The Development and Use of Radiolabeled Antitumor Antibodies

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Abstract

The use of radioactive tumor-localizing antibodies is developed historically, starting with the demonstration that antibodies can be radioiodinated without destroying antibody activity. Then, antibodies against normal organs were shown to contain antibodies which localize in the normal organ. Subsequently, antibodies capable of localizing in tumors were demonstrated, and these localized well enough to permit their use diagnostically by scanning for radioactivity and their use therapeutically by localizing sufficient radiiodine. Various relevant problems are discussed.

Historical Background

Attempts to use antibodies made against malignant tissues for cancer treatment can be traced back to the turn of the century. In 1895, Hericourt and Richet (17) prepared antisera against a human osteogenic sarcoma in an ass and 2 dogs and claimed that these sera were effective in reducing 2 different neoplasms, a fibrosarcoma of the chest wall and a cancer of the stomach. Subsequently, 50 cases treated similarly by themselves or their colleagues were reported to give excellent results in general, whereas normal serum was ineffective (18). Although many attempts to use heterologous antitumor antisera have been made since that time, this mode of therapy still awaits more convincing experimental proof. The failure of investigators to obtain consistent positive results may have been due to one or more of the following reasons: (a) the sera contained no antibodies which would react with any part of the tumor tissue; (b) the sera contained antibodies which cross-reacted with other tissues and were completely bound by the other tissues, thus being unavailable to the tumor; (c) the sera contained antibodies for specific constituents within the tumor cells but could not reach these intracellular components and affect them; (d) the sera contained antibodies which did localize in the tumors but did not produce an observable physiological effect, thus being recorded as negative. It therefore became obvious that it first had to be proven that there are antibodies in an antiserum prepared against a tumor which do in fact go to the tumor. I reasoned that this could be achieved by using radioactive tracers coupled to the antibodies. If it were possible to demonstrate that antibodies did go to the tumor, then it should be possible to use them to carry sufficient amounts of radioactivity to be effective therapeutically or diagnostically, even when the original antibodies are physiologically ineffective by themselves (35). By means of this approach, we were able to show that antibodies prepared in rabbits against certain animal tumors contained antibodies which do get to the tumor (24, 45). This was subsequently confirmed by experiments by Bale et al. (5). In 1958, we were able to show that radiiodinated antibodies could be used diagnostically, since antibodies localized in a tumor could be detected by scanning (47). Bale et al., in 1960 (4), succeeded in showing that tumor-localizing antibodies could carry a therapeutic dose of radioactivity to the tumor. Since then, several groups of investigators have used radiolabeled antitumor antibodies both diagnostically and therapeutically, as is documented in the papers of this workshop (see also Refs. 11 and 36).

Preliminary Experiments

The first step in the problem of radiolabeling antibodies was to choose the proper label and the technique of labeling the antibody which does not destroy antibody activity. Earlier work had already indicated that various substances can be coupled to antibodies without destroying the ability of the latter to combine with antigen (36). This was not too surprising, since the antibody molecule is very large, with a molecular weight of about 160,000, and the antibody-specific region is merely a small part of the surface of the molecule. I decided on the use of 131I as a tracer, since it has the advantages that iodine forms a definite chemical bond with the protein molecule, it is easily obtained, is easily counted, has an appropriate half-life, and is not reincorporated into any tissue except thyroid; also, localization in the thyroid can be prevented by feeding nonradioactive potassium iodide.

The first iodine I used was the short-lived, 25-min half-life, 128I. Dr. Charles C. Lauritsen made it for me by the Szilard-Chalmers nuclear excitation reaction by bombardment of ordinary ethyl [127I]iodide with neutrons from the Van de Graaff machine at the California Institute of Technology. My first 131I came from the nuclear facility at Oakridge, Tenn., as the neutron-irradiated tellurium target from which I had to isolate the 131I. Using the short-life 128I, I was able to determine the conditions for coupling iodine to globulin and show that antibodies could be made radioactive by iodination. Subsequently, with 131I, I was able to show that the iodination did not destroy antibody activity in the case of anti-ovalbumin antibody (43).

For the next step, we showed that antibodies to a normal tissue, kidney, when radioiodinated and injected i.v. showed localization in the kidney. We chose kidney because Masugi (28) had shown that antisera raised in rabbits against rat kidney were nephrotoxic and produced physiological changes in the structure of the rat kidney. In 1948, we prepared such an antiserum against kidney tissue, separated out the globulin fraction by alcohol precipitation, and then iodinated with iodine containing tracer concentrations of 131I (43). We gave injections of this radioiodinated globulin fraction of anti-kidney serum to several rats and gave others a similarly prepared radioiodinated globulin fraction of anti-ovalbumin serum as a control, waited for 4 days, and then sacrificed the animals. On analyzing the various tissues, we found evidence for specific localization. With the anti-kidney preparation, there was appreciable accumulation of radioactivity in the kidney; whereas with the anti-
ovalbumin, there was practically no accumulation in the kidney. This showed definitely that there were antibodies in the anti-kidney serum which localized in the kidney. No localization was seen for the anti-ovalbumin preparation.

