unique among pathogenic fungi, because it possesses a polysaccharide capsule that is essential for virulence. *C. neoformans* has a worldwide distribution and does not require a mammalian host for survival. Infection occurs early in life but is rarely associated with clinically apparent disease (15). Cryptococcosis can result from reactivation of a latent infection (14, 39) or a newly acquired infection (33) but occurs predominantly in immunocompromised patients (discussed in reference 8). Recent studies suggest that 20 to 60% of cases of cryptococcosis in patients who do not have human immunodeficiency virus (HIV) or AIDS occur in solid-organ transplant recipients (16). The incidence of cryptococcosis in this patient group is 1% to 5% (18, 20), with reported mortality rates from 20 to 42% (18). Hence, cryptococcosis is an emerging and important infectious complication of solid-organ transplantation. Immunological factors that contribute to the risk for transplant-associated cryptococcosis have not been identified.

Intact T-cell-mediated immunity is required for resistance to *C. neoformans* (5), but T-cell deficiency is insufficient to account for the high incidence of disease in HIV-infected individuals (discussed in reference 8). In contrast to the incontro-

vertible role of CD4⁺ T cells in immunity to *C. neoformans*, the importance of B cells and antibody is unknown. Nonetheless, there are several lines of evidence that suggest antibody immunity could contribute to resistance to cryptococcosis. First, glucuronoxylomannan (GXM)-reactive mouse and human monoclonal antibodies (MAbs) prolong survival in lethal experimental cryptococcosis in mice, and B-cell deficiency alters the pathogenesis of *C. neoformans* in mice (reviewed in reference 7). Second, in humans, GXM-reactive and nonspecific antibody profiles differ between groups that are at high and low risk for cryptococcosis, namely, HIV-infected subjects and HIV-uninfected subjects, respectively (11, 13, 17, 40). Third, the risk for cryptococcosis is increased in patients with immunoglobulin disorders and deficiency, including hyperimmunoglobulin M (hyper-IgM), hypogammaglobulinemia, X-linked immunodeficiency, common variable immunodeficiency, and B-cell-associated malignancy (19, 21, 34, 38, 42). Fourth, vaccines that induce antibodies to cryptococcal polysaccharide determinants enhance resistance to experimental cryptococcosis (discussed in reference 10). In aggregate, these observations suggest that defects in antibody immunity could contribute to susceptibility to cryptococcosis in certain individuals. The aim of this study was to analyze the total and GXM-reactive antibody repertoires of solid-organ transplant recipients who did and did not develop cryptococcosis.

MATERIALS AND METHODS

Sera and subjects. Sera from 49 subjects who underwent solid-organ transplantation were studied. These 49 subjects included 25 from whom serum was obtained before transplantation and 24 from whom serum was obtained after

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transplantation, including 9 subjects from whom serum was also collected before transplantation. The primary immunosuppressive regimen of these individuals comprised tacrolimus in 46 patients, tacrolimus plus azathioprine in 2 patients, and tacrolimus plus sirolimus in 1 patient. The pretransplant cohort included 15 subjects who developed cryptococcosis (*C. neoformans* positive) and 10 subjects who did not develop cryptococcosis (*C. neoformans* negative). The posttransplant cohort included 13 subjects who developed cryptococcosis, including 9 who were also part of the pretransplant cohort, and 11 who did not develop cryptococcosis. These subjects were identified from a larger cohort of organ transplant recipients with cryptococcosis in a prospective, multicenter study (37). The types of underlying liver, lung, and kidney disease were similar in the *C. neoformans*-positive and *C. neoformans*-negative cohorts. Cryptococcosis was defined as the growth of *C. neoformans* in a clinical specimen or a positive cryptococcal antigen in the blood or cerebrospinal fluid of a patient with compatible clinical presentation (18). In transplant recipients with cryptococcosis, sera were collected at the time of diagnosis. The sera from the subjects who did not develop cryptococcosis were collected at the same time or as close after transplantation as the time elapsed from transplantation in the patients who developed cryptococcosis. Sera were separated from whole-blood samples by centrifugation, stored at -70°C , and heated for 30 min at 56°C before use. Sera were studied in a blinded-coded fashion.

