Editorial: F(ab′)$_2$ Fragments Versus Intact Antibody—An Isodose Comparison

An important objective of any new form of radiation therapy must be to establish a dose response relation and then to understand which parameters can be changed to improve it. The experience in radioimmunotherapy (RIT) at most centers is still limited to a few diseases with small numbers of patients, so it is perhaps still premature to discuss dose response. Indeed, most centers have observed severe dose limiting tissue toxicities, in particular bone marrow, at activities of radioimmunoglobulin sufficient to produce radiation dose levels of only 10–25 Gy to tumor. This dose range is considered insufficient to expect a significant therapeutic response when given by conventional external beam therapy. The future of RIT, as a therapeutic modality, depends upon the development of methods to improve the tumor-to-normal tissue dose ratios. These activities include techniques to increase target cytotoxicity, e.g., more lethal isotopes (*1–2*), increase tolerance of normal organs to radiation allowing higher isotope doses to be given, e.g., bone marrow transplantation (*3–4*), and maneuvers to obtain more favorable antibody (Ab) distribution, e.g., Ab fragments (*5–6*), bifunctional Ab's (*7*), and 2nd Ab techniques (*8*). Most of the studies find their testing ground in the experimental nude mouse with human tumor xenografts. Although there are many shortcomings to the xenograft model for evaluating the clinical potential of radiolabeled Abs, it remains perhaps the best current in-vivo model for investigating treatment variables one by one, and assessing therapeutic gains and losses involved in each manipulation.

If one wishes to obtain a dose-response relation, then an accurate description of both dose and effect is mandatory. In external beam therapy, the dose to the treatment area is usually accurate and can be determined to better than 5%, but it is usually problematic to quantify the biologic response. In RIT, both may be equally complex. The purpose of measurement units is to provide a standard basis for intercomparison. One meter of cloth in Washington, D.C. should be the same as one meter of cloth in San Francisco. However, with standard units of radiation dose (1 Gy = 1 Joule energy absorbed per kilogram), one may be misled into believing that the same radiation dose always produces the same biologic effect. This is not true. The biologic effect produced from a radiation insult will depend on factors such as the quality of the radiation, the dose rate, the number of fractions, and the inherent radioresponsiveness of the target tissue.

Buchegger et al. (*9*) are to be commended on their highly original comparative study of the therapeutic efficacy of the treatment of a human colon xenograft model T380 with intact iodine-131- (^{131}I)labeled Ab (a mixture of four distinct monoclonal antibodies of subclass IgG$_1$ directed against different carcinoembryonic antigen (CEA) epitopes versus treatment with the ^{131}I-F(ab′)$_2$ fragments of the same antibody pool. Several studies have been published using the colon xenograft model, using whole immunoglobulin G (IgG) (*10,11*), and fragments (*12–13*). The usual basis of comparison of tumor response involves the injection of equal activities of the radiolabeled proteins. What makes the current work unique is the authors' attempts at a tumor isodose comparison. The logistics of such a study are not straightforward. Unlike external beam radiotherapy where one can accurately prescribe a radiation dose to a given treatment volume, the tumor dose received by an injected radiolabeled Ab depends on the unique set of pharmacokinetics of that molecule for each mouse. The level of tumor uptake will depend on (a) the properties of the tumor, e.g., its mass, the vascularity of the xenograft, the degree of antigen expression, etc., and (b) the properties of the radiolabeled Ab administered, e.g., the molecular weight of the Ab/fragment, the amount of Ab administered, the degree of retention of immunospecificity after radiolabeling, the immune response of the mouse (in particular after repeated injections), etc. In addition, there is the inherent statistical mouse-to-mouse variability, as well as a number of more trivial practical problems.

Buchegger and his colleagues have attempted in their study to compensate for two of these factors to achieve equal tumor dose: (a) the dependence of uptake on tumor mass, and (b) the dependence of the tumor retention time between the intact IgG and the F(ab′)$_2$ fragment. The first compensation technique involves administering an activity of radiolabeled protein adjusted by the expected percentage uptake per gram of tumor interpolated from studies of tumor uptake as a function of tumor mass. The second technique involves measurements of the biologic half-lives of IgG and F(ab′)$_2$ in the tumor, which are then used to scale the respective administered activities of each to yield equal areas under the specific tumor activity versus time plot. In summary, the dosing protocol is determined for each individual animal in the hope of achieving an equal average absorbed total radiation dose to each tumor.

