Discussion

Dr. Richard E. Wilson (Boston): I'd like to first discuss the question of disease and syndrome incidence.

Dr. Simmons attempted to perform what is called by the epidemiologists a prospective cohort study in 19 control patients which he mentions in the manuscript; here one starts with exposure and looks for disease.

We would like to know what percentage of transplant patients exposed to CMV disease developed it, and what percentage of these identified as having CMV developed the true syndrome that he mentions? Unfortunately, the 19 patients is a small group, but maybe he can tell us what has actually happened to those 19 people that he looked at prior to transplant.

It's difficult to compare those with 110 patients who actually had CMV disease, and then were looked at in retrospect.

The other approach is to do what's called a case control study, which starts with the syndrome, looking for CMV disease; in this you need a control group of transplant patients with the same complaints to determine whether or not they actually have this disease.

I don't think these data can answer these questions exactly, as to whether CMV is the cause of the complaint, rejection or lethality. I think a much larger prospective look at their transplant population will be necessary, and I hope they will do this, because they have developed these investigative tools, which are so necessary.

I'd like to focus on the question of the adjuvant effect of CMV infection on allograft rejection. We and others have noticed for many years that reducing or stopping immunosuppressive therapy to permit survival from severe pulmonary infection of all etiologies has almost always been associated with a rejection episode, while, interestingly, cessation of Imuran for hepatic toxicity or hepatitis rarely results in such a rejection. From the diagrams he shows in the manuscript, the cessation of Imuran, followed by rejection and the onset of CMV titer could all be the result of reduction of Imuran alone, with some unidentified viral agent giving the complaint, not necessarily the CMV virus.

Also in the chart of the lethal patient that he showed, it does not necessarily follow that this was due to CMV death; rather, a marked increase in immunosuppression, with wasting and lethality related to that, and the CMV virus being a concomitant.

What I'm really asking is: Is there a difference in the way the patients were treated, especially the ones who got the rejection episodes? Were their immunosuppressive agents reduced significantly, and, conversity were all of the patients who died those who had immunosuppression greatly increased, as part of the management of their disease?

Dr. Thomas C. Moore (Torrance, California): We, too, have been concerned with the problem of the high incidence of cyto megalovirus—CMV—infection in renal transplant patients. Dr. Milan Fiala, of the Infectious Disease Service of the Harbor General Hospital, has been conducting a collaborative study relating to this problem for some time now with our renal transplant unit at UCLA Harbor, and the unit of Drs. Berne and Payne at USC County.

(Slide) In a retrospective six-month-long study of 35 recipients, viremia was found in 43%, viruria in 66%, and complement fixing antibody rise in 26%, with an overall evidence of active CMV infection of 76% of recipients.

The incidence of viremia was significantly higher, 52%, in patients studied within one year of transplantation in comparison with those studied two years after transplantation, where it was only 16%. All 15 patients with arthralgia had active CMV infection. The incidence of other viral infections was comparatively low in this retrospective study.

Since February of 1973 a prospective study, currently involving 26 recipients, has been carried out, starting with the period of pretransplant hemodialysis, and continuing at monthly intervals for four to thirteen months after transplantation.

After transplantation, 38% of these patients had CMV viremia, 50% had viruria, and 77% had a rise in complement fixing antibody, for an overall incidence of active infection of 88%. One additional patient had autopsy evidence of CMV infection in the transplant, but no identification of virus or of seroconversion. Perhaps this patient corresponds to the fatal syndrome referred to by Dr. Simmons.

Reactivation of other viral infections, such as *Herpes simplex, varicella zoster,* adenovirus, measles, Epstein-Barr virus and hepatitis B virus were comparatively low, in comparison to CMV, in this prospective study, but were higher than was encountered in the retrospective study I have just cited.

Of the 26 recipients 20 of the 26 recipients in the prospective study, or 77%, had fever and arthralgia and a combination of other symptoms; chest infiltrate, SGOT rise, with elevated bilirubin levels 2 to 6 mg/%, and transplant rejection episodes.