Serum GXM measurements. Serum GXM levels were determined as described previously (26), with some modifications. Microtiter plates (96-well plates) were coated for 3 h at room temperature with $10\ \mu\text{g}/\text{ml}$ of G19, a human IgM MAb to GXM (25), blocked overnight with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (1% BSA-PBS), washed with PBS containing 0.05% Tween 20 (Sigma, St. Louis, MO), and incubated with twofold dilutions of purified GXM from *C. neoformans* strain 24067 to generate a standard curve as described previously (12, 26). The plates were incubated for 1 h at 37°C with serum samples serially diluted after an initial dilution of 1:100, washed, and incubated for 1 h at 37°C with $10\ \mu\text{g}/\text{ml}$ of a mouse IgG1 MAb to GXM (2H1; provided by A. Casadevall, Albert Einstein College of Medicine). Bound 2H1 was detected by incubating the plates with alkaline phosphatase (AP)-conjugated goat anti-mouse IgG1 (Southern Biotechnology) (diluted 1:5,000) for 1 h at 37°C . The plates were developed with *p*-nitrophenyl phosphate (Sigma) in bicarbonate buffer (pH 9.8) and read at 405 nm using the MRX enzyme-linked immunosorbent assay (ELISA) reader (Dyex Technologies, Chantilly, VA). The GXM concentrations in test samples were calculated from the absorbance values in the linear portion of the sample titration curves by nonlinear regression to the standard curve (Prism 4.02; GraphPad Software Inc., San Diego, CA).

Measurement of total immunoglobulin concentrations. Total serum IgM, IgG, and IgA concentrations were measured using anti-human IgM, IgG, and IgA radial immunodiffusion kits (Kent Laboratories, Bellingham, WA) according to the manufacturer's instructions. All samples were analyzed without dilution, and samples that were not within the range of the reference sera were reanalyzed at consecutive dilutions from 1:2 to 1:5.

Measurement of antibodies to GXM. The titers of IgM and IgG to GXM (GXM-reactive IgM and IgG) were determined by ELISAs as described previously (13, 40). IgA to GXM was not determined in this study due to limited serum availability and the lack of difference in titers between high-risk (HIV-infected) and low-risk (HIV-uninfected) individuals in a previous study (40). Polystyrene plates (96-well plates) (Costar; Corning Glass Works, Corning, NY) were coated for 3 h at room temperature with $1\ \mu\text{g}/\text{ml}$ of SB4, a serotype A isolate of *C. neoformans* (provided by A. Casadevall). The plates were then blocked overnight at 4°C with 1% BSA-PBS and washed with PBS containing 0.05% Tween 20. Serum samples were applied to the plates in duplicate at an initial dilution of 1:50 and then serially diluted 1:3 in 1% BSA-PBS. The plates were then incubated for 1 h at 37°C , washed, and incubated with AP-labeled goat anti-human IgM or IgG (Southern Biotech, Birmingham, AL) (diluted 1:5,000) for 1 h at 37°C . After the plates were washed, they were developed as described above, and absorbance at 405 nm was measured. 2H1 MAb (mentioned above) was used as a positive control, and wells with no sera were included as a negative control to determine the background absorbance of the reagents. For each sample, the average of duplicate wells at each dilution was determined after subtracting 1.5 times the background (the absorbance of wells without serum), and inverse titers were determined as the point at which the titration curve crossed an optical density of 0.1.

Measurement of antibodies to PPS. The antibody determinations were performed by ELISAs using a pneumococcal capsular polysaccharide (PPS) vaccine (Pneumovax; Merck, West Point, PA) as described previously (1, 41). Plates were coated with $10\ \mu\text{g}/\text{ml}$ of the vaccine diluted in PBS and incubated overnight at 4°C . After the plates were blocked, they were washed and incubated with serial dilutions of sera from subjects beginning at a dilution of 1:50 for 1 h at 37°C ,

washed, and incubated with AP-labeled goat anti-human IgM or IgG (Southern Biotech). The positive control was a standard antipneumococcal serum (89SF; FDA). The negative control was wells without sera. After incubation with the secondary or tertiary antibodies, the plates were developed, and inverse titers were determined as described above.

