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EDITORIAL

Optimizing Antibodies for Use in Nuclear Medicine

Monoclonal antibodies (Mab) by virtue of their unique in vitro avidity for their antigen have been considered particularly attractive as selective carriers of diagnostic/therapeutic agents in vivo. This expectation is based on the fact that Mab (a) show high specificity and affinity for their intended target, (b) are generally nontoxic, and (c) can transport such agents. Their application to both diagnosis—labeled with ^{123}I or ^{131}I (*1–6*), ^{99m}Tc (*6,7*) and ^{111}In (*6,8–12*)—and therapy—labeled with the β-emitters ^{131}I (*13–17*), ^{186}Re (*18*), ^{90}Y (*19, 20*), and ^{67}Cu (*21*) and the α-emitters ^{211}At (*22–24*) and ^{212}Bi (*25–27*)— is the focus of attention in many nuclear medicine research facilities.

In all of these studies, the basic assumption continues to be that radiolabeled Mab have a role in radioimmunodiagnosis (RID) and radioimmunotherapy (RIT). How justifiable is this assumption? Certainly, several characteristics (e.g., the low percent injected dose per gram of target tissue (≤0.01%), low tumor-to-normal-tissue ratios, slow clearance, nonuniform distribution within the tumor, long biologic half-life that may be unsuitable for short-lived isotopes) could lead one to conclude that antibodies do not possess the intrinsic qualities necessary for their utilization in RID and RIT. In fact, the early enthusiasm of a decade ago has been dampened with some investigators questioning the very future of Mab in nuclear medicine (*28,29*). Despite various opinions on the subject (*30–35*), it is clear that there is a pressing need to enhance the diagnostic and/or therapeutic potential of radiolabeled Mab while maintaining their immunointegrity and minimizing structural/conformational changes that might limit their uptake and retention within the intended target.

A review of the nuclear medicine literature on radiolabeled antibodies indicates that in general there is no methodical examination of cause and effect with respect to the various inadvertent modifications that antibodies undergo during radiolabeling. Most studies have been limited to finding a technique to radiolabel the Mab with the radionuclide of interest, examining its in vitro immunoreactiv-

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ity with the target tissue, and finally determining its in vivo stability, kinetics of biodistribution, and targeting. In developing radiolabeling methods, particular attention is paid to minimizing changes mainly at these endpoints. This approach however fails to consider a number of variables that may be of importance in the efficacy and potential of the final product in RID and RIT since: (a) the conditions needed for labeling Mab with different radionuclides, chelates, or radiolabeled molecules differ dramatically (*1–27*), (b) during radiolabeling, the susceptibility of various Mab to the same set of conditions and of a particular Mab to different set of conditions may vary greatly (*36–40*), and (c) current in vitro assessments of radiolabeled antibodies do not necessarily predict in vivo behavior (*39*). For example, the electrophoretic mobility of antibodies may be altered following radiolabeling. These changes have been shown to depend on the number of iodine atoms per antibody molecule (*40*), the oxidant used and the molar concentration of the latter (*40,41*). Furthermore, the conjugation of a molecule to an antibody may alter the conformation of its combining site, especially if the agent is distinctly hydrophobic, possesses multiple charged groups, or causes stearic hindrance of antigen binding (*42*). These effects are also likely to become more pronounced as the degree of radiolabel incorporation increases. It is also worth noting that although the sites of covalent modification of antibodies during radiolabeling are numerous (*43*), each of these groups may not be equally available for conjugation. This has been clearly demonstrated in studies where the coupling of only a few molecules to the amino groups of a particular Mab has been reported to decrease its antigen-binding activity (*43*), suggesting that amino groups important for antigen-binding activity are in certain antibodies more reactive than other groups and therefore undergo covalent coupling first.

It is clear from the foregoing that the identification and characterization of common structural and physical changes in radiolabeled Mab molecules resulting from the various manipulations that occur during their radiolabeling, purification, etc., is the first step towards the successful use of Mabs in nuclear medicine. Once the effects of these alterations in radiolabeled antibody structure have been identified, quantified, and related to in vivo behavior, the purposeful manipulation of antibody molecules to systematically modify and control the kinetics of their biodistribution in a desirable manner becomes achievable.

The article by Khaw et al. (*44*) reports the results of a novel approach in which an antimyosin Mab has been modified to carry a high negative charge. The authors theorized that the nonspecific ionic interactions between positively charged antibody molecules and negatively charged cell surfaces could be decreased without affecting the avid binding of the Mab to its intended target. They characterized the pI of the thus negatively charged Mab and demonstrated that its immunoreactivity had not been altered. In vivo studies showed decreased background activity in normal myocardium and nontarget tissues and enhanced target (necrotic myocardium) visualization.

This well-designed and well-executed study clearly demonstrates why the nuclear medicine community should continue the active support of investigations that examine the potential diagnostic and therapeutic roles of Mab. The authors have carried out one particular modification of a Mab, the manipulation of its pI. In doing so, they achieved several highly desirable endpoints: (a) reduction of background activity and consequently a decrease in the dose to normal organs and tissues and an enhancement of target visualization; and (b) development of an approach that lends itself to the production of a radiolabeled Mab with remarkably higher specific activity, thus making it possible to use substantially less antibody; as such, they predict a reduction in the administered xeno-protein dose which should also reduce the antimurine antibody response.

A single modification of a Mab and all these highly significant advantages! Is this an anomaly or do antibody molecules have intrinsic qualities that can be modified to enhance their potential in RID and RIT? Mab molecules, which have been studied and characterized extensively by investigators in many fields, do in fact have several highly advantageous attributes:

1. Being polypeptide molecules, they possess several sites for modification.
2. By virtue of their high molecular weight, the covalent binding of a radionuclide or a radiolabeled molecule will not alter their size substantially.
3. They have characteristics (e.g., charge and size) that can be easily altered.
4. They can be radiolabeled intrinsically (e.g., ^{14}C, ^{35}S, ^{75}Se), thereby providing a baseline to which extrinsically radiolabeled and/or modified Mab can be compared.

Based on the above, it is apparent that the identification of changes induced in these versatile protein molecules that are secondary to any systematic modification and radiolabeling procedures with subsequent correlation to desirable in vivo behavior is essential for bypassing the known limitations (and those to be identified) of radiolabeled antibodies. The novel approach reported by Khaw et al. in this issue of the *Journal* (*44*), those published recently (e.g., *40,45–50*), as well as those yet to come, are certainly paving the way for the successful and eventual common use of Mabs in the field of nuclear medicine.

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