# FUNGAL INFECTIONS COMPLICATING ORTHOTOPIC LIVER TRANSPLANTATION

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Invasive fungal infections, which are caused primarily by *Candida* species and *Aspergillus* species, have occurred in 5 to 42% of patients undergoing orthotopic liver transplantation (1–9) and have been associated with case-fatality rates ranging from 25 to 69% (2,8,9). Not unexpectedly, fungal infection was noted to be a significant cause of morbidity and mortality among our patients undergoing liver transplantation. To develop strategies to reduce this problem, we have studied the clinical presentation, epidemiology, and risk factors associated with strictly defined invasive fungal infection that occurred among patients undergoing orthotopic liver transplantation (OLT).

## Materials and Methods

We retrospectively reviewed the inpatient and outpatient records of all patients undergoing OLT at the New England Deaconess Hospital who survived more than 48 hours after surgery (except one patient whose record was lost). We first studied those patients with transplants performed from July 1983 through June 1990; subsequently we studied those with transplants done from January 1990 through September 1992. Donor management, organ retrieval and transplantation were performed in a standard manner (10). The biliary tract was reconstructed by using choledochocholedochostomy or, when this was not feasible, by using a Roux-en-Y choledochojejunostomy. Antibiotic prophylaxis was initiated immediately prior to surgery and continued for 48 hours thereafter. Oral nystatin was begun postoperatively and continued for 3 to 6 months but no other antifungal prophylaxis was administered routinely. Prophylaxis for herpes simplex virus or cytomegalovirus (CMV) infection was not given routinely. Induction im-

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munosuppression included intravenous cyclosporin A, tapering corticosteroids and azathioprine; as soon as feasible, immunosuppressive therapy was administered orally. From December 1986 to April 1989 as part of a randomized trial, patients received either cyclosporin A or OKT3 (Orthoclone, Ortho Biotech, Raritan, NJ) as induction therapy and from March 1991 through October 1991 and May 1992 through September 1992 nonazotemic patients participated in a randomized comparison of cyclosporin A and tacrolimus (FK506, Fujisawa, Deerfield, IL). Rejection episodes were treated initially by corticosteroid boluses (100–1000 mg) and when the response was not prompt, this was followed by a repeat cycle of induction corticosteroid therapy. Refractory rejection was treated with 10 to 14 days of OKT3 or, on rare occasions, with tacrolimus. Patients were evaluated for infection as clinically indicated; surveillance cultures were not performed.

Fungal infections were defined as follows: Fungemia required isolation of the same organism from two or more peripheral blood cultures or from one peripheral blood culture and either a blood culture drawn from a central venous catheter or from the catheter tip. Invasive surgical site infection required a positive intra-abdominal culture and either fungemia or microscopic or gross pathologic evidence of infection. Invasive nonsurgical site infection required microbiologic evidence as well as either clinical or histologic evidence of fungal infection at a normally sterile site. Microbiologic and histologic evidence of fungal infection in two noncontiguous sterile organs or the combination of candidemia and endophthalmitis indicated disseminated fungal infection.

Extensive clinical and laboratory data were extracted from medical records for the analyses. Included were the causes of liver failure, urgency of transplantation, preoperative dysfunction of organs other than the liver, details of the transplant procedure and intraoperative support requirements, intraoperative complications, preoperative and postoperative specialized life support requirements (including intensive care unit days), need for subsequent surgery or repeat transplantation, details of immunosuppressive therapy, antimicrobial therapy, complicating cytomegalovirus infection, and clinical, microbiologic, and chemical laboratory results from the preoperative and postoperative periods.

## Results

From July 1983 through June 1990, 187 OLT were performed in 158 patients. Nineteen patients underwent an early retransplantation (median 7 days; range 1 to 12 days after OLT) and 10 patients under-

went retransplantation more than 100 days after the initial OLT. The mean age of the patients was 46 years (range 17 to 69 years) and 53% were men.