Measurement of antibodies to TT. The antibody determinations were performed by ELISAs using a tetanus toxoid (TT) vaccine (Aventis Pasteur, Swiftwater, PA). Plates were coated with $10\ \mu\text{g}/\text{ml}$ of the vaccine diluted in PBS and incubated overnight at 4°C . After the plates were blocked, they were washed and incubated with serial dilutions of sera from subjects beginning at a dilution of 1:50 for 1 h at 37°C , washed, and incubated with AP-labeled goat antihuman IgM or IgG (Southern Biotech). After incubation with the secondary or tertiary antibodies, the plates were developed and read, and inverse titers were determined as described above.

Statistical analysis. Differences in the concentrations of serum IgM, IgA, and IgG, antibodies to GXM, and idiotype titers between groups (i.e., *C. neoformans*-positive group compared to *C. neoformans*-negative group for pre- and posttransplant samples) were evaluated with the nonparametric Mann-Whitney U test; pre- and posttransplant values for nine paired samples were evaluated with the Wilcoxon signed-rank test for the significance of differences between paired observations. A *P* value of <0.05 was considered significant. Statistical analyses were performed using Prism version 4.02 (GraphPad Software Inc.).

RESULTS

Study population. The characteristics of the patients, including patient age, organ transplanted, immunosuppressive regimen, history of prior organ rejection, and site of cryptococcal infection, are shown in Table 1. The median time to the onset of cryptococcosis was 533 days after transplantation (range, 40 to 1,338 days). The sites of cryptococcal infection for the 24 posttransplant subjects were as follows: pulmonary in 15 patients; pulmonary and central nervous system (CNS) in 2 patients; pulmonary and cutaneous in 2 patients; CNS and cutaneous in 2 patients; and CNS, osteoarticular, and cutaneous alone in 1 subject each. The patients were 22 to 65 years of age (median, 51 years), and 80% were male. For pretransplant subjects, the rates of rejection preceding the current transplant were 26% for those who subsequently developed cryptococcosis and 0% for those who did not. For the posttransplant subjects, the rates of prior rejection were 23% for the subjects who developed cryptococcosis and 63% for the subjects who did not develop cryptococcosis. Differences in the ages of the subjects in the groups that did or did not develop cryptococcosis and in the pre- and posttransplant groups were not significant.

Serum GXM levels. All pretransplant samples and posttransplant samples from subjects who did not develop cryptococcosis had undetectable levels of GXM. GXM was detected in samples from 8 of 13 posttransplant subjects who developed cryptococcosis. The GXM concentration in these samples ranged from 0.2 to $28\ \mu\text{g}/\text{ml}$ (data not shown).

Total immunoglobulin levels. Total IgM was higher in posttransplant samples than in pretransplant samples, with a trend towards significance ($P = 0.07$), while total IgA was significantly lower in posttransplant samples than in pretransplant samples ($P < 0.0001$) (Fig. 1). No significant differences in total IgG were observed. Total IgM, IgG, and IgA did not differ significantly in nine paired pre- and posttransplant samples or between *C. neoformans*-positive and *C. neoformans*-negative subjects (data not shown).

Antibody to GXM. All subjects had detectable antibody to GXM. The levels of IgM to GXM were significantly lower ($P = 0.0003$) in pretransplant samples (Fig. 2A) from *C. neoformans*-

TABLE 1. Characteristics of the cohorts of organ transplant recipients who did and did not develop cryptococcosis after transplantation

Characteristic	Total	Pretransplantation sera from patients ^a		Posttransplantation sera from patients ^a	
		CN-	CN+	CN-	CN+
No. of subjects	49	10	15 (9) ^b	11	13 (9) ^b
No. of males		10	14	5	11
Mean age (yr) (range)	51	44 (39–49)	53 (37–64)	53 (45–63)	54 (22–65)
No. of patients with the indicated organ transplanted	13 liver			5 lung	7 liver
	6 lung 4 kidney 1 heart			6 liver	4 kidney 1 lung 1 heart
Prior rejection rate (%) ^c		0	26	63	23
Primary immunosuppressive drug (no. of patients)		Tacrolimus (10)	Tacrolimus (15)	Tacrolimus (8), tacrolimus + sirolimus (1), tacrolimus + azathioprine (2)	Tacrolimus (13)
No. of patients with the indicated site infected with <i>C. neoformans</i>			8 lung		9 lung (includes 6 subjects also studied pretransplant) 2 lung + CNS 1 CNS 1 CNS + skin
			2 lung + skin 1 bone 1 skin 1 CNS 2 lung + CNS		
Mean time to cryptococcosis (days) (range) ^d				246 (37–476)	533 (40–1,338)

^a Pre- and posttransplantation sera were obtained from organ transplant recipients who developed cryptococcosis (CN+) and from those who did not develop cryptococcosis (CN-) after transplantation.

