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DISCUSSION

DR. ISRAEL PENN (Denver, Colorado): I would like to confine my comments to one aspect of the authors' findings, and that is the development of lymphomas in two of their 24 patients. A well recognized complication of various forms of conventional immunosuppression is an increased incidence of certain malignancies. (slide) Lymphomas, mostly of non-Hodgkin's type, have an incidence 45 to 100 times greater than that seen in aged-matched controls. Skin cancers are increased about sevenfold in areas of low sunshine exposure, and are increased about twenty-onefold in areas of high sunshine exposure. The other type of common malignancy in these patients is *in-situ* carcinomas of the cervix of the uterus, which are increased approximately fourteenfold over their incidence in the general population.

(slide) In the last 1229 patients that were reported to the Denver Transplant Tumor Registry, skin and lip cancers made up 501 malignancies, solid lymphomas 248, and carcinomas of the cervix, most of which are *in-situ* carcinomas, made up 97 of the neoplasms.

If we take these crude figures, the lymphomas make up approximately 18% of the malignancies. However, if we exclude nonmelanoma skin cancers and *in-situ* carcinomas of the cervix of the uterus, which are excluded from most cancer statistics, then the lymphomas become the most important single group, making up approximately 26% of all cancers. This contrasts with a 3 to 4% incidence in the general population.

There has been some experience with the development of malignancies following the use of TLI in patients with Hodgkin's disease, and this was reported by Kaplan and his colleagues at Stanford (*Transplant Proc* 1981; 13: 425-428.). They found that if TLI only was used in the treatment of more than 300 patients with Hodgkin's disease, there were no cases of leukemia or lymphoma. However, when TLI was used in conjunction with cancer chemotherapy in nearly 700 patients, there was a 3 to 5% incidence of leukemia or lymphoma.

A similar situation may be present in renal transplant recipients who receive not only TLI but other forms of immunosuppression, as you have heard, such as splenectomy, Imuran, and prednisone.

At first glance, the incidence of lymphoma in Dr. Najarian's series may appear to be rather high. However, the series is a small one, and with further experience it is quite possible that the incidence of lymphomas will be no higher than that seen with other forms of immunosuppressive therapy.

DR. PAUL S. RUSSELL (Boston, Massachusetts): As I see it, clinical transplantation is in an extraordinarily interesting phase right now, with at least five major possibilities for early advances in the control of rejection reactions. Those five are the following: the management of blood transfusions from the donor or from other individuals—it is not clear which is better; the possibility that the alteration of what are called "passenger leukocytes" in the donor tissue, and particularly from among that class of cells, the dendritic cell population, which have been found to be ubiquitous through many of the organs of the body, and are believed to be strongly immunogenic. Perhaps eliminating these cells in certain ways will make quite a difference to the antigenicity of transplants.

Third, Cyclosporin A you have heard quite a lot about, and for my lights, properly so; I think it is important, but I am not so sure it will be all important.

Fourth, TLI is a very interesting possibility that you have just heard raised for immunosuppression. Finally, our particular interest has been in the use of antibodies, especially monoclonal antibodies, directed toward lymphoid cells, and, in particular, subclasses of T-cells.

Now, the Minnesota group has reported in this paper the use of TLI for what are termed "immunoreactive" patients. I share their belief that there probably are such individuals. One could imagine that there are at least two reasons why patients might be particularly immunoreactive. One is a non-specific and poorly understood status of greater reactivity. Perhaps a lot of things may be involved in this, nutritional status, and other things must have a lot to do with one's inert immunoreactivity. Genetic factors are also known to play a role.

Also previous exposure to transplantation antigens will, of course, make an individual specifically more immunoreactive to those same antigens seen again, and we make every effort to avoid that kind of reactivity by appropriate cross-match tests before transplantation.

So this group of patients selected by John Najarian and his colleagues may be quite a mixed bag; if, in fact, previous immunity is part of their increased reactivity, cyclosporin A or TLI will be relatively ineffective as they are not very active against preexisting immune reactions.

Now, in our small studies with primates using TLI in cynomolgus monkeys, Dr. Gary Haas and Ben Cosimi have been looking carefully at what happens to T cells in the course of treatment with 2000 rads over three weeks. These cells do plunge right down in numbers, as John Najarian showed. It is interesting that in returning back up toward normal levels at the cessation of the radiation, the suppressor cells seem to come up quite a lot faster than do the helper/inducer subset cells. Whether that is important or not, I do not know, but I do know that if we put heart transplants into those animals during the period of their lymphopenia, they will do a great deal better than if you wait a few days until after the cells start to return again to the circulation. This raises the question I would like to ask Dr. Najarian.

Does he think that the timing of the last dose of radiation makes very much difference in regard to the time when the transplant is put in?

DR. ANTHONY P. MONACO (Boston, Massachusetts): I would like to follow up on the remarks of Dr. Wilson and Dr. Russell.