Invasive fungal infection occurred in 40 (25%) of 158 patients. Among the 43 organisms causing these infections, *Candida* species accounted for 31 infections (27 due to *Candida albicans* and 4 due to *Candida tropicalis*). Seven patients were infected by *Aspergillus* species. In two patients candida and aspergillus infection occurred concurrently and in a third patient aspergillus infection followed candida infection. Each of the following organisms infected a single patient: *Torulopsis glabrata, Pseudoallescheria boydii, Histoplasma capsulatum, Cryptococcus neoformans,* and *Exophiala jeanselmei.*

The distribution of fungal isolates by category of infection is shown in Figure 1. Sixteen fungi were isolated from 15 patients with intra-abdominal surgical site infection. All 15 patients were infected by *Candida* species; one had a concurrent infection with *Aspergillus* species as well. Candidemia was documented in 11 of these 15 patients. Twenty fungi caused disseminated infection in 18 patients. Disseminated candidiasis was noted in 12 patients, 5 of whom had candidemia and endophthalmitis. Disseminated aspergillosis was noted in 6 patients; two of these patients also had disseminated candidiasis. Aspergillosis was diagnosed antemortem in only one patient; this diagnosis was based upon cultures of pleural fluid and intracranial lesions. Of the 5 patients with a post mortem diagnosis of aspergillosis, only 3 had ante mortem cultures that yielded *Aspergillus* species (all isolates

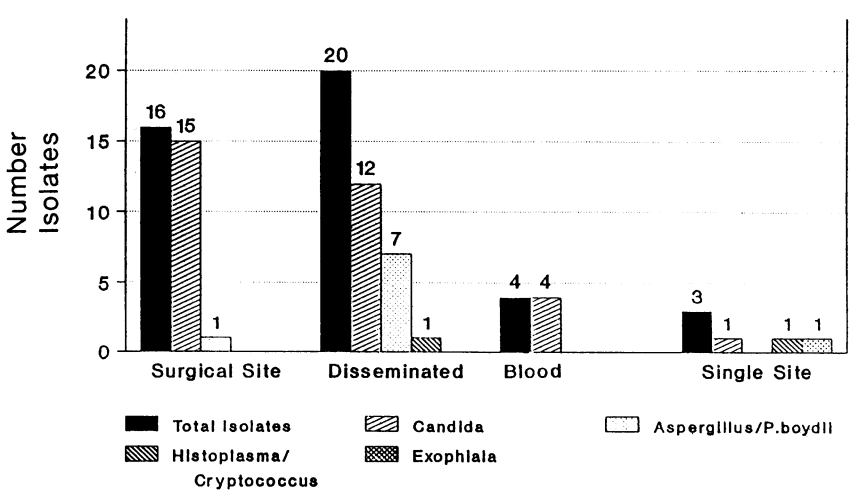


FIG. 1. The distribution of Fungal isolates is shown by defined categories of infection

were from respiratory secretions). Disseminated *P. boydii* infection, suspected ante mortem because of fungemia and echocardiographic abnormalities, was confirmed at autopsy in one patient. One patient had disseminated histoplasmosis. Four patients had candidemia only. Three patients had a single nonsurgical site infection: cryptococcal pneumonia, candida pneumonia, and invasive infection of skin and subcutaneous tissue in the distal leg caused by *Exophiala jeanselmei*.

The interval from the most recent OLT to the diagnosis of the first invasive fungal infection is shown in Figure 2. Of 40 patients with fungal infection, 34 (85%) were diagnosed within 100 days after the proximate OLT. In 31 of these patients, fungal infection occurred during the admission for the OLT. Of 32 infections caused by *Candida* species or *T. glabrata*, 13 occurred within one week and 29 within 100 days after transplantation. *Aspergillus* species or *P. boydii* caused the first diagnosed fungal infection in 5 patients; all occurred within the initial 100 days after OLT. Two patients initially noted to have candida infection within the first 100 days after OLT were subsequently found to have concomitant aspergillosis. Six patients had invasive fungal infection more than 100 days after OLT; these infections were caused by *Candida* species in 2 patients, concurrent *Candida* and *Aspergillus* species in one patient, and by *H. capsulatum, C. neoformans*, and *E. jeanselmei* each in one patient.

Overall, 28 (70%) of the 40 patients with invasive fungal infection died. Among the 33 patients who received antifungal therapy, 21 (64%)

