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Discussion

DR. CHARLES M. BALCH (Houston, Texas): Dr. Rosenberg has made major contributions in this important and evolving area of research involving biologic therapy. His research represents a blend of both excellent preclinical models and well-controlled clinical trials. I have a few comments relevant to the lymphokines he discussed today and would like to ask two questions.

(Slide) The battlefield for host–tumor relationships is within the tumor, and here again Dr. Rosenberg has done pioneering work in examining TIL and exploring their use as a treatment strategy. There is a profound defect of the lymphocytes that are recovered from such cancer as melanoma and renal cell carcinoma in that they cannot bind or kill autologous tumor cells. However this deficit can be corrected *in vitro* by adding Interleukin-2, and Dr. Rosenberg proposes that at least in some patients you can augment cellular immune responses *in vivo* as well as by administering Interleukin-2 in combination with other lymphokines.

We have studied these TIL extensively and found an extraordinary diversity of the immune responses. However some patterns emerge as shown in this study of more than 120 human tumors in which we classified the subtypes of lymphocytes that emigrated into the tumor and their growth rate with Interleukin-2. In melanoma most of these cells are T-cells with a cytotoxic phenotype ($CD8^+$). There are almost no NK cells. Renal cell carcinomas, on the other hand, have both T-cell subsets and NK-cells, as do sarcomas. Breast cancers and colon cancers are different yet again. The cytotoxic capacity of these cells is also quite different among various human cancers. Thus TIL from distant metastatic melanoma has an efficient level of cytotoxicity that is restricted to the patient's own tumor (*i.e.*, they cannot kill allogeneic cells). TIL from lymph node metastases from melanomas are very inefficient cytotoxic effector cells. TIL from renal cell carcinomas are different from those in melanomas because these lymphocytes have the capacity to kill both autologous and allogenic tumor target cells.

Because biologic therapy is an indirect approach to eliminate cancers by augmenting an immune rejection response, one would expect that there would be some variations from tumor to tumor and site to site, and would also emphasize an important part of Dr. Rosenberg's treatment strategy in that he used multiagent immunotherapy using agents with different mechanisms of action.

I would like to ask two questions. First do you have any idea about the nature of the functional defect of these tumor-infiltrating lymphocytes that appear to be overcome *in vitro* by adding back pharmacologic doses of Interleukin-2? Second what is the relative contribution of the expanded lymphocytes that are included in the Interleukin-2 regimens? That is how do you know that the TIL or LAK cells are contributing significantly to these tumor responses *in vivo*? Do you have data now from your studies, either by trafficking or clinical trials, showing the relative contribution of adoptive immunotherapy compared to the therapeutic effect of using the lymphokines alone?

DR. DONALD L. MORTON (Los Angeles, California): To Dr. Rosenberg must go the credit for ushering in the modern era of immunotherapy with cytokines, in combination with adoptive immunotherapy with lympoid cells. I admire Dr. Rosenberg, not only for his scientific advances, but especially for his tenacity and hard work in dealing with 652 critically ill patients with hopeless malignancy who have undergone the toxicity he has described with IL-2. The response rates of 21% to 35% in disseminated melanoma and renal cell cancer are impressive. The even higher response rates of 35% to 50% with alpha interferon and IL-2 and TIL-IL-2 are even more significant when one considers that these tumors are refractory to chemotherapy.

It is perhaps significant that these responses are all or none, which is different from chemotherapy in which there are often only partial responses in some metastatic sites. I want to ask Dr. Rosenberg two questions: Does he have an explanation for this all-or-none phenomenon, and does he know the target structure to which the IL-2 LAK cells are directed?

Our own work has concentrated on active specific immunotherapy with tumor cell vaccines, and I thought this might be a chance to give the Association a brief update. We have used a whole cell vaccine composed of three allogeneic melanoma cell lines irradiated to 10,000 rads and administered intradermally. Low-dose cyclophosphamide has been used as an immunomodulator and compared this with tumor cell vaccine alone. The early results in the 300 mg/M^2 dosage shows no difference between tumor cell vaccine alone and tumor cell vaccine with cyclophosphamide.

However if one compares the results we have seen with vaccine immunotherapy with those previously seen with chemotherapy in disseminated melanoma, we see that the patients receiving vaccine with or without cyclophosphamide do significantly better. The median survival for chemotherapy is 6 to 9 months *versus* 16 months for immunotherapy; the 40-month survival for immunotherapy is 30% *versus* 5% for chemotherapy.

We began to see a rise in antibody titer to one or more of the seven melanoma-associated cell surface antigens 4 to 8 weeks after the start of immunotherapy. Regression of evaluable disease begins at about 3 months.

