Editorial: In Vivo Fate of Monoclonal Antibody B72.3

In the current issue of the *Journal of Nuclear Medicine*, Colcher et al. (*1*) present data concerning the in vivo fate of monoclonal antibody (MAb) B72.3 in patients with colorectal cancer. The authors follow the serum disappearance curve of the iodine-B1 (^{131}I) label and simultaneously assess the integrity of the B72.3 molecule by electrophoretic and column technique. In the process, they determined the effect of carrier MAb on the kinetics of the injectate, the quantity and possible clinical implications of circulating antigen in the serum, the effect of this antigen on the formation of intravascular antigen:antibody complexes, and the formation of human anti-mouse antibody (HAMA) at various times following the injection of the radiopharmaceutical. Finally, the authors discuss these data relative to the known literature. The study was well done and the results are interesting.

The radiolabel in the vascular compartment stayed attached to the immunoglobulin molecule at virtually all time points studied. In only one case was free iodine actually found in the patient's serum. This was also our observation when we tested iodinated and indium labeled MAbs in Balb/c and tumor-bearing nude mice. The blood disappearance curve of the ^{125}I- and indium-111- (^{111}In) MAbs were virtually identical (*2*). This was not the case in other tissues of the mice. The radioiodine was lost from solid tissue relative to the indium label (*3*). This is probably due to the fact that dehalogenation takes place in solid tissue but minimally in the vascular compartment. Interestingly enough, one MAb we iodinated did not dehalogenate in *any* tissue. Perhaps in this particular case, the iodine was applied to a "protected" site on the immunoglobulin. Folding of the molecule could theoretically allow for this.

Colcher et al. used a careful approach to determine the integrity of the circulating MAb. There seems little doubt that if the long-feared translocation of the radioiodine to another protein (other than an IgG) had taken place that it would have been found. Further, the fact that minimal small species were observed adds evidence for the long-held theory that what is circulating in the vascular compartment, during the first few days following intact MAb injection, is the same species that was administered. This is important information and, while it deals with only a single intact antibody, it implies that circulating radiolabeled material in patients administered with other intact MAbs really is radiolabeled MAb. In our own laboratory, we have produced data by similar techniques (with a different antibody) which corroborate that of Colcher et al. We have also used a bioassay to further prove that the molecule had good integrity. In the latter case, we removed serum from patients at various times after ^{111}In-MAb injection and administered it intravenously to Balb/c mice. The kinetics and biodistribution of the labeled anti-carcinoembryonic antigen (CEA) MAb were nearly identical with the kinetics and distribution of the material injected into the patient (*4*).

Many investigators have now shown that increasing MAb mass can slow the removal of radiolabeled murine MAb from the vascular compartment (*5–8*). The liver appears to be a major factor in the mass-effect phenomenon and is often seen to become quite radioactive when the mass of injectate is small. Similar uptake is seen in other organs and is, to some extent, dependent on the particular MAb administered. The usual organs involved, other than the liver, include the spleen, bone marrow, and gastrointestinal tract. Antibody mass can also, at times, alter the distribution of a radiolabeled MAb. The data of Colcher et al. indicate that B72.3, while sensitive to MAb mass, appears to be less so than several other radiolabeled antibodies that have been studied. The questions that remain are: why should a mass-effect happen at all and why should it vary from one MAb to another? A hunt for specific antigen on these tissues may or may not show them to be present. In fact, the presence of small amounts of antigen in a variety of normal tissues has not, in our experience, been the cause of unusual uptake of the radiolabeled MAb by that tissue. For example, CEA exists in normal colon mucosa (*9*). The administration of the anti-CEA MAb, ZCE-025, does not result in a dramatic uptake of the radiopharmaceutical by this organ. In short, this normal-colon CEA appears to be inaccessible to the MAb. Does the lack of access have to do with the vascular integrity of the capillaries in normal tissues or perhaps the integrity of the cell membranes of normal tissue. Whatever the reason, and regardless of the dose, the colon does not acquire much of the MAb.

Thus, while it is unclear how MAb mass actually changes the distribution and pharmacokinetics of a murine MAb, the chances are that the mechanism involved is not antigen:antibody dependent in the classic sense.