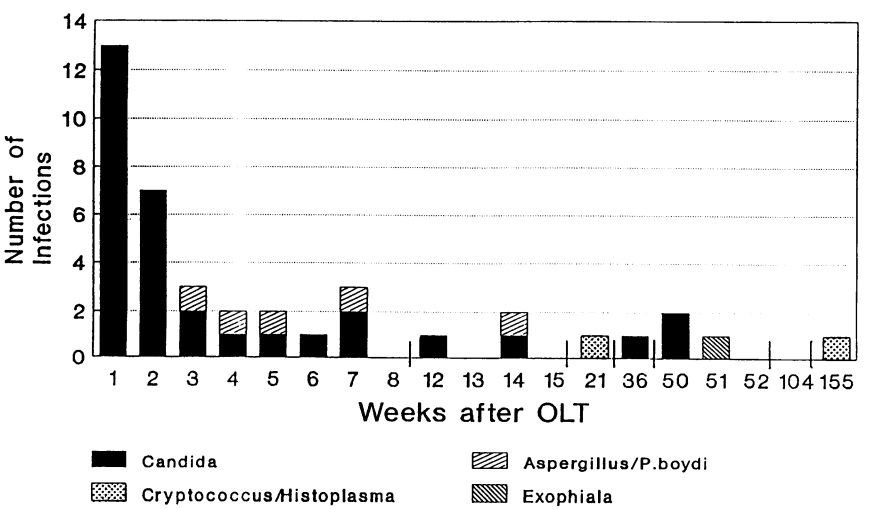


FIG. 2. The interval in weeks from the most recent OLT to the diagnosis of the first invasive fungal infection

died. Fungal infection was judged to have contributed to death in 18 of these 21 patients. All patients infected by Aspergillus species or *P. boydii* died. Twelve patients (36%) survived. Nine of 29 patients with infection caused only by *Candida* species or *T. glabrata* survived as did the patients with histoplasmosis, cryptococcosis, and *E. jeanselmei* infection. Among the survivors of candida infection, 5 had surgical site infection, 2 had candidemia, and one each had disseminated infection and pneumonia. Among survivors with candida infection, 8 were treated with amphotericin B (mean dose 920 mg; range 455 to 1870 mg) and one with fluconazole. The patients with histoplasmosis and cryptococcosis were treated with amphotericin B (2810 mg and 1440 mg, respectively) and the patient with *E. jeanselmei* infection was treated with amphotericin B (1090 mg) and surgical excision.

Crude (unadjusted) and multivariate analyses of 70 variables describing the pretransplant, intraoperative, and posttransplant state of the patients were performed using Cox proportional hazard regression (Systat for DOS, Evanston, IL) to generate hazard ratios as the measure of effect (relative risk). The date of invasive fungal infection was that of histologic or microbiologic diagnosis. Data for uninfected patients were censored at death or 100 days after OLT. Pretransplant and intraoperative observations were considered baseline covariates; posttransplant factors were time-dependent covariates and considered in the model according to days after transplantation only if the factor antedated fungal infection. Patients undergoing early retransplantation were considered a single OLT using the initial transplant to assess baseline factors. For patients undergoing late retransplantation, each procedure was analyzed separately. Hazard ratios (HR) over time after OLT were not constant for fungal colonization; therefore, this variable was analyzed using sequential Cox regression, with relative risk determined separately for $< 10$ days after OLT and $\geq 10$ days after OLT.

Fungal colonization of urine, sputum, abdominal and biliary drains, or the wound was a significant predictor of infection occurring $< 10$ days after OLT (16 colonized of 17 with infection vs. 60 colonized of 151 without infection) but was not a predictor of infection occurring $\geq 10$ days after OLT (12 colonized of 17 with infection vs. 86 colonized of 130 without infection). The risk of infection was not increased by increasing numbers of colonized sites nor by the specific site that was colonized. The associations between colonization and risk of infection are undermined in part because colonization was not determined prospectively.

In the multivariate analysis five variables were noted to be independent predictors of invasive fungal infection that occurred within 100

days of OLT: preoperative creatinine (HR, 1.4; 95% confidence interval [CI], 1.2–1.6) duration of transplant surgery (HR 1.2; 75% CI, 1.1–1.4), repeat OLT (HR 3.2; 95% CI, 1.5–6.5) abdominal or thoracic nontransplant surgery (HR 2.5; 95% CI, 1.6–3.8) and CMV infection (HR 8.5; 95% CI, 3.3–21.7). Notably, 81% of CMV infections occurred 2 or more weeks after OLT; whereas 20 (59%) of 34 fungal infections were diagnosed within 2 weeks after OLT. Nevertheless, among the 14 patients with fungal infection occurring more than 2 weeks after OLT, 12 had prior CMV infection. The average interval from the diagnosis of CMV infection to onset of fungal infection was 17 days.

