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Report on the Discussion of the Fourth Session

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DR. YALOW: I was impressed by the absence from the data you presented of the use of radioactively labeled antibodies in immunotherapy. It has been tried for 20 years and failed. Presumably, this is the reason you did not include it in your data. The reasons for the failure might be interesting.

DR. PARKER: I agree this has been an idea that has been around for a long time, but I am not so sure that it is, by any means, a hopeless idea. I think my question about its efficacy has to do with our experiments with the iodination system *in vitro*, where we could put cold iodine into cells and kill them very nicely. When we make the iodine radioactive instead, we do not really kill the cells any better; so at least with the particular cell lives we are looking at *in vitro*, we are unable to amplify the system in any way by having the iodine radioactive. Of course, as to why it does not work, I presume there are problems in localization. The iodine distributes in various places in the body, and it is radiating systemically while the antibody is being localized. This places limitations on the amount of antibody that can be used with a given level of radioactivity. These are problems associated with alteration of the distribution of the antibody; if you iodinate the antibody too heavily, *i.e.*, 50 atoms per antibody, the latter does not circulate as long as it should. That is one problem. An approach that may be better is an idea that Dr. Hawthorne has suggested and which has interested us. This is to conjugate bornanes to antibodies. The bornanes when radiated with slow neutrons, give off *alpha* particles. This allows systemic injection of the antibody coupled to the bornanes; the conjugate is allowed to localize, and then with a non-toxic external dose of slow neutrons, it is fed into the area of the tumor to activate the tissue-damaging radiation.

DR. PRESSMAN: The problem with radioiodinated antitumor antibodies is getting the antibody into the tumor, and I think we will have the same problem with bornane-coupled antibody, with enzyme-coupled antibody, etc. With radioiodinated antibodies, we do know that the latter does get into tumors and there is localization, although not in as large concentrations as we would like to see it. I think that Dr. Parker has a slide explaining what the problem may be, namely the presence of antigens in circulation. The antibody may well be neutralized by circulating antigens, and if a means can be obtained for removing the circulating antigen, one may be able to get a reasonable localization of antitumor antibodies coupled with anything that would be cytotoxic to the tumor cells.

In connection with the removal of tumor antigen from the circulation, Dr. Parker has suggested the use of a solid absorbent connected with the venous system. I think it might be worthwhile to move the position of the solid absorbent over to the thoracic duct and perhaps use various intravenous pressures to bring all macromolecules being generated out the thoracic duct. In this way, the tumor antigen molecules could be removed as they are liberated by the tumor.

DR. PARKER: That is an interesting idea. To go back to the radioactive antibody question, most of the work in the past has been done with ^{131}I, and relatively little with ^{125}I. In addition to the iodinated molecules, other ways exist by which one could couple other radioactive substances to proteins; this is an area that deserves careful exploration

DR. EISEN: Dr Hook, do you think that sickle cell traits in newly born individuals could be treated by antisickle cell hemoglobin antibody?

DR. HOOK: I do not know. It is an interesting possibility. Is there an antibody that will distinguish sickle cell hemoglobin from normal? I would not be surprised. I had always thought that unlike the immunoglobulin system, a given cell with a sickle cell trait will express both sickle cell and normal hemoglobin. You are suggesting the possibility of using antisickle cell hemoglobin to knock out the cell in the homozygous state, if one knew that there really is a homozygous state. One would destroy those cells and perhaps, encourage the outgrowth of one of the minor hemoglobin genes. This seems a reasonable suggestion. Obviously, one cannot try this in man. It would be interesting to know whether there are any animal models which could be used, because I think there is an outside chance that one might see something interesting.

DR. YALOW: I would like to ask Dr. Siskind whether he has ever considered immunizing the same animal with large antigens and with small antigens, to see whether the response with respect to the change in antibody concentration and the change in the equilibrium constants would be the same for both types of antigens. It has been our impression from the studies we have done, that there really has been no significant change in equilibrium constant with repeated immunizations or following a single administration. Usually, we use two or three immunizations and follow the antibody concentration over a period of time.