^b The nine subjects who were studied pre- and posttransplantation.

^c Prior rejection is defined here as one or more episodes of rejection 6 months prior to the transplant.

^d Time blood was drawn after transplant. For CN+ subjects, it is the length of time after the transplant before the cryptococcosis diagnosis; for CN- subjects, it is the same length of time or as close after transplantation as the time elapsed from transplantation in CN+ subjects.

mans-positive subjects than from *C. neoformans*-negative subjects; there was no significant difference in IgG to GXM in pretransplant samples on the basis of *C. neoformans* status. On the other hand, IgM and IgG to GXM were significantly higher in posttransplant samples (Fig. 2B) from *C. neoformans*-posi-

tive subjects ($P < 0.003$) than from *C. neoformans*-negative subjects. Comparing pre- to posttransplant samples, the levels of IgM and IgG to GXM were similar in samples from *C. neoformans*-positive subjects but lower in posttransplant samples than in pretransplant samples from *C. neoformans*-negative subjects (compare Fig. 2A and B). For the nine paired pre- and posttransplant samples from *C. neoformans*-positive subjects, median IgM levels were higher posttransplantation, but this difference was not statistically significant (Fig. 3).

Levels of antibodies to PPS and TT. Pretransplant samples from *C. neoformans*-negative subjects had a significantly higher median level of IgM to PPS ($P < 0.043$) than those from *C. neoformans*-positive subjects (Fig. 4). There were no cases of pneumococcal disease in either cohort. Posttransplant samples from *C. neoformans*-positive subjects had significantly higher median levels of IgG to TT ($P < 0.033$) (Fig. 5). There was no other significant difference between samples from *C. neoformans*-positive and *C. neoformans*-negative subjects in the levels of IgM and IgG to PPS in posttransplant samples or of IgM and IgG to TT in pretransplant samples.

DISCUSSION

To the best of our knowledge, this is the first study of the human antibody response to GXM in solid-organ transplant recipients. Remarkably, we found that the levels of GXM-reactive IgM were significantly lower in pretransplant samples from subjects who subsequently developed cryptococcosis (*C. neoformans* positive) than in samples from subjects who did not develop cryptococcosis (*C. neoformans* negative). The levels of total IgM were similar in both groups. Hence, lower levels in

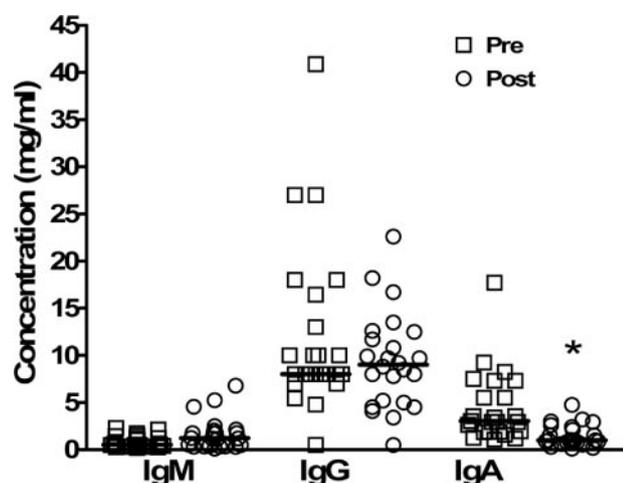


FIG. 1. Total immunoglobulin concentrations in pre- and posttransplant serum samples from solid-organ transplant recipients. Scatterplots of concentrations of IgM, IgG, and IgA for subjects before (Pre) and after (Post) the transplant are shown. Each point shows the value for one individual, and the median values are indicated by black bars. All comparisons were made by the Mann-Whitney U test. The asterisk indicated that the values were significantly different ($P < 0.0001$) for IgA levels in pretransplant samples compared to those in posttransplant samples.