The second study of patients undergoing liver transplantation was based on an independent retrospective review of medical records from all patients with transplant from January 1990 through September 1992. Data collection and definitions were as noted for the initial study. From January 1990 through September 1992, 133 OLT were performed on 118 patients. Nine patients had early retransplant (within 5 days of the initial OLT). These patients were analyzed as a single OLT dating from the initial surgery. Six patients underwent late retransplantation and are analyzed as separate OLT episodes. Thus, there were 124 OLT episodes studied. Cyclosporin A was used as primary immunosuppression in 102 patients and tacrolimus in 22 patients.

Of the 124 OLT episodes, 19 (15%) were complicated by invasive fungal infection. Seventeen patients had intra-abdominal surgical site infection; 6 of these patients had concomitant fungemia. Two other patients had isolated fungemia. Invasive fungal infection in the 19 patients was associated with 21 fungal organisms including *C. albicans* in 13 patients, *C. tropicalis* in 5 patients (2 of whom had concurrent infection with *C. albicans*). *C. lusitaniae, T. glabrata* and an unidentified yeast (histologically documented at autopsy) each caused infection in one patient. The median interval from OLT to onset of invasive fungal infection was 21 days (range, 1–69 days).

Crude and multivariate analyses to estimate the hazard ratio related to pretransplantation, intraoperative and posttransplantation factors were performed using the Cox proportional hazard regression as noted earlier. As before, fungal colonization was omitted from the model and examined independently.

Fungal colonization was associated with an increased risk of infection; this effect was not restricted to the initial two weeks after OLT. Of 19 patients with invasive fungal infection, 15 had one or more sites colonized prior to onset of infection (HR, 13.2; 95% CI, 3.3–52.8). The risk of fungal infection in this cohort of OLT patients did increase with increasing number of sites colonized but risk did not vary significantly according to the anatomic site that was colonized. In the multivariate

Cox proportional hazards regression, three variables were independent predictors of risk for invasive fungal infection: choledochojejunostomy (HR 4.9; 95% CI, 1.8–13.8), number units of blood and platelets transfused intraoperatively (HR 2.2; 95% CI, 2.1–4.4), and CMV infection (HR 3.4; 95% CI, 1.1–10.2). Duration of intensive care unit stay after OLT approached significance as an independent risk factor for fungal infection (HR 1.9; 95% CI, 0.99–3.8, p m=.052). Fungal infection occurred in 17 (17%) patients receiving cyclosporin A and 2 (9%) of those receiving tacrolimus as primary immunosuppressive therapy. The trend toward reduced risk among tacrolimus recipients was not significant when adjusted for other variables with the Cox model (HR m=.58; 95% CI, 0.13–2.6). This observation, however, is limited by the small population that was studied.

After eliminating duplicate data sets resulting from the January 1990 through June 1990 overlap of the two reviews, the data describing the pretransplant, intraoperative and 100 day posttransplant experience of 265 OLT episodes were combined and a repeat multivariate analysis was performed. Using the Cox proportional hazard regression, we developed a model predictive of fungal infection using variables that are detectable at the time of transplantation or within 4 to 5 days thereafter. Five variables, some of which were converted from continuous to dichotomous variables to maximize discriminability and simplify use, were identified as independent predictors: intraoperative transfusions (≥ 40 units of blood or platelets), intensive care unit stay after OLT (> 3 days), choledochojejunostomy, early or late retransplantation, and fungal colonization within 3 days after OLT (Table 1).

The Kaplan-Meier curve showing freedom from invasive fungal infection suggests that OLT patients can be divided into groups with low,

TABLE 1

*Factors Dectectable Before or Shortly After OLT That Independently Predict Invasive Fungal Infection. Risk Factors Determined Using a Multivariate Cox Proportional Hazard Model and 265 Patients Undergoing OLT from July 1983-September 1992*

| Risk Factor | Hazard Ratio | 95 % Confidence Interval | P |
|---|---|---|---|
| Intraoperative transfusions* | 2.6 | 1.4-4.9 | .003 |
| Intensive care unit† | 4.5 | 1.7-12.1 | .002 |
| Choledochojejunostomy | 2.8 | 1.5-5.2 | .001 |
| Retransplantation (early or late) | 2.9 | 1.5-5.7 | .001 |
| Fungal colonization‡ | 4.7 | 2.5-8.9 | <.001 |

*> 40 units red cells and platelets

† >3 days

‡ Deleted before or ≤ 3 days after OLT

moderate and high risk for fungal infection based upon the number of predictors present (Figure 3). The incidence of fungal infection was 4% (5/130) in patients with zero or one predictor, 22% (18/63) in patients with 2 predictors, and 51% (25/49) in patients with 3 or more predictors.