The fundamental issue up to this time for those patients who have rapidly rejected their transplants within 12 months, statistically, in North America, and within six months in European studies, is the fact that these individuals reject their transplants with the formation of broadly reactive cytotoxic antibodies to a large panel of HL-A antigens represented in the human population. Therefore, these individuals become untransplantable by virtue of the fact that, first, they reject their kidneys, and secondly, up until this time, we have not been able to find a kidney for them that theoretically we could transplant because of broadly reactive antibody to most donors.

Now, Dr. Najarian has clearly shown that when patients like this are subjected to TLI, they do not change their antibody titer during

that period. Therefore, my question is, in those 17 cadaver transplant recipients, (1) I presume they had broadly reactive antibody; (2) was this broadly reactive antibody such that it contained antibodies against the antigens of donated kidney?

If this is true, then this is a most important observation, because it would then permit us to transplant individuals who up to this time have been classified as untransplantable. I think this is the fundamental issue of this outstanding paper.

DR. JOHN S. NAJARIAN (Closing discussion): First, we are all, in transplantation, indebted to Sol Penn and the group at Denver for keeping a transplant tumor registry. It is extremely valuable, providing us with precise figures on the frequency of various tumors in these patients to compare with tumors in nonimmunosuppressed patients.

Both of our patients who eventually developed lymphomas, had the longest interval between completion of irradiation and transplantation. In answer to Dr. Monaco's question, an extended wait from radiation to the finding of a suitable transplant usually means the presence of positive antibodies. As a result we gave small doses of irradiation, so that both these patients who developed lymphomas had "excessive" TLI: one had a cumulative dose of 3200 rads, and the other had almost 4100 rads. This is too much irradiation; 2500 rads is adequate.

In addition, we feel that anything that will immunosuppress (whether it be cyclosporine, azathioprine or prednisone), will, if used in excess, make the patient susceptible to tumor formation, and lymphoma in particular, as Sol Penn has so nicely shown.

Paul Russell talked about the subclasses of T cells. We too have been interested in following the subclasses of T cells in our transplant patients. And even though the T cells do recover in number, those that do (as he found in his primates and we found in the human primate) show a reversal of the OKT-4/OKT-8 with a depression of helper cells, and an increase in "suppressor" cells.

It is important that the transplant be done as rapidly as possible following the full course of irradiation. As I pointed out, the ideal preparation is no more than 2500 rads given no more than two weeks before transplantation. If you go beyond two weeks, you lose some of the effect.

How long does the TLI effect last? Some effect lasts as long as one year to two years with depression of MLC reactivity of the T cells, but in some patients this returns more rapidly. Recently, in the *New England Journal*, two articles appeared (back-to-back) on the use TLI in the treatment of arthritis. One group, from Boston, showed that the immunoreactivity returned within the year. The group at Stanford reported that the effect was sustained for much longer. What the actual answer is, I do not know, but I would suspect that individual variation does occur.

To Richard Wilson, the preformed antibodies that were present in these patients delayed us from getting suitable donors promptly. In

one patient a gastrointestinal hemorrhage forced us to remove the kidney at about two weeks post-transplant. Incidentally, this kidney showed marked vascular rejection—very few cells were seen on histologic section. In fact, we know that the vascular (humoral) response is not effected by TLI. The kidney was not being rejected, but the vascular component of rejection, the complement and antibodies were present within the kidney. TLI only affects, as far as we can see, cellular components of rejection.

Finally, Tony Monaco asked an important question about cytotoxic antibodies. That is one of our major problems. We have difficulty finding a donor on these patients because, obviously, they have high numbers of cytotoxic antibodies to the panel of unselected target cells. This has been the major problem of effecting the logistics of this technique.

We have never successfully transplanted one of these patients against a positive T-cell crossmatch. It is not helpful in that regard. We must find a negative T-cell crossmatch before we can transplant.

I think the role of TLI is established in transplantation. It should be part of the armamentarium of transplant surgeons. TLI is very effective in those patients who are highly immunoreactive. Cyclosporine may also be effective in this group of patients (early results). If this proves to be true then TLI can be used for those patients that need a second graft after having rapidly rejected their graft while on cyclosporine. Thus, if cyclosporine fails, TLI is a good thing to have in your back pocket.

DR. RICHARD E. WILSON (Boston, Massachusetts): As an ex-transplanter, I want to congratulate John for this very neat exposition of the use of a selective form of total body radiation.

I think there is one problem that Dr. Russell did not list in his group of five, and that Dr. Najarian mentions, but I would like to ask him more about it—these immunoreactive patients. Do they have either preformed antibodies, or are they making an anamnestic response so quickly that it is the vascular antibody-mediated injury that is getting them?

If so, I think that he is well aware of the work that we did in the past with the Fab₂ material that we presented at the transplant conference here in Boston a year or two ago. We did a randomized trial and showed that in those patients receiving multidose FAB from multiple individuals sensitized against humans, we were able to totally prevent the anamnestic type of rejection. None of those patients, all cadaver recipients, had a rejection in the first five months after transplant, and none of them ever had a vascular type of injury.

I do not think that what you are treating at this time is protective against that preformed vascular injury, and I would like to know if you have any plans to try to use something such as the Fab₂ to add to your present protocol. All of those survival slopes still show the early loss in this type of patient.