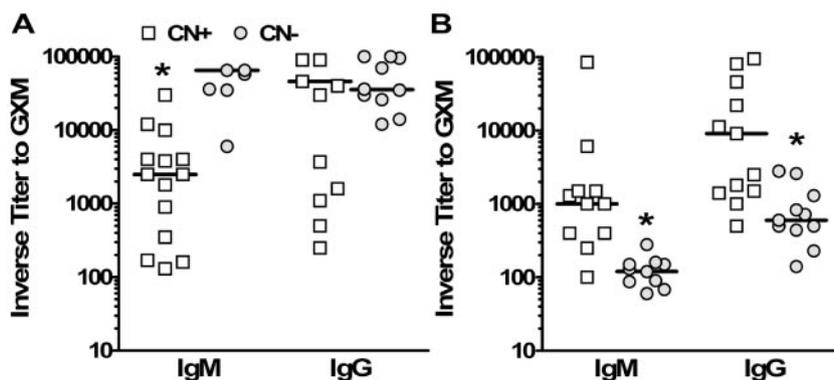


FIG. 2. Levels of IgM and IgG to GXM in sera of solid-organ transplant recipients. Scatterplots of inverse titers of IgM and IgG to GXM in sera from subjects who developed cryptococcosis (CN+) or who did not develop cryptococcosis (CN-) in samples obtained pretransplantation (A) and posttransplantation (B). Each point shows the value for one individual, and the median values are indicated by black bars. In panel A, the asterisk indicates that the values for IgM to GXM in subjects who or did not develop cryptococcosis in pretransplant samples were significantly different ($P = 0.0003$). In panel B, the asterisk indicates that the values for GXM-specific IgM and IgG in subjects who or did not develop cryptococcosis in posttransplant samples were significantly different ($P < 0.003$).

pretransplant samples did not reflect a global decrease in IgM. The possible roles of antibodies to GXM in resistance and susceptibility to cryptococcosis are unknown, but available data suggest that the naturally occurring GXM-reactive repertoire may contribute to resistance (discussed in references 8 and 10). Studies comparing HIV-infected and HIV-uninfected individuals, groups that are at very high and low risk for cryptococcosis, respectively, have also found lower levels of GXM-reactive IgM in HIV-infected subjects than in HIV-uninfected subjects (13, 17, 40), including individuals with relatively intact T-cell immunity (13). The data presented herein suggest that a reduced level of GXM-reactive IgM could portend a risk of cryptococcosis in the setting of immunosuppression, although whether it reflects previously unrecognized variation in the population or is induced by HIV infection or transplantation is unknown.

The reason some subjects who subsequently developed cryptococcosis had lower levels of GXM-reactive IgM before transplantation is unknown. One possibility is that there is variability in the population due to unknown genetic or other factors.

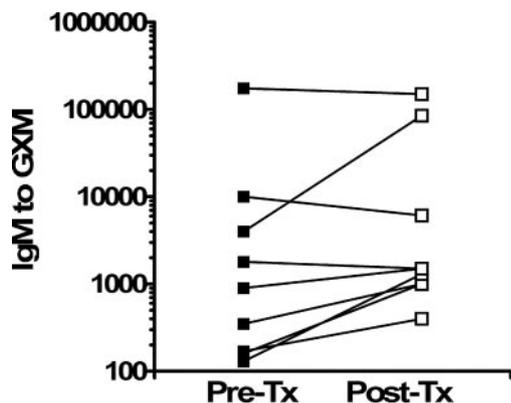


FIG. 3. Effect of transplantation on IgM to GXM. The levels of IgM to GXM in paired serum samples from nine *C. neoformans*-positive subjects before transplantation (Pre-Tx) and after transplantation (Post-Tx) are shown.

If diseases associated with reduced antibody levels are rare, they might come to light only in the setting of another defect, such as HIV- or transplant-induced immunosuppression. Notably, and similar to GXM-reactive IgM, pretransplant sera from the subjects who developed cryptococcosis also had lower levels of PPS-reactive IgM while posttransplant sera had higher levels (albeit an order of magnitude lower than pretransplant sera) than sera from subjects who did not develop cryptococcosis did. Solid-organ transplant recipients are also predisposed to disease with pneumococcus (30). A loss of IgM memory B cells was implicated in susceptibility to pneumococcus and impaired antibody responses to PPS (23, 36, 44) and in infections with encapsulated bacteria in patients with common variable immunodeficiency (6). There were no cases of pneumococcal disease in either the *C. neoformans*-positive or *C. neoformans*-negative cohort, and a previous study found no association between GXM- and PPS-reactive antibodies (40). However, the foregoing studies suggest an intriguing link to cryptococcal pathogenesis, since reduced memory IgM is a central defect in patients with increased risk for cryptococcosis, such as those with common variable immunodeficiency (2, 38), hyper-IgM (3, 19, 21, 42), and HIV (32). Since serological studies cannot distinguish between memory and naïve IgM, B-cell analysis is needed to determine whether GXM-reactive IgM is derived from memory IgM. B-cell numbers were decreased in children with renal transplantation in one study (28), and memory B-cell reconstitution was markedly delayed in patients after stem cell transplantation (4). The effects of regimens used to treat organ rejection or the underlying diseases that prompt transplantation on the memory and naïve B-cell repertoire have not been studied.