Other models can be constructed from these data, e.g. using baseline creatinine ≥ 2.0 mg/dl in lieu of a postoperative intensive care unit stay exceeding 3 days or retaining both of these variables and having a model with 6 predictors. The Kaplan-Meier curves generated by these alternate models are generally similar to that shown in Figure 3.

## Comments

Three studies have used multivariate analyses in the evaluation of risk factors for fungal infection after OLT. Although questions can be raised about the analytic methods or definitions of invasive fungal infection employed in these studies, their findings are similar to ours. In one study, independently significant risk factors included retransplantation, reintubation, bacterial infection, intraoperative transfusion requirement, pretransplant status (urgent status), steroid dose, vascular complication and antibiotic use (8). In a second study, significant risk factors for fungal infection occurring more than 10 days after transplantation were retransplantation, longer operative procedures (transplantation) and high transfusion requirements (4). Lastly, in a

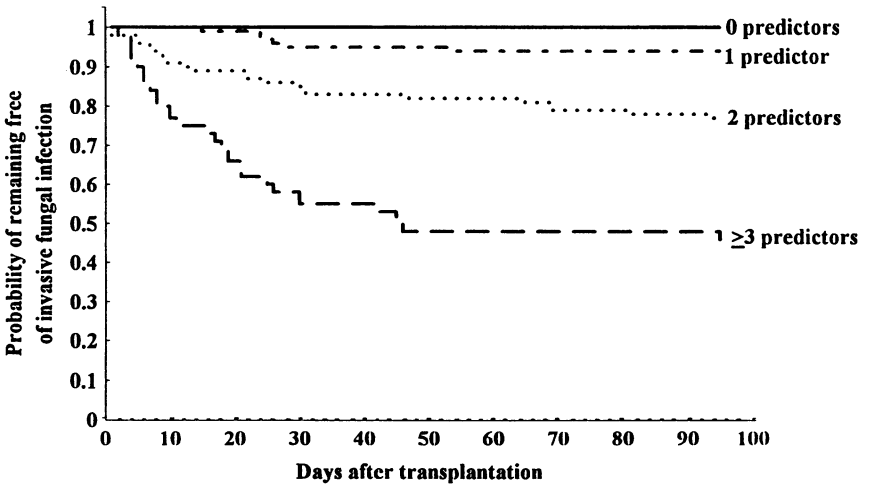


FIG. 3: Kaplan-Meier curve showing freedom from invasive fungal infection among OLT patients stratified by baseline predictors (posttransplant intensive care unit stay > 3 days, retransplantation, intraoperative transfusion [red blood cells and platelets] ≥40 units, early fungal colonization (≤ 3 days), and choledochojejunostomy).

study of aspergillosis after OLT, only elevated creatinine and use of OKT3 were found to be significant risk factors for infection (11). Independent risk factors identified in these studies as well as our studies, such as transplant operation time, repeat transplantation, intraoperative transfusion requirement, and choledochojejunostomy, suggest that difficult surgery constitutes a significant risk for fungal infection. Factors such as elevated preoperative creatinine, increased days in the intensive care unit after OLT, and additional abdominal and thoracic surgery reflect the likely role of increased severity of illness preoperatively and of a complicated postoperative course in the development of fungal infection. Although the impact of primary immunosuppression cannot be evaluated because all patients receive this treatment, enhanced immunosuppression, as utilized to treat rejection, was not an independent risk factor in our studies.

In summary, we have confirmed that invasive fungal infection is a major source of morbidity and mortality in patients undergoing OLT, that these infections occur primarily during the early months after transplantation, and that *Candida* species are the major pathogens causing these infections. Risk factor analyses suggest that difficult surgery performed on patients with increased severity of illness who experience complicated postoperative courses results in a high risk for fungal infection.

In addition, several potential strategies for reducing invasive fungal infection after OLT are suggested. The very strong association of CMV infection with fungal infection in both of our studies suggest that CMV infection may predispose patients to fungal infections. If so, prevention of CMV infection might reduce the number of fungal infections, particularly those that occur 2 or more weeks after OLT. A randomized double-blind, placebo-controlled trial evaluating prophylaxis with anti-CMV immune globulin in liver transplant patients suggested such an effect (12).

