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Discussion

DR. FRANCIS D. MOORE, SR. (Boston, Massachusetts): I would like to ask two questions and make one suggestion. The first question is one of histology. Dr. Rosenberg, do you have any needle biopsies or information on your patients while they are under treatment to demonstrate increased infiltration with lymphocytes or any change in the staining of those lymphocytes or in the activity of those lymphocytes to indicate that it is truly lymphocytic activation which is producing these responses? That seems like a simple, naive question, but I would like to know if you can confirm histologically that the response to IL-2 stimultes lymphocyte attack on the neoplasms.

The other question has to do with the illness produced by giving IL-2. You naturally didn't have time to go into that in detail. Several of your patients were in the intensive care ward while they were under treatment. I would like to know the nature of the response to IL-2. How sick does it make patients? And in what way does it make them sick?

Now, my suggestion is based on the fact that in the second half of this century, two new approaches to malignancy have been very encouraging. And they have both been pursued from their initial discovery to their present-day use by Fellows of this Association. One is the immunologic approach pursued by Dr. Rosenberg and several other of our colleagues. The other is based on neovascularization in tumors, an approach first described by Dr. Folkman, who is here this morning. I would just express the hope and the suggestion that Rosenberg and Folkman would study the two modalities together. A tumor which has been compromised by immunotherapy might be especially vulnerable to treatment directed toward its neovascularization. Such might be done with endostatin or somatostatin. Vice versa, a tumor that has had its vascular supply compromised might be very sensitive to immunological therapy. I hope they will get together and work out a combined approach. Thank you for a wonderful paper.

DR. STEVEN A. ROSENBERG (Bethesda, Maryland): Thank you. When I came to the Peter Bent Brigham Hospital as an intern in 1963, Dr. Moore was the chief there. He was then and remains now the smartest person I know. As surgeons, we all stand taller because of his accomplishments. Thank you, Dr. Moore, for all you have done for me.

In answer to the questions: We have had the opportunity to biopsy lesions sequentially during the course of treatment in patients that have multiple subcutaneous or cutaneous nodules and in those patients we can obtain snapshots of what is occurring as the tumor disappears. We see increasing infiltration of lymphocytes into the lesions as they regress. Recently we have developed techniques for growing lymphocytes and tumor from fine needle aspirates so we can study the same lesions sequentially during the course of treatment.

Interleukin-2 administration is associated with toxicity. The earliest physiologic change we see is a decrease in systemic vascular resistance which can be associated with hypotension and tachycardia. We have learned to effectively control these side effects. In the first 157 patients tested we had four treatment-related deaths but, recently we have treated over 800 consecutive patients with no treatment related mortality. I believe treatment with high-dose IL-2 can be administered safely by those with experience, certainly as safely as most combination chemotherapies in common use.

I agree that combining these immunotherapeutic approaches with the anti-angiogenic approaches that have been pioneered so eloquently by Dr. Folkman is an extremely fruitful area for investigation. Some of the effects of cytokines, such as Interleukin-12, appear to be mediated by inhibiting angiogenesis. Dr. Folkman and I are in frequent contact and I think the conjunction of these two approaches will be quite productive in the future.

DR. JUDAH M. FOLKMAN (Boston, Massachusetts): This is a very important paper by a pioneering surgeon scientist. Why is it important? Well, for one, there is no other therapy today that can provide such prolonged, durable tumor regression of melanoma or renal cell carcinoma. Secondly, this approach depends upon therapy that does not attack the tumor cells directly. And thirdly, this paper forces us to ask two additional questions: What is the mechanism for the durable response in the 6% to 8% of patients? And what is the mechanism of the nonresponders?

Dr. Rosenberg has emphasized that the detailed mechanism of Interleukin-2 for its ability to cause tumor regression and durable response is not clear. His hypothesis, a very good one, is that Interleukin-2 expands the population of T cells capable of recognizing specific tumor antigens on the surface of melanoma cells or renal cells. This is very likely, but there could also be an additional response that is working; that is, T cell attacks against the endothelial cells on their way into the tumor. One clue in his manuscript is that Interleukin-2 causes a transient decrease of lymphocytes in the circulation and a rapid rebound when Interleukin-2 is discontinued.