In posttransplant samples, the levels of GXM-reactive IgM and IgG were significantly higher among subjects with cryptococcosis than among those without cryptococcosis. However, there was no difference between the antibody levels in pretransplant samples from subjects who subsequently developed cryptococcosis and posttransplant samples from subjects with cryptococcosis. In contrast, the levels of GXM-reactive IgM and IgG from subjects who did not develop cryptococcosis

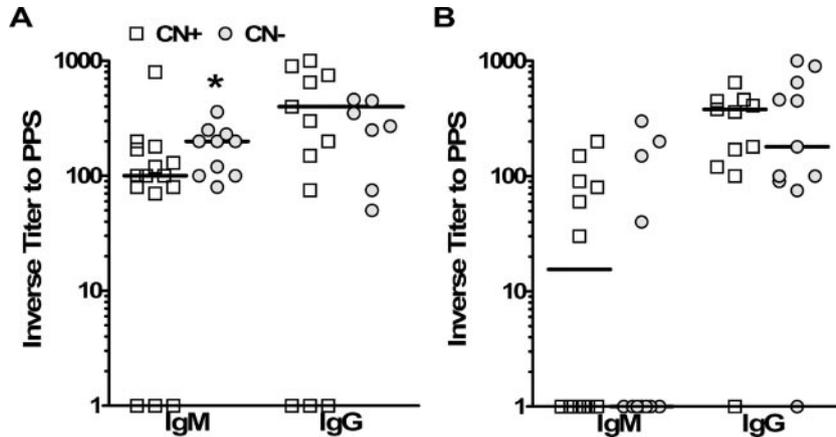


FIG. 4. Levels of antibody to PPS in sera from solid-organ transplant recipients. Scatterplots of inverse titers of IgM and IgG to PPS in pretransplant (A) and posttransplant (B) samples from subjects who developed cryptococcosis (CN+) or who did not develop cryptococcosis (CN-) are shown. Each point shows the value for one individual, and the median values are indicated by black bars. The asterisk indicates that the level of IgM was significantly higher in pretransplant samples in subjects who did not develop cryptococcosis than in subjects who developed cryptococcosis ($P < 0.04$).

were significantly lower in posttransplant samples than in pretransplant samples. The reason that subjects who developed cryptococcosis had higher levels of antibody after transplantation is unknown. One possibility is that preexisting pretransplant antibody levels could have been maintained or increased by antigenic stimulation from *C. neoformans* in the subjects with cryptococcosis. Notably, 11 of 13 posttransplant samples from subjects with cryptococcosis were from subjects with pulmonary disease. Serum GXM is often not detected in patients with pulmonary or nondisseminated cryptococcosis, and making the diagnosis of pneumonia often requires culture or histopathological examination of tissue (24, 31). Subramaniam et al. found that HIV-infected subjects with a clinical history of pneumonia had higher levels of GXM-reactive IgM and IgG than those who did not have a history of pneumonia (40). Hence, our observations lead us to wonder whether GXM-reactive antibody after transplantation could be associated with the risk for pulmonary or nondisseminated cryptococco-

sis. We believe prospective studies to evaluate this hypothesis are warranted in view of the difficulty in diagnosing cryptococcal pulmonary disease.