The ability to use baseline and early postoperative information to identify a population of liver transplant recipients at high risk for invasive fungal infection provides an opportunity to develop and evaluate the strategy of targeted antifungal prophylaxis or preemptive (presumptive) therapy. By targeting the use of antifungal agents to high risk patients, the approximately 50% of patients who are at low risk of fungal infection could be spared exposure to antifungal agents and thus avoid potential toxicities (including drug-drug interactions). In contrast to universal use, targeted use of antifungal agents in OLT patients, if effective, would reduce the pressure that selects and sustains resistant fungi and could possibly be a more cost effective strategy for prevention of fungal infection in liver transplant recipients.

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## DISCUSSION

**Dismukes,** Birmingham: In your comments on this very nice study on a large cohort of patients, you alluded at the end to the issue of fungal prophylaxis. In terms of risk factors for invasive fungal disease, fungal colonization was a major risk factor in the hazards analysis. Could you tell us what has happened since 1992 in your own hospital and other centers which do a lot of liver transplants? You were using only Nystatin suspension as a prophylactic measure. Have you gone to an oral azole agent like Fluconazole? You hinted about more discriminate use, perhaps no use in low risk patients, but tell us what is being done now.

**Karchmer:** Actually, we are not doing anything routinely at this point in time, although in the interval between completing this study and the present, we have participated in a randomized placebo-controlled trial of Fluconazole prophylaxis. One of the points of these data was well illustrated by a study from the Mayo Clinic and investigators in Spain that was presented at the ICAAC meetings just this past month. They did use Fluconazole in a placebo-controlled prophylaxis trial and, in fact, showed

benefit only in preventing superficial thrush infection and other superficial fungal infections. One possible reason for the outcome was that they had, in fact, excluded high risk patients from the prophylaxis trial and were prophylaxing all of them with Fluconazole. At this point in time we really have little data on the efficacy of more modern agents for fungal prophylaxis from randomized comparative trials. Nevertheless, the situation is that some centers are using it.

**Douglas,** Whitehouse Station: I was curious with regard to the suggestion. If you look at the multivariant analysis, it appears to me that what is really going on is that these are terribly sick people. The ones that are getting subsequent fungal infections are undergoing complex procedures and if they get in trouble during surgery, or later, they are the ones that more likely have longer procedures and are thus more likely to have fungal infections. If you give prophylaxis to that group with something specific for Candida, what is going to pop up next? What is your speculation on that?

Secondly, the CMV relationship is fascinating. In the renal transplant situation, it has been shown that CMV actually is the culprit in terms of damage to the kidney. I wonder whether CMV is merely a barometer of the immune status, indicating suppression both of the antibody system and the patient's immunity, white cell count, etc., that allows CMV and fungi to proliferate. Does CMV actually do something to enhance fungal infection in some other way and, therefore, will suppression of CMV, in fact, lead to fewer fungal infections? It is a very curious relationship and at least to my knowledge has not been adequately worked out.

**Karchmer:** I think you are absolutely right in summarizing the data that these are very sick people that have complicated operations and who have postoperative complications and this seems to place them at an inordinate risk of these invasive fungal infections. Whether one can prevent that scenario and enhance survival through prophylaxis, I think, is a question to be answered. Whether other fungi will move into that gap if you eliminate Candida is yet another question. Since much of what else has been seen is environmental, we have some strategies to apply. Initially we can try to reduce aspergillus and other micelial fungal exposure in the early post-transplant interface within the hospital environment. The role of CMV is very complicated and I don't really think that it can be understood until we have an effective way to intervene and prevent CMV infection. Then only will we be able to knock CMV out of the picture and find out whether this relationship due to severe immunosuppression and complicated postoperative care with CMV just another one of the emerging pathogens or whether CMV is, in fact, a causative immunosuppressive agent in this process. The antiglobulin prophylaxis data are a suggestion that CMV may play a causative role in enhancing immunosuppression and weakening of the host against these pathogens.

**Duma,** Bethesda: I did want to ask you if there are any data available or being obtained concerning receptors for Candida, particularly mucus membrane receptors? It seems as though for many years now we have gone through this agony of trying to utilize various antifungals, particularly Nystatin mouthwashes, to reduce yeast colonization, but what about some other approaches, such as interfering with or blocking receptors? What is taking place in this particular area?

**Karchmer:** Dick, I don't really know the answer to the question and I'm not sure that anyone does. Unfortunately, Jack Edwards who might be one of the few people who could respond to that knowledgeably isn't in the audience. Investigations in this area are in their infancy. We are really only beginning to look at the basic pathogenesis of Candida infection in the human at this point. It seems as though with this particular infection, as with many others, that colonization appears to be the first step. If we can interfere at this level, maybe we can begin to control some of these problems.