DR. SISKIND: It should be mentioned that if one boosts animals repeatedly, a decrease in affinity which one sees would occur. If guinea pigs are given repeated injections . . . three or four injections . . . during the course of an immunization scheme, one gets lower affinity antibody, and generally less antibody than if only one injection were given. Repeated injections do tend to dampen out this maturation sequence. I do not know whether that has to do with the conditions of immunization, or various subtleties of the whole procedure. Faber and Oppenheimer found that they can immunize rabbits with pneumococcal vaccines but every rabbit, if repeatedly immunized, gave homogenous antibody at certain times of their course. Sometimes, such a homogenous peak disappears and a new one arises. When a new one arises, it is of a higher affinity than the one that went away. So there are subtleties as to whether one stimulates, in some way, the appearance of a rather restricted zone of cells which elaborate antibody of specific affinity. As shown in some of the slides, 3 months after immunization, animals showed 85% of their antibody to be of a type with a relatively restricted spectrum of affinity. If one were not very careful to look at that other 15%, especially since that 15% is of a lower affinity, one could very well miss it and say that those animals had only this particular antibody. There are a lot of subtleties involved, but I do think that the question of simultaneous immunization of an animal with several different types of antigens under somewhat comparable conditions is an experiment which has not been looked at adequately It really should be looked at since it is an important question.

QUESTION: In order to reduce the number of animals that are immunized to prepare antibodies for radioimmunoassay, we generally immunize simultaneously with a number of antigens and find that the responses to the different antigens are completely variable with respect to the time at which they reach their peak titers, etc. We had hoped to find that all animals would be a good antibody producer for all antigens. We have not found that with multiple immunization with the small antigens.

DR. PRESSMAN: Since we just dusted off radioiodinated antitumor antibodies, I would like to recall some experiments in which there were definite toxic side effects due to radio-

activity when antitumor antibodies were used. Of course, these effects were in systems in which the antibody got to the tumor cell as in the HeLa experiments you quoted. For example, Bayle and Spaar were able to get definite killing of tumor cells so that the tumors would not grow when the tumor cells were treated with radioiodinated antibody, whereas treating with the same amount of uniodinated antibody did not prevent tumor growth. Others have reported definite effects of the radioiodine on the tumor.

DR. LEVINE: Dr Bloom, on your slide, with respect to the anti-S100, you showed fluorescence inside of the plasma cell. Did you notice any fluorescence in the nuclei of neurons as Hudan originally described?

REPLY: No, we did not. The anti-S100 that we had was generated against some very recently harvested S100 obtained elsewhere. It is a different species of anti-S100 than the material used by others in 1967. We saw no staining of the nuclei.

DR. SILVERSTEIN: Dr. Siskind, do you have any information on species differences in antibody affinities?

DR. SISKIND: Rather limited information. Rabbits, guinea pigs, mice, rats all showed a progressive increase in affinity to the DNP determinant which we described here for rabbits. Generally, it appears that rabbits make the highest affinity antibodies in this type of sequence. I think rats give a slightly higher response than guinea pigs. It is a very risky thing to make such comparisons because, obviously, the details of what you see in terms of the affinity depend very much upon the details of how the immunization is done. In a dose-response study, there seems to be some difference in the affinity of the antibody produced by these different species. But I am not convinced that if the immune regimen were modified a little bit, one could not find a more optimum immune regimen to immunize one of these other species and do just as well. There have been some differences reported among different strains of mice and among different strains of guinea pigs but they are of rather small magnitude, and one would suspect that some of these differences might well be details of the immunization routine which require slight differences in the different animals. There are, however, differences among individual animals which do appear to be real, though we cannot be completely certain about all of this yet.

DR. LA DU: I think we have had a very loyal audience and perhaps it is time to adjourn. Before doing this, however, I want to thank all the speakers. It has been, I think, a meeting of high quality all the way through from the very beginning to the end. The speakers stuck to their topics and gave us a very good insight into what immunopharmacology is all about. It is hard to imagine that anyone going into pharmacology today can ignore what is going on in immunopharmacology and immunology. This applies to people other than those in pharmacology, too. As is the case with genetics, it is a field that is now spreading into all aspects of experimental medicine, and we are seeing the growth phase. I agree with Dr. Eisen that it would be criminal if this cannot expand at the proper rate in the next few years, because it has so much to offer in understanding of biological processes and in therapeutic possibilities.

Thank you all.