The species specificity of the Fc portion of the murine MAb is often blamed for the mass effect. If that were true, turning intact antibody into an $F(ab')_2$ should reduce or eliminate the carrier-effect in humans. It does not, at least not in the case of ZCE-025 (*5*). In the mouse, however, removal of the Fc dramatically de-

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creased the liver uptake of at least one anti-CEA MAb we studied (*10*). Why should it do so in a mouse and not in a human? Perhaps chimeric antibodies will answer these questions.

The presence of antigen on normal tissue is important when it is on a circulating entity in the blood, a white cell for instance. When this occurs, the radiolabeled MAb adheres to the leukocyte following, in which both the leukocyte and labeled MAb are quickly acquired by the reticuloendothelial system (RES) (*11–13*). This is frequently accompanied by shaking chills, fever, nausea, vomiting, and a drop in blood pressure if sufficient MAb mass is administered. We have theorized that the symptoms are due to destruction of these cells with cytosol being liberated into the blood. If the MAb mass is kept low, the cells still appear to be removed but there is no reaction.

As the authors indicated, circulating antigen will result in the formation of immune complexes following the injection of MAb. The authors also indicated that immune complexes formed between the MAb and circulating Tag-72 antigen did not preclude the targeting of tumor. This happens in the CEA as well as in the Tag-72 system. One patient we studied had CEA levels of ~5,000 ng/ml. Over half of the injectate (~10 mg total MAb mass) was bound into a high molecular weight molecule within a few hours following administration of the dose. From that point onward, these (presumed) immune complexes continued to circulate in the patient. In fact, the patient had one of the longest circulating MAb half-times of any individual that we have studied. Why weren't the complexes removed by some organ as one would expect and how can MAb bind antigen on the tumor if it is already bound to circulating antigen? One answer to the removal question may be the size of the complexes. Very large complexes or aggregates are likely to be removed from the blood by the RES. Complexes such as those formed between MAbs and antigens like Tag-72 and CEA are small. To begin with, each has only two sites for specific localization. Further, each theoretically targets the same epitope on the antigen. The maximum size of the complex will be limited to the size of one MAb and two antigen molecules. Given the volume of serum that the molecules reside in, the distance between them will be large even at a 5,000 ng/ml concentration. The odds of one MAb complexing with two antigens will be miniscule. These tiny complexes should continue to circulate. If small amounts of labeled MAb are administered, interactions will occur but it will take time. If carrier MAb is added to this system, it will compete with the labeled MAb for circulating antigen and further diminish the chances of a labeled MAb:antigen complex. Indeed, the authors' data suggests that while a high percentage of the MAb eventually circulated as an immune complex it was a relatively slow process which took place over several hours. During this time, most of the MAb molecules administered floated "free" in the plasma giving the tumor at least an early shot at them. A further possible explanation of tumor targeting in the face of immune complex formation is that the complex might disassociate long enough while circulating through the tumor for the radiolabeled MAb to translocate onto an antigen in the tumor. It should also be remembered that a 1:1 MAb:antigen complex would have a second binding site available if it managed to reach the tumor. Once on the tumor, the MAb should be less likely to escape because of the abundance of the antigen. Then again, no one knows how much labeled MAb leaves the tumor after having been acquired by it.

Antigen produced by tumor invading a normal organ may result in MAb uptake by that organ. We have observed normal liver tissue in patients with a high liver-tumor burden to become intensely radioactive (very quickly) after the administration of an anti-CEA MAb. The tumor sites can appear as "cold" areas in a "hot" field. We postulate that the tumor is producing large amounts of CEA, which in some manner attaches to the normal liver cells. When the labeled MAb is injected, these "primed" cells act as a sump for the radiopharmaceutical. We have observed this phenomenon only in the liver but it has occurred with both intact MAb and fragments. Since the normal liver has a blood supply of at least an order of magnitude higher than the tumor tissue, it is not hard to understand why it would acquire a greater amount of the MAb than the tumor if the antigen were present on both. Interestingly, while the complexes formed with circulating specific antigen are not removed rapidly from the serum of the human, they are removed quickly by the liver of a mouse (*14*). So is *uncomplexed* CEA (*15*). The mouse is useful as a model in this work but one must carefully define not only its job, but more importantly, its limitations.