Now, a rapid rebound of leukocytes after discontinuing therapy usually means that it is not the bone marrow that is suppressed, because the recovery is too rapid. It usually means that the lymphocytes have marginated to the side walls, where they either stick or roll slowly. And this has been seen also for interferon alpha, but on a smaller scale. For a large population of leukocytes to disappear and reappear, there has to be a large surface area. In fact, the surface area of the endothelium is about 1000 square meters, about a tennis court. Proliferating endothelial cells may be particularly vulnerable to activated T cells by IL-2. And this is testable.

Another clue that Interleukin-2 activated T cells may attack the endothelium is the vascular leak syndrome. Big vascular leaks such as Dr. Rosenberg has described in some patients could be transient damage to endothelial cells leaving spaces between them. In fact, a professor at the Mass General showed in a recent paper by video microscopy in transparent skin chambers in animals that wherever T cells adhere to endothelium there was pouring out of plasma into the tissue between those endothelial cells and that this could be reversed. He also showed that when Interleukin-2 was given to tumor bearing animals, that lymphocytes began to roll toward the tumor and then stick in the endothelium, and some extravasated but many simply damaged the tumor vessels.

Finally, why the non-responders? Could the tumors in those patients somehow prevent T lymphocytes from adhering to tumor vessels, thus causing local tolerance? It turns out that most human tumors secrete for their beginning antigenic factors either fibroblast growth factor or vascular endothelial growth factor, one or the other. Those that secrete basic fibroblast growth factors do in fact prevent lymphocytes from sticking, and lymphocytes stream right through, they don't stop in the tumor vessels. Those tumors that use the antigenic factor VEGF increase lymphocyte sticking.

Could you tell the difference in advance? Well, these two antigenic proteins can be measured in blood or urine. But this would have to be done prospectively. And Dr. Rosenberg and I have discussed this. A better way would be to look in the biopsies of tumors in the paraffin blocks. However things turn out, I congratulate Dr. Rosenberg because he has changed our thinking about cancer therapy in a fundamental way. And that is, not to attack the cancer cell directly, which gives you toxicity and drug resistance virtually always, but to instruct the host cells as best we can to dispose of tumor cells.

Both immunotherapy and anti-antigenic therapy are headed in this direction. And as Moore suggests, they may soon join each other. And as Dr. Moore suggests, Dr. Rosenberg and I discussed more and more, even though I was trained at the Mass General and he at the Brigham, now that the two hospitals are combined, it is okay to do this.

DR. STEVEN A. ROSENBERG (Bethesda, Maryland): One of the areas that we are actively pursuing now involves attempts to generate immune reactions not against the tumor itself, but, rather, against some of the unique molecules that Dr. Folkman has described on the tumor microvasculature such as the receptor for vascular endothelial growth factor, which is up-regulated in those vessels. Perhaps specific immune reactions attacking those molecules can lead to tumor destruction.

DR. DONALD L. MORTON (Santa Monica, California): I just wanted to share with you a little different approach to stage IV melanoma, in which we induce a complete surgical regression by resecting all metastatic sites and then give the patient a polyvalent melanoma vaccine as a postoperative adjuvant. And in 113 patients with surgery alone we have a median survival of seven months and a 5-year survival of 15%; whereas in 150 patients with postoperative polyvalent vaccine, we have a median 39 months and a 5-year survival of 42%, which in our experience is extraordinary. Based upon these findings, we have just initiated a multi-center randomized trial with this approach.

I would like to ask you what your overall median survival is with IL-2? It is very clear that the durable responses are extraordinary. But what is your overall median survival in melanoma patients? Secondly, I would like to ask how you can be sure that the particular clones of TILs that you have identified are the ones that are mediating the regression *in vivo*? Have you been able to expand specific clones of CTLs and show that when a single clone is administered back into the patient, you get a regression?

Finally, that fascinating observation that active immunotherapy with specific antigens appears to work when combined with IL-2 but not alone or with IL-12. I wonder if you have any ideas as to the reason for this dramatic difference in responses?