There was some accumulation of radioactivity by organs other than kidney when radiiodinated anti-kidney antibody was administered, but to a lower extent than was observed with the kidney. Some cross-reaction was seen due to the presence of common types of tissue in the various organs, such as blood vessels, etc. The cross-reacting antibodies could be removed by suitable absorption with preparations from other organs. Subsequently, we showed that the actual localization was in the glomerular tuft by making radioautographs of sections of the kidney. Kidneys from animals receiving normal rabbit globulin labeled with iodine showed no such localization (40, 42).

The rate of removal of anti-kidney antibodies from the circulation was determined, and it was found that they were essentially completely removed from the blood as it passed through the kidney (40, 41) and that it took a tremendous amount of antibody to saturate the kidney (39).

Antibody remained fixed in kidney for over 2 months, being removed with a half-life of 20 days in the case of the mouse (34).

Antibodies with specificities for other tissues and organs have been found by radioidine methods; for example, antibodies favoring localization in adrenal, liver, lung, and placenta (19, 23, 46, 56, 59, 60).

The Iodination Reaction

Oxidized iodine in the form of hypoiodous acid reacts with the tyrosines and histidines of the antibody molecule. At the present time, a popular method is the use of chloramine-T (16) to oxidize iodide to hypoiodous acid.

Another popular method of iodination involves the use of peroxidase and hydrogen peroxide (27, 31). By this method, iodide is oxidized and attached, in the oxidized state, to the active site of the peroxidase, which then transfers it to a tyrosine on the antibody. This is a very mild reaction and is used to iodinate surface proteins on cells.

The number of iodines incorporated per molecule of antibody is not the same for all molecules present, except where the average number of iodines per molecule is much less than 1 (49). Then, essentially all iodinated molecules bear only one iodine. When the average number is 1 or more, there is a distribution of iodine such that various molecules of antibody have different numbers of iodine on a probability basis. Molecules with the same number of iodines may well differ from each other, since they would have the iodines distributed over different tyrosines. In our studies of the effect of iodination on antibody activity, we first used an anti-hapten system, and found that anti-benzoate and anti-benzenearsenolate antibodies lose their ability to precipitate when iodinated extensively (25 iodines/antibody molecule) and that the loss can be prevented by the presence of the specific hapten protecting the site during iodination (50). The destruction is interpreted as being due to iodination of a particular group, probably tyrosine, in the antibody-specific site which may be more reactive or less reactive than other tyrosines in the molecule. Iodination below 2 atoms/molecule does not affect antibody activity of antibodies requiring only one site for activity, such as for localizing antibodies. The localizing activity with anti-rat kidney antibodies was still 30% of the original localizing activity in the kidney when 19 atoms of iodine were present per molecule of globulin, while the liver-localizing activity was 50% of the original value at this ratio.

Preparation of Antibodies

Of paramount concern to the field of tumor localization with radiolabeled antibodies is the isolation of antibodies which localize specifically in the tumor. Hence, it was necessary from the outset to develop methods for purifying antibodies. Use was made of specific absorbents to concentrate antibodies capable of localizing in the tumor, as well as other specific absorbents to remove cross-localizing antibodies which localize elsewhere than in the tumor. Effort was also made to produce more specific antibodies by using a purer tumor antigen preparation in the first place. In some earlier investigations, antibodies were purified by injecting the radiiodinated antibody into tumor-bearing animals, and the antibodies fixed in the tumor (or normal tissue of interest) were eluted and then used for assay in a second animal with increased localizing activity (3, 48). Conversely, the cross-reactive determinants can be removed by injecting the radiiodinated antibody into normal animals to remove the antibodies capable of reacting with normal tissue constituents, and then the antibodies remaining in the circulation are assayed for localizing activity. In the case of the acetaminolfluorene-induced hepatoma of rats, this method showed a specific localization of radioantibody in the tumor (8, 9, 25).

Major progress was made when purified antigens were used for raising antibody, such as was the case for CEA3 purified from human colonic cancers. The anti-CEA antibodies raised against such preparations resulted in specific and significant localization of human colonic tumors xenografted in hamsters and nude mice and still producing CEA (12, 15, 53). Subsequently, Primus et al. (54) demonstrated that affinity-purified antibody to CEA had even higher localization ratios than did the conventionally purified antibody. These studies then led to CEA radioiodolocalization studies in humans. Kim et al. (21) demonstrated, despite earlier failures by Reif et al. (55) and Mach et al. (26), successful localization of human cancers with radiiodinated, affinity-purified goat IgG raised against purified CEA. These studies also point to the realization that the previously held view, that the presence of the antigen in question in the circulation might bind to the antibody and thus render it nonlocalizing, is not warranted. Even the problem of sensitization by the heteroantiserum did not hinder these clinical studies, probably because such minute amounts of purified goat IgG were used. Similar techniques have been used for tumor localization with antibodies specific for Hodgkin ferritin (32), a-fetoprotein (13), and human chorionic gonadotropin (14).