Eighty-five per cent of the 20 recipients with fever and arthralgia, 100% of the four with chest infiltrates, 100% of the four with elevated SGOT and bilirubin levels, and 95% of the 19 with a rejection episode had active CMV infection. Only one of the four with elevated SGOT and bilirubin levels was positive for hepatitis B antigen.

Eighteen of the 19 recipients with rejection episodes had evidence of active CMV infection. Nine of the 19 with positive viremia studies were studied prior to rejection episodes. Two of these were positive at four and ten days prior to the rejection episodes, and seven were positive only after rejection episodes, these at eight, 11, 13, 28 and 47 days after the onset of rejection.

The fatal syndrome of the six cases cited by Dr. Simmons was encountered in only one of the recipients in our study as I mentioned earlier.

We did have three patients in our study with CMV viremia who had many atypical lymphocytes, with virtual disappearance, to less than five %, of both T- and B-cells. These three recipients did not experience a fatal outcome.

In order to investigate more thoroughly the source of CMV infection in renal transplant recipients, we recently have begun to augment the studies cited above by a study of cadaver donor blood and tissues and a study of Belzer machine plasma perfusates both before and at the end of machine perfusion preservation.

Dr. G. Melville Williams (Baltimore): We have had 16 patients with a diagnosis of cytomegalovirus disease in whom the cytomegalovirus was the chief pathogen involved. In this group of 16 patients, out of the 200 that have been transplanted in the past four years, we have had three deaths. The only death from infection occurring at Johns Hopkins Hospital in the last two years has been in one of these patients. In this group of 16 recipients of cadaveric transplants, only four continue to have functional kidneys. The remainder have had to have immunosuppression stopped and the kidneys have been lost.

What has interested us was the fortuitous finding present in open biopsies in all of these 16 transplants. Drs. Robert Wyllie and Norman Anderson in our laboratory have been fascinated by applying histochemical techniques in the study of transplant rejection. They noted, in the first patient of this series, little red dots which could be viral bodies in the proximal tubules when frozen tissue was stained with methyl green-saphronin. They suggested in this particular patient that we rebiopsy the patient in one month; while this lesion was at first focal, it became apparent that more tubules were involved with time. These little red spots stained positively for DNA, using DNA stains, suggesting that these cytoplasmic inclusions contained DNA and, therefore, added weight to the idea that this was a cytoplasmic phase of a DNA virus.

(Slide) This slide simply shows the degree to which tubular degeneration can be associated with this particular kind of red dot disease. We think that this is probably cytomegalovirus; for in 12 of 12 patients in whom we had sequential sera, there was a very good correlation between this finding and subsequent elevations in antibody two to three weeks later. In eight of the 12 cases, the virus was cultured from the specimen.

We have also worried about the seriousness of this problem. For example, we have screened dialysis patients and have found, not infrequently, patients with rising antibody titers. Should they or should they not be transplanted? One patient in particular was disturbing, for he developed "uremic" pericarditis and tamponade. His pericardium was resected and cytomegalovirus could be cultured from pericardial tissue but not from pericardial fluid.

This is an ubiquitous virus. Therefore, it's very hard to pin down with certainty whether this is indeed the chief pathogenic agent in the viral syndromes reported. However, I think studies such as those performed by the group at Minnesota are contributing to increasing the evidence favoring the concept that this virus in these patients is a major pathogen.

DR. C. W. PUTNAM (Denver, Colorado): We can confirm some aspects of Dr. Simmons' very interesting paper.

For example, we have documented that almost every transplant recipient under immunosuppression has evidence, either serologic or by culture, of viral infection, which is often chronic. We would tend to emphasize that this evidence may develop without fever, leukopenia, rejection, or any other significant clinical manifestation. This does not mean, as was Dr. Simmons' main message, that these are harmless infestations.

I hope in the future that at least three questions, which are subtle ones, may be answered: First, does the development of antigen-antibody complexes in these patients occur? And if so, do they contribute to the glomerulonephritis mentioned earlier this afternoon?