(Slide) This is a patient with extensive recurrent melanoma over the ear, face, and neck, refractory to chemotherapy and radiation therapy treated with this vaccine. After 4 months, partial regression is observed. After 10 months it is almost complete, except for some small, residual disease on the cheek, which is completely gone at 19 months. This patient is free of disease at 36 months.

Of 25 patients treated with evaluable disease who were treated, we have had two complete regressions and an overall response rate of 25%. It is interesting that the responses we see with active specific immunotherapy are slower in their evolution, but are of more durable duration than the responses we see with chemotherapy.

DR. JEROME J. DECOSSE (New York, New York): May I ask Dr. Rosenberg a point of clarification. Were any of the patients described in your talk also treated with either radiation therapy or chemotherapy and can you exclude an effect of these other modalities?

DR. JONATHAN L. MEAKINS (Montreal, Canada): Last year Dr. Wilmore presented a paper that showed that ibuprofin could control some of the symptoms associated with IL-2, and the question was raised at that time whether that would affect any of the antitumor effects of LAK cells and IL-2 or other cytokines.

I wonder if Dr. Rosenberg could tell us whether he has been looking at this. It may have very real implications for the acceptability of this form of therapy as well as its more general applicability in other than very highly specialized centers.

DR. THOMAS C. MOORE (Los Angeles, California): Dr. Rosenberg's report of his innovative work with lymphokines alone and in combination and his use of tumor-infiltrating lymphocytes is most impressive. It is not unreasonable to assume that these lymphocytes are in tumors for a reason and Dr. Rosenberg and his associate have made important advances in exploring this intriguing potential.

I wish to ask Dr. Rosenberg if he and his associates have considered

the involvement and use of other types of soluble mediators such as potent vasoactive neurotransmitter substances? I am thinking of immunostimulative substances such as bradykinin and substance P, which are involved also in inflammation and pain transmission.

We have observed and reported that intralesional administration of bradykinin in hamsters has produced malignant tumor growth slowing and regression. Bradykinin *in vitro* has increased the antitumor cell cytolytic activity of spleen cells from tumor-bearing animals whose tumor growth has been slowed or stopped by intralesional administration of bradykinin.

Professor Robert Fauve, of the Pasteur Institute in Paris, has made the important observation that malignant tumors in Lewis rats may produce an anti-bradykinin substance that inhibits bradykinin-induced macrophage mobility.

Because these and other vasoactive neurotransmitter substances influence lymphocyte levels of cyclic nucleotides, lymphocyte transformation by mitogens, and antigen and lymphocyte production and release of lymphokines such as interleukin 2 (IL-2), their usefulness in clinical cancer control is worthy of serious consideration.

DR. STEVEN A. ROSENBERG (Closing discussion): Studies of these tumor-infiltrating lymphocytes (TIL) reveal that they are heterogeneous. Cells that exhibit major histocompatability complex (MHC), restricted lysis, and nonrestricted lysis, are present. The demonstration that these TIL can have specific lytic reactivity against the tumor from which they are derived and not against other tumors or normal cells from that patient, probably represents the best available evidence we have today that at least some patients with cancer do mount immunologic reactions against their own established cancers.

There are several possible reasons that the TIL already present in growing tumors do not inhibit tumor growth in the original host. We give about 2 to 3 $\times$ 10^{11} lymphocytes. That is probably equivalent to the entire lymphocyte pool of the individual. These cells are grown to those large numbers in culture and part of the explanation might well be a quantitative one. The second is that there are suppressive influences that exist in the body that may affect the differentiation of these TILs that do not exist when we educate them to react against tumors outside the body.

We have recently completed a prospective randomized trial of 181 patients randomized to receive either IL-2 alone or LAK cells and IL-2. The incidence of complete responses is statistically significantly increased in those patients receiving LAK cells.

We do not now understand why some patients respond and others do not and have been unsuccessful in identifying correlates of response either in the patient or in the patient's tumor cells. We don't know the target structure that is recognized by these immune cells. We do know, however, that the lysis can be MHC restricted and that antibodies against class I histocompatibility antigens can inhibit lysis completely.

We have treated 19 patients with cyclophosphamide and IL-2 and saw two partial responses. We also treated 32 patients with monoclonal antibodies. The toxicity data, however, included all of the 652 patients. I should mention that no patient received any other form of therapy other than the immunotherapy for the 1 month before or throughout the protocol follow-up period, so all of the responses are, in fact, due to the immunotherapy and not to other concurrent treatments.

In terms of abrogating the toxicity due to IL-2 administration, we have looked at the ability of steroids to overcome many of these side effects. Unfortunately the administration of high-dose steroids also aborts the therapeutic effects of this immunotherapy.

Finally, we have not yet begun looking at other vasoactive substances administered in conjunction with IL-2, but that is probably an area worth investigating.