Localizing Activity of Normal Globulin

When normal globulin is passed through the same specific purification procedure used to purify antitumor antibodies, the isolates can act like localizing antibody, since normal globulin is a mixture of globulins, some of which may be able to bind to

\[3\text{ The abbreviation used is: CEA, carcinoembryonic antigen.}\]
Experimental Studies

That antibodies prepared against a tumor and injected into the tumor-bearing host can be fixed in the tumor was first shown in our laboratory in 1953 in the case of the Wagner melanoma of rats (24). Fixation of iodine-labeled antitumor antibodies raised in rabbits has been found with the 2-acetaminofluorene-induced hepatoma of rats (8, 25), the L1210 lymphoma of mice (22), and the MOPC 104E plasmacytoma of mice (51). Iodine-labeled antibodies raised in the same species as the tumor were found to localize in a transplantable methylcholanthrene-induced tumor of rats, as reported in the papers of Bale et al. (1) and Izzo et al. (20).

A complication in the determination of localization of antibody in a tumor became apparent in our earlier work. Tumors can accumulate globulins and other macromolecules nonspecifically, as first reported by Duran-Reynolds in 1939 (10). Therefore, we developed a paired radiodiode label technique in which the antitumor antibodies are labeled with one radioactive isotope of iodine, e.g., $^{125}\text{I}$, and normal control globulins are labeled with another, such as $^{131}\text{I}$. The 2 preparations are mixed and injected into tumor-bearing animals, and in this way the tumors can be evaluated at the end of the experiment to determine how much antibody and how much control protein is present (38). Indeed, the paired-label technique has been extended to the triad-label technique, in which the localization of 3 protein preparations, i.e., antitumor antibody, anti-normal tissue antibody, and control globulin, can be determined simultaneously, using 3 different isotopes of iodine, a different one to label each protein (47).

Quite early in our investigations, we made the observation that antibody to fibrin or fibrinogen will localize in fast-growing tumors such as the Murphy lymphosarcoma of rats, or wherever fibrin is deposited (6, 7). At the same time, Spar et al. (58) reported the localization of anti-rat fibrin antibodies in the Murphy lymphosarcoma of rats. Thus, a rapidly growing tumor induces fibrin deposition and will cause localization of the anti-fibrin antibodies. These observations led to the use of anti-fibrin antibody to locate animal and human tumors by Spar et al. (57) and by McCordie et al. (29). This method has also been used to locate blood clots in the body.

Mechanism of Localization

In order for radiolabeled antibody to localize on the tumor cell, it must react with an antigen in the cell surface. If this antigen and the corresponding part of the cell surface are part of the lumen of the blood vessel, then localization is most rapid, although still probably slow because of the relatively small fraction of the cardiac output received by the tumor. If the tumor cells are not exposed directly to the blood, then the antibody must be carried to the cell by the extravascular fluid, which thus reduces the rate of localization and limits the fraction of specific antibody present which can be fixed on the cell before the antibody is metabolized (2). Thus, localization can be fostered by increasing the antibody coming to the tumor, such as with a circulation-isolation technique or by perfusion of the involved organ, as described by Order (32).

If the antibody is not directed to a cell surface constituent but is against an interior component and depends upon pinoctosis, uptake would be much too slow for effective diagnostic or therapeutic use.

Weissman et al. (61) have measured the fixation of radiolabeled tumor-localizing antibodies by determining the reduction of tumor-reactive radioactivity remaining in the circulation at various times. They found that Moloney lymphoma-bearing mice, regardless of the site of tumor growth, showed appreciable loss of in vivo-filtered rat antitumor antibodies from the circulation. This indicates good contact of the circulating antibody with tumor substances. However, these authors did not determine whether or not the localization was on tumor tissue or as fixation of antigen-antibody complexes in various tissues. The presence of tumor substances which may be circulating in the blood of the host is a problem of concern with the use of antitumor antibodies. It was originally believed that this would be a serious hindrance for CEA tumor radioimmunodetection in humans, but the work of Primus et al. (52) proved that very high blood titers of CEA did not prevent tumor detection and localization in humans after the administration of radioantibodies against CEA. Subsequent work by Goldberg et al. (13, 14) have shown that this phenomenon is also true for radiolocalization involving $\alpha$-fetoprotein and human chorionic gonadotropin as markers.

Concluding Remarks

The opportunities for tumor localization using antitumor radioantibodies were realized after the ability of antibodies to localize selectively in certain normal organs was investigated. Subsequently, a number of studies showed that animal tumors could be localized with radiolabeled antibodies made against what we can now consider quite crude antigen preparations. The development of animal models for human tumors containing tumor-associated products provided a renewed impetus and interest in tumor radiolocalization, and it is gratifying that these studies have led to clinical trials and interest, as exemplified by this workshop. The stage has been set for the immunologist, experimental oncologist, and clinical oncologist to address common objectives and problems in the improved detection and, possibly, therapy of neoplasms with specific antitumor antibodies, yet we must not lose sight of the need for much more fundamental investigation of the mechanisms and dynamics of antibody localization and binding to tumor and tumor constituents.

References

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History and Use of Radioantibodies

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