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Their results show that 8 of 10 and 6 of 10 mice given the ^{131}I-F(ab')$_2$ suffered no recurrence after 6 mo and 10 mo, respectively. In contrast, 7 of 8 mice given the ^{131}I-intact anti-CEA relapsed between 1–3.5 mo. Controls were performed with nonspecific intact Ab and F(ab')$_2$ fragments, but all animals died from tumor or radiation toxicity. The conclusions drawn are that, for the same tumor dose, ^{131}I-F(ab')$_2$ is more tumoricidal than ^{131}I-intact IgG antibody. Although this conclusion seems to be straightforward, it should be interpreted with extreme caution.

There are three major pitfalls in this isodose comparison to which Buchegger and co-authors have alluded, but not amplified sufficiently to clarify their results.

1. How well have the investigators overcome the technical difficulties of truly achieving an equal radiation dose to the tumor of each animal? The dosimetry calculations involve a number of gross simplications. In particular, it is assumed that the tumor retention from repeated administrations of both ^{131}I-intact Ab or the ^{131}I-F(ab')$_2$ is 30% reduced on second and third administrations. It is quite feasible that a higher percentage uptake per gram of tumor results with repeated injections of the F(ab')$_2$ fragment and that the higher tumor response in this group is merely the result of a higher tumor dose. This issue must be addressed experimentally.
2. In order to obtain an equal tumor dose for both intact and Ab fragment, the activity of radiolabeled F(ab')$_2$ administered has to be ~4–5 times higher than the intact anti-CEA to compensate for its more rapid clearance. This implies very different delivery dose rates between the two studies. In the low dose rate realm of 0–30 cGy/hr, typical in RIT, small changes in dose rate may result in large changes in tumor control, since the slow rates of cell killing may be significantly augmented by tumor repopulation kinetics (*14*). Tumor control may thus have been as good with the ^{131}I-intact anti-CEA if the tumor dose had been delivered at the same dose rate.
3. The microdistribution of ^{131}I-F(ab')$_2$ and ^{131}I-intact anti-CEA in the tumor may be dissimilar for several reasons. In particular, differences in molecular weight and peptide structure between the two molecules may alter tissue penetration properties. Tumor areas of poor Ab localization will receive lower radiation doses than expected by the standard MIRD dose protocol (*15*), and the magnitude of cold regions may thus impact upon the tumoricidal effect of a given tumor dose. MIRD equidose calculations do not necessarily imply equi-effect. Intact Ab may localize only on the tumor periphery resulting in considerable overkill to the peripheral cells, while Ab fragments may diffuse more deeply into the tumor core and result in a more uniform dose distribution. Autoradiography may help resolve this issue.

These points demonstrate both the difficulty of obtaining equi-tumor doses and difficulty of interpreting the results even when they are achieved. The objective of Buchegger and colleagues may well have been to compare normal tissue toxicities for an equi-control of the tumor. But in view of the above concerns, the only clear conclusion is that an improved tumor control was achieved with ^{131}I-F(ab')$_2$ at perhaps lower normal tissue toxicities than with ^{131}I-intact Ab. The underlying reason for this is not easily identified.

If one wishes to intercompare results between different experiments with radiolabeled targeting molecules, then there are two general approaches. The simplest is to compare experiments based on equal amounts of injected specific activity. This is a phenomenologic approach and possesses the disadvantage that one must evaluate individually each administered dose-response relation for each radiopharmaceutical, because total radiation doses to the relevant tissues will differ. Alternatively, intercomparison may be based on the radiation tumor dose. This approach is more useful although more difficult, since it assumes a meaningful measure of the radiation dose to the tumor and other dose limiting tissues can be achieved. However, even this approach is not complete, because one is comparing the total area under each of two curves and assuming that the shape of the curve does not matter. The spatial and temporal distributions of radiation doses in RIT are extremely important, but difficult to assess. The usual approach to incorporated radionuclide dosimetry is the method set out by the MIRD committee (*15*). Because the tissue doses derived by this protocol implicitly assume a uniform distribution of sources through the target tissue, the relevance as an indicator of tissue radiotoxicity will depend in part on the degree to which the activity is truly uniformly localized relative to the range of the prevalent nonpenetrating radionuclide emissions.

True radiobiologic equi-effect experiments will require attention to all the standard indices of radioresponsiveness, including radiation quality, total dose, dose rate, dose heterogeneity, target vascularity and oxygenation status, and inherent target-tissue radiation damage repair capacity. Advances in tumor modeling and dosimetry over the next few years will hopefully result in an improved description of the microdistribution and fluctuation of radiation dose from the administration of radiolabeled molecules and the consequences of these fluctuations for target and normal tissue radiotoxicity. New experimental techniques are evolving to quantify source heterogeneities in tissue (e.g., using implanted micro-TLDs (*16*), quantitative autoradiography using scanning densitometry (*17*),

phosphor imaging plates (*18*), and image analysis), but these techniques give only partial information and are at present too time consuming to ever become of routine value in the clinic.

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