The observations concerning immune complex pharmacology is somewhat different when the immune complexes are formed with HAMA. Immune complexes formed with HAMA are more likely to grow larger than those formed with tumor-associated antigen. They should be rapidly removed from the vascular compartment by the RES, principally by the liver if the concentrations of antigen and HAMA are sufficient to allow production of a big complex. Images taken under these circumstances sometimes show a very radioactive RES shortly after injection with little of the radiopharmaceutical remaining in the vascular compartment. Interestingly enough, this distribution is not necessarily the case if the mass of MAb administered to the patient is adequate. In a fascinating study Abdel-Nabi et al. (*16*) have administered an anti-CEA MAb to a series of patients at multiple times with minimal reaction and

has been able to target tumor even in the presence of HAMA. In Nabi's study, reactions have not been severe and have been well controlled with simple techniques. Studies of this type should be explored further.

Colcher et al. (*1*) observed a correlation between the formation of HAMA and the quantity of antibody administered. When one looks at their data, however, the turning point for HAMA formation occurs at very low levels of administered MAb. Further, the data are not linear above a 1-mg dose. Indeed, two patients who received the highest mass of MAb failed to mount a HAMA response. One wonders how much immunogenicity varies with the MAb administered. Neither has the uniqueness of each patient been defined as a factor in HAMA formation. Certainly normal people vary in their response to an immunogenic stimulus. Some cancer patients, such as those with lymphoma, hardly mount a HAMA response at all. Thus, there are multiple possible controlling factors in the production of HAMA. The important clinical point (not to be forgotten here) is that the desired result of our protocols is to achieve the best imaging (or therapy) situations possible. There is evidence that higher amounts of carrier results in higher imaging sensitivity (at least for some MAbs) than if the mass of MAb administered is kept low. If the data from radioimmunodetection and radioimmunotherapy of cancer is really worthwhile, the objective of our efforts should be optimization of the procedure followed by vigilance during a second administration of the MAb rather than risking the standardization of an ineffective technique in the hope that it might minimize HAMA formation in a few specific patients.

As the authors indicated, HAMA formation may become less of a problem with the use of fragments, however, one of the authors from this very group (*17*) has shown that Fab, at ~50,000 daltons will mount an immune response in patients following intravenous administration. In fact, we should be very surprised if it did not since a 50,000-dalton protein molecule should be immunogenic.

One wonders how the type of HAMA response will affect the second injection of a MAb. If the HAMA is a circulating IgG that is not there in abundance, one could expect the serum half-life of the injected MAb to be close to normal, especially if sufficient carrier accompanied the labeled MAb. However, if the protein produced is an IgM, with its many possible binding sites, an injected MAb might be rapidly bound and removed from the vascular compartment simply because IgM molecules have a short serum life.

The authors observed that they were better able to target tumors when the Tag-72 serum levels were high, as opposed to when circulating Tag-72 levels were low. This may be due to the fact that the high levels were associated with larger tumor masses which might be more easily imaged than small masses. Interpretation of these data could also be difficult if production of the Tag-72 antigen parallels the findings in CEA production. Some tumors apparently produce CEA in abundance and secrete nearly all that they make while others make the antigen and secrete little of it (*15*). A high secretor could actually become a poor target under these circumstances. Further, there is some evidence that the CEA production and secretory rate of a tumor may be a stable characteristic of that tumor. We have been working with a human colon tumor (T-380) for ~10 yr and have passed it from animal to animal literally hundreds of times and it has retained the same CEA production and secretory pattern that it had at the time we first began working with it. Also, in a small series of patients that we have studied with primary colon cancers, we have achieved excellent concentrations of the ^{111}In-anti-CEA MAb in tumors in which the circulating levels of CEA were normal or near normal and the concentrations of CEA on the tumor was as low as 500 ng/g of tumor. Without actually harvesting the tumor and measuring the amount of antigen in it per gram vis a vis its MAb uptake and serum concentration, it is hard to know exactly what circulating antigen levels indicate as far as imaging potential is concerned.

Finally, the authors should be congratulated for producing an excellent piece of work that gives valuable insight into mechanisms which will help in the optimization of radioimmunodetection and radioimmunotherapy.

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