EDITORIAL

Radiolabeled Monoclonal Antibodies: Radiochemical Pharmacokinetic and Clinical Challenges

Four basic science articles and two special contributions included in this issue of the Journal address some of the persistent challenges that continue to moderate the progress of radioimmunoimaging and therapy (*1-6*). The reports are important because of the new data they provide about current methodologies for radiolabeling antibodies for imaging and therapy. This editorial will review, and expand upon, several points brought out in these experiments. One focus will be on the chemistry issues now before us, thereby supplementing the excellent review article on monoclonal antibodies presented by Keenan (*5*), as well as some discussion about clinical considerations.

The importance of radiolabeling antibodies which have been chemically activated by the addition of a chelating agent should be emphasized. The prototype method utilized a bidentate chelate to couple heavy metals to proteins (*7*). Subsequently diethylenetriaminepentaacetic acid (DTPA) was identified as a more desirable alternative (*8*) since synthesis of intermediates is not required and the chemistry is less complex. Though the basic methodology of using chelate-coupled antibody has changed little, many variations now exist among investigators. For example, Goodwin (*1*) employs bromoacetamidobenzyl ethylenediaminetetraacetic acid, while Paik et al. (*2*) and Fawwaz et al. (*4*) employ DTPA anhydride. Reported radiolabeling efficiencies among these investigators range from 35-95%, figures which depend upon two variables for proper interpretation: (a) whether postlabeling purification methods were used; and (b) the statistical variability involved in these procedures. The explanation for the first is obvious. If postlabeling unbound radioactivity is removed, reported labeling efficiencies will be higher. An example of the second variable is the situation wherein an average of three chelators are attached to each of a large population of antibody molecules. The use of Poisson statistics (*9*) reveals that, though we may wish to target three chelate molecules per antibody, 5% of the resulting antibody population will fail to couple any chelate, 23% will possess three chelates, and 13% will possess five. This variability is very difficult to control but may be a factor in suboptimal images, as will be seen below. Establishing reaction conditions which skew the degree of coupling in the desired direction would be a useful approach to minimizing this source of variability. Regardless of the variability, it is clear that optimizing the integrity of the final product depends upon carefully controlling the molar ratio of DTPA to antibody prior to performing the labeling step. The importance of a well-documented inverse relationship between the average number of chelate molecules per antibody and the subsequent immunoreactivity of the final labeled product also deserves emphasis. Just as important are efforts now underway to site-direct DTPA coupling so that antibody binding sites will not be masked.

Effective radiolabels have been developed but their half-lives and residence times must be carefully matched with the needs of both imaging and therapy. Choosing among radiolabels is currently a complicated task. The concern about deiodination is a valid one. Indium does have the advantage that once it binds to tumor, it remains there. However, if it is removed from the antibody in vivo, it may be reutilized.

Many obstacles have been overcome in the endeavor to image tumors with monoclonal antibodies, but much more work is needed to create a stronger distinction between tumors and surrounding normal tissues. It appears largely a pharmacological problem to find the right combination of antibody type, fragment, and amount for the equilibrium kinetics, where there is partition between target and nontarget tissues.

The theme of annoying background levels of radioactivity which persist for 24 or more hours after administration of the radiolabeled antibody continues. Goodwin et al. (*1*) and Hnatowich et al. (*3*) stress the importance of using alternative sites of administration wherever possible,

such as subcutaneous and intracavitary routes. In this way, tumor to background ratios may be doubled at 24 hr postadministration. Goodwin (*1*) also notes that, in mice, blood background may be significantly reduced by injection of a nonradiolabeled polyclonal antiserum just prior to imaging. Even under optimum conditions, however, about 4% per day of the indium-111 (^{111}In) antibody is lost by transchelation to transferrin (overall loss is 11%/day), while for yttrium-90 this value is 2% per day (overall loss, 13%/day). These high background levels interfere with imaging; but they make it difficult to obtain meaningful dosimetry data prior to initiating a course of radioimmunotherapy, and more time-consuming to identify the most appropriate radionuclide to use for radioimmunotherapy.

Perhaps the most significant attribute of this developing technology, when combined with the recent advances described in the production of monoclonal antibodies and their fragments (*5*), is the feasibility of preparing and lyophilizing the coupled antibody complex in sterile, pyrogen-free unit doses for long-term storage. When required, the appropriate radionuclide activity may be added for instant radiolabeling. Such products are now available for limited investigational use and routinely exhibit labeling efficiencies in excess of 85%.

As with the production of antibody, many radiolabeling issues must be solved before this modality becomes routine. For instance, it has been observed that more heavily radiolabeled proteins exhibit shorter plasma half-lives and that the stability of the system is increased when the reactive site residues on the antibody are not radiolabeled. This concept takes on added significance in light of the observation (*9*) that the most heavily labeled fraction of antibody molecules (the upper 3.7%) may account for 26% of the radioactive counts. These heavily labeled proteins are least likely to retain their native biological properties. Such considerations point to the importance of ongoing studies which have as their goal the development of site-directed labeling procedures.

The articles appearing in this issue paint a cautious picture of the present state of radioimmunoimaging and therapy. Although difficulties continue to be resolved in the basic science arena, it is questionable whether we have moved far in tumor detection efficacy. The successful image depends on such factors as tumor size and location and specificity of the antibody. Using a specific monoclonal and label, one might succeed with radioimmunotherapy while failing to image microscopic tumors. In addition, in vivo transschelation of indium-111 to transferrin, deiodination (up to 50% excretion of elemental radioiodine within 24 hr of dose administration), persistent liver uptake of radioactivity, and uncertainty about the optimum method of radiolabeling and its effect upon antibody specificity continue to challenge investigators in this field. More optimism may be experienced when experimental results obtained in animals can be consistently obtained in patients.

The importance of the research reported in this issue of the Journal is the insight provided about where nuclear medicine stands in the ongoing development of this modality. In discussions with potential users, our referring physicians, we should now be better able to appreciate the difficulty of the work being performed. A glimpse back along the chain of experiments which has led us to this point means not only that we cannot deny the progress already made, but also that there should be a resolve to develop a clearer picture of its clinical utility and practicality.

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