Although there was a higher rate of previous organ rejection among the pretransplant subjects who developed cryptococcosis, this trend was reversed in posttransplant subjects who developed cryptococcosis. Rejection has not been shown to be a risk factor for *C. neoformans* in organ transplant recipients (29), and the effect of rejection on antibody levels is not known. Posttransplant subjects who developed cryptococcosis had higher levels of GXM-reactive antibody than the subjects without cryptococcosis. The lower levels of GXM-reactive antibody could reflect the influence of a regimen used to treat rejection. Interestingly, in both pre- and posttransplantation sera, the level of GXM-reactive antibody was lower in sera from the group with the higher rate of previous organ rejection, the *C. neoformans*-positive group for pretransplant sera and the *C. neoformans*-negative group for posttransplant sam-

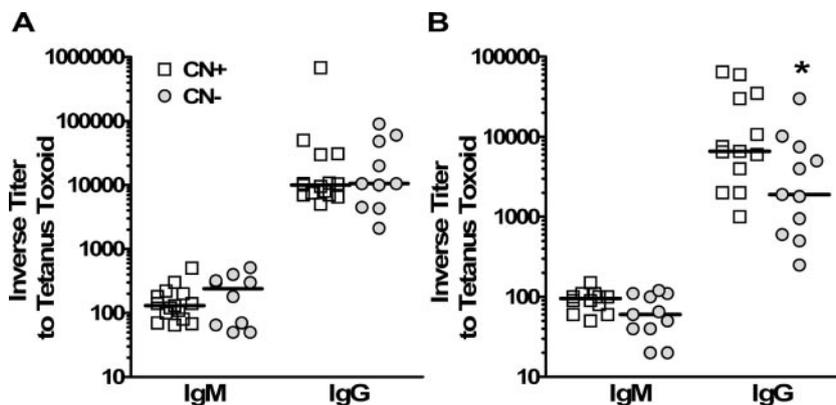


FIG. 5. Levels of antibody to TT in sera from solid-organ transplant recipients. Scatterplots of inverse titers of IgM and IgG to TT in pretransplant (A) and posttransplant (B) samples from subjects who developed cryptococcosis (CN+) or who did not develop cryptococcosis (CN-) are shown. Each point shows the value for one individual, and the median values are indicated by black bars. The asterisk indicates that the level of IgG in posttransplant samples was significantly higher ($P < 0.03$) in subjects who developed cryptococcosis than in subjects who did not develop cryptococcosis.

ples. This observation calls for further studies of the relationship between treatments for rejection and antibody levels, since our study was not designed to evaluate the effects of previous rejection on the levels of total or GXM-reactive antibodies. Irrespective of the cause, our data suggest an association between lower levels of pretransplant IgM, but higher levels of posttransplant IgM and IgG to GXM, and the risk for transplant-associated cryptococcosis. This dichotomy may not be as paradoxical as it seems; high levels of antibodies to GXM have been associated with a prozone-like phenomenon that enhances mortality in experimental cryptococcosis (26, 43). Higher levels of IgG to GXM were also found among HIV-infected subjects than in HIV-uninfected subjects (13, 17, 40).

We found that total IgA was lower in posttransplant samples than in pretransplant samples. This could implicate the immunosuppressive regimens used and/or other medications and/or surgery itself in perturbing the immunoglobulin repertoire. The effects of immunosuppressive regimens used in organ transplantation on total immunoglobulin levels have not been directly examined. However, the possible influence of these regimens on antibody levels is suggested by reports of hypogammaglobulinemia and infectious complications in organ transplant recipients (9, 22, 35). Interestingly, tacrolimus decreased the number of B1-a cells in mice (45). Antibodies to PPS are derived from B-1 cells (27), but the derivation of antibodies to GXM is unknown. Together with the data reported herein, these observations suggest that the immunosuppressive regimens used in transplantation could result in antibody repertoire defects. This possibility requires validation, but if it is confirmed, monitoring immunoglobulin levels might identify patients who are at risk for cryptococcosis.

In summary, our data suggest that alterations in the levels of GXM-reactive antibodies before and after transplantation could be associated with susceptibility to cryptococcosis. Our study had certain methodological limitations, including that it was retrospective, we were not able to examine paired pre- and posttransplant samples from most subjects, and we could not control for previous rejection or evaluate the effect of immunosuppressive therapy on antibody levels. As such, we consider our data to be hypothesis generating in suggesting that reduced levels of GXM-reactive IgM before transplantation could contribute to the risk for transplant-associated cryptococcosis. We believe this question warrants further investigation, since currently, immunological risk factors for transplant-associated cryptococcosis are unknown.

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