Second, what role, if any, does chronic viremia, including viruses other than the Herpes group, play in the increased incidence of malignancy in chronically immunosuppressed patients, particularly the skin cancers mentioned by Professor Ewing of Melbourne?

Finally, I draw your attention to the speculation, published by Corman and others from the Colorado group, that CMV infection of the bile duct epithelium has caused at least some of the biliary obstructions that have plagued efforts at liver transplantation.

Dr. Simmons' paper opens a Pandora's box of possibilities, of which none has been absolutely established, but all of which deserve exploration. The transplant fraternity owes him a debt of gratitude for doing this.

DR. RICHARD L. SIMMONS (Closing discussion): To Dr. Wilson, who wants to know whether these patients are retrospective; of course, it is in part retrospective and partly prospective. At first you study the patients who are ill, then you study the patients who have been transplanted but before they become ill, and finally you start studying those who are not yet transplanted. That's why I focused on the 46 patients who had no evidence of infection in the posttransplant period and who were, in a sense, prospective, but not classically so.

To answer your direct question: What per cent of the patients who were studied prior to transplantation got the virus? Sixteen of 19, 84.5%, of the prospective patients developed the viral infection. Notice that 83.5% of the 132 patients studies over all also had the infection. Thus, the prospective and retrospective studies show remarkably similar results similar to Dr. Moore's 88%, and to 70–90% in the literature. The patients without infections are very important. We almost never see fever or leukopenia in patients without infection despite the fact that there are many other potential causes of fever.

Which comes first, the rejection or the infection? There are a number of pieces of evidence in the literature that rejection can stimulate lymphoproliferations, and that lymphoproliferation can lead to reactivation of a latent endogenous virus. For example, leukemia viruses can be activated by a graft-versus-host disease. Thus, we must assume that rejection can stimulate infection.

But it is also well known that infections can trigger rejection. A beautiful study in our laboratory by Dr. Richard Howard has shown that graft rejection can be accelerated by infusing murine cytomegalovirus into a mouse who is immune to CMV. On the other hand, CMV is a strongly immunosuppressive viral infection if the animal is not previously immune.

Dr. Moore brings up a lot of other problems. We have rarely found other viruses. Unfortunately, we have not looked for Epstein-Barr virus, which has been found very frequently in England. Clinical hepatitis, we think, is very commonly caused by cytomegalovirus, especially in a chronic form long after transplantation even in patients who show high titers of complement fixing antibody against CMV.

Again, the source of the virus is very important. We have cultured bank blood and everybody that has cultured bank blood finds that it's very difficult to culture CMV from bank blood. The virus is in the buffy coat, and can be cultured from fresh blood. The postperfusion syndrome in cardiac patients may be due to CMV in fresh blood transfusion. Culturing of kidney biopsies has failed to reveal the virus, and culturing of the preservation fluid has failed in every case to reveal the virus.

The source of infection may well determine the clinical syndrome seen. It is possible, for example, that the first infection from an exogenous source in a non-immune patient results in profound immunosuppression (as in mice) leading to death. However, if the patient is immune to the cytomegalovirus and reactivates the endogenous virus, the infection may act as a terrific immunologic adjuvant leading to a rejection episode.

Dr. Williams has been smart enough to use a special stain on his kidney biopsies, and probably is demonstrating the cytoplasmic phase of the cytomegalovirus. We're going to try that as well.

Dr. Putnam again raises the question of how frequently we see the virus, and how rarely we see a clinically important syndrome. I think the syndromes are frequently very minor. I do think that if you do the prospective study, it's very likely that you will find fever, at least, very frequently appearing with the virus. He also brings up the possibility that these viruses may be involved in the pathogenesis of cancer. Herpesviruses like CMV are known carcinogens in animals, and are highly suspect as carcinogens in man. Herpesviruses persist in latent form, they cause cancer, and transplant patients have a high incidence of cancer. This relationship obviously needs to be studied further.