DR. STEVEN A. ROSENBERG (Bethesda, Maryland): Dr. Morton has been one of the early pioneers in the field of immunotherapy, and I appreciate his comments. The long-term survival in this patient population, many of whom have fairly bulky metastatic cancer, is about 20%. As you suggest, if one surgically debulked these patients to make them clinically disease free prior to administering the treatment, one might well be able to improve upon those results.

We have actively studied whether the cells that we administered traffic to the tumor site are responsible for the tumor regression. In earlier experiments with these TIL we inserted a marker gene, neomycin phosphotransferase, that would enable us to traffic these

cells and identify them at various locations in the body. We found localization of these cells to tumors.

These cells can now be cloned, and in a planned protocol submitted to our Investigational Review Board we will study the administration of these cloned populations of TIL to patients. That trial will hopefully start within the next three to 4 months.

Why does IL-2 work and not IL-12? IL-2 is probably the most potent factor we have for growing activated lymphocytes, not only in the laboratory but also *in vivo.* We can often see diffuse lymphocytic infiltrates throughout tissues when we give Interleukin-2. We hypothesize that the combination of vaccination and Interleukin-2 works in two stages. The immunization results in an increase in the number of precursor cells capable of recognizing the tumor, and the Interleukin-2 then activates and further increases those populations *in vivo.*

DR. CHARLES M. BALCH (Duarte, California): As all of you know, stage IV melanomas are a very heterogeneous disease. And there are small subsets of patients who have very long-term survival, particularly those who have surgery. In fact, I have patients who have been referred to me for surgery who now have 5 to 10, even 15 years survival, even with these multiple visceral metastases that were surgically resected.

In a series of patients treated at the M. D. Anderson Cancer Center with a variety of drugs and immunotherapy protocols over 10 years, the only common denominator of the long-term survivors were those who had surgery as part of their treatment for stage IV disease. So there must be some kind of selection for those patients referred for surgery that yields long-term results as a part of the natural history of their disease.

My question, Dr. Rosenberg: In your experience through the years, is there anything that you could identify that may be different about patients who come to the NCI or that you select for these immunotherapy trials that might influence their incidence of complete responses and the duration of survival that is higher than that experienced at other centers who may be less selective or who have not otherwise been able to reproduce these results with IL-2 with or without LAK cells or TIL cells?

The second question: Have you or do you plan to do any controlled comparison of IL-2 with other biological agents like interferon? And the third question is: How many of your patients had surgery as a component of their therapy at any time in the course of their metastatic disease which may comprrise a subset with more favorable outcome compared to those whose natural history of metastatic melanoma did allow for surgical intervention?

DR. FRANK C. SPENCER (New York, New York): All of these questions about this exciting paper have a similar theme: are the changes seen in molecular biology "cause and effect" or a "spectator" phenomenon? Are these chance association? The paper opens an exciting and important frontier; important questions remain for future studies.

DR. STEVEN A. ROSENBERG (Bethesda, Maryland): The referral of patients to the National Cancer Institute is by physicians who generally have treated these patients unsuccessfully with standard available methods. We are very aggressive in surgically resecting metastatic disease. But, the great majority of the patients we accept in these trials have widespread disease not amenable to surgical resection. Our results with high dose Interleukin-2 for the treatment of patients with metastatic melanoma have been repeated by many groups and on the basis of those studies, the Food and Drug Administration just three months ago approved high dose Interleukin-2 for the treatment of patients with metastatic cancer.

Surgery for the treatment of metastatic melanoma certainly has a role. Surgery is in fact the best method for treating metastatic cancer in many patients with solid tumors. Chemotherapies can cause regressions but are rarely curative for patients with bulky solid tumors. I agree that surgery should be aggressively pursued in patients who have resectable metastatic melanoma. We have tried combining Interleukin-2 with interferon in a prospective study and saw no improvement over the use of Interleukin-2 alone but it did add to the toxicity. As we explore these immunotherapies and as new biologic molecules become available, I think studies of combination immunotherapies with varied reagents and cells, will be very productive in the future.