

## Second antibody clearance of radiolabeled antibody in cancer radioimmunodetection

(tumor localization/tumor imaging/colonic cancer/human tumor xenografts/carcinoembryonic antigen)

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**ABSTRACT** The imaging of tumors using radiolabeled antibodies previously has required the implementation of computer-assisted subtraction techniques to reduce background radioactivity. A decrease in radioactivity in the blood of hamsters bearing human colonic tumor xenografts has been achieved by administering a second antibody directed against a radiolabeled primary antibody to carcinoembryonic antigen (CEA). This method was found to reduce the level of blood radioactivity by a factor of 4 within 2 hr after injection of the second antibody and to enhance tumor/nontumor ratios within 24 hr. Unlike liposomally entrapped second antibody, the primary anti-CEA antibody did not show increased accretion of radioactivity in the liver, spleen, or other major organs. These results suggest that administration of a second antibody alone may improve tumor imaging with a radiolabeled antitumor antibody.

The concept of using radiolabeled antibodies to localize tumors *in vivo* was proposed three decades ago by Pressman and Korngold (1). However, only within the past 5 yr has this method, called radioimmunodetection (2), been found to be effective for imaging cancer by external scintigraphy (3-7). Although radioimmunodetection was relatively slow in development, there were several advances that enabled its clinical application. The development of high-titer antisera against tumor-associated antigens, such as carcinoembryonic antigen (CEA), and the refinement of techniques for specific antibody purification contributed toward improved radioimmunodetection of tumors (3, 6). Initially, tumor imaging by external photoscanning could only be achieved several days after the injection of radiolabeled antibody because radioactivity in the circulation and nontarget tissues interfered with the delineation of tumor sites. In order to circumvent this problem, Goldenberg *et al.* (4) successfully used computer-assisted subtraction techniques to image primary and secondary tumor sites within 48 hr of injection of radiolabeled anti-CEA antibody. Other manipulations have been shown to enhance tumor imaging by radiolabeled antibodies in experimental animals models. Solter *et al.* (8) reported that F(ab')<sub>2</sub> fragments were superior to whole antibody for tumor localization because the fragments were cleared more rapidly from the body than was whole IgG. Ryman and Barratt (9) and Begent *et al.* (10) showed that liposomally entrapped second antibody (LESA) directed against radioiodinated anti-tumor primary antibody accelerated the clearance of the radiolabeled antibody to 2- to 4-fold within 2 hr after administering LESA. Although accelerated clearance was observed from the blood within 2 hr, the radioactivity accumulated in the liver for 24 hr. The concentration of radioactivity in the liver can be attributed to the use of liposomes as a carrier for the second antibody because liposomes are

known to accumulate in tissues rich in reticuloendothelial cells (9). The use of liposomes containing second antibody fosters other problems, such as inefficiency of incorporating functionally active antibodies into liposomes by sonication, the possibility of causing aggregation of the IgG molecules, and *in vivo* destabilization of the lipid bilayer that could ultimately result in the dissociation of the liposomally bound antibodies (11-12). It was our intention to determine if the injection of second antibody by itself would also accelerate the clearance of a primary anti-tumor antibody, thereby avoiding the problems associated with LESA. In this report, we provide evidence that an injection of a second antibody alone gives similar radioactivity clearance rates as reported for LESA, but without accretion in the liver.

### MATERIALS AND METHODS

**Antibody Preparation.** Goat anti-CEA antibody was purified by affinity chromatography as described (6). The antibody was radioiodinated with <sup>131</sup>I by the chloramine-T method (13) and yielded a specific activity of 13  $\mu$ Ci/ $\mu$ g (1 Ci = 37 GBq). Sixty-five percent of the radiolabeled antibody bound to a CEA immunoabsorbent. Normal goat IgG (Miles) was radioiodinated with <sup>125</sup>I in a similar fashion, resulting in a specific activity of 12  $\mu$ Ci/ $\mu$ g. Less than 2% of the radiolabeled IgG was aggregated, as determined by gel filtration over Sephacryl-300 (Pharmacia). The specific IgG fraction from donkey anti-goat IgG antiserum was isolated by ammonium sulfate precipitation and affinity purification by passage over a goat IgG immunoabsorbent. The adsorbed fraction was shown by immunoelectrophoresis to be free of extraneous serum proteins, and only 8% of the radiolabeled donkey anti-goat IgG was aggregated as determined by gel filtration. By radioimmunoassay, 70 ng of the purified donkey anti-goat IgG bound 5 ng of <sup>125</sup>I-labeled goat IgG.

**Animal Studies.** Adult female golden hamsters (70-90 g) were given 0.5 ml of a 20% suspension of freshly excised GW-39 (a CEA-producing human tumor xenograft; ref. 14) intramuscularly in both hind legs. After 7 days, each animal was injected *i.p.* with a mixture containing 5  $\mu$ g of <sup>131</sup>I-labeled anti-CEA antibody and 5  $\mu$ g of <sup>125</sup>I-labeled goat IgG. An *i.p.* injection of 250  $\mu$ g of donkey anti-goat IgG (second antibody) was given to one group of animals 24 hr later, while a second group was not injected with the second antibody (control group). At 2, 6, 24, and 51 hr after injection of the second antibody, three animals from each group were sacrificed, and the organs were removed and counted. All animals were bled by cardiac puncture immediately before injection of the second antibody and before sacrificing. The average tumor weight was 0.38  $\pm$  0.13 g. Differences between the groups were tested by a one-way analysis of variance using a two-tailed F distribution.

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Abbreviations: CEA, carcinoembryonic antigen; LESA, liposomally entrapped second antibody.

## RESULTS

A comparison of the percentage of  $^{131}\text{I}$ -labeled goat anti-CEA antibody in the blood of animals that were injected with donkey anti-goat IgG (second antibody) to those animals that did not receive the second antibody is shown in Fig. 1. The animals that received the second antibody had less radioactivity by a factor of 4 in their blood than the control animals had 2 hr after the injection of the second antibody. This difference between the two groups increased by factors of 16 and 48 by 24 and 51 hr, respectively. The enhanced clearance of radiolabeled antibody from the circulation resulted in higher tumor/blood ratios as early as 2 hr after injection of the second antibody (Table 1). After 6 hr, animals that were injected with the second antibody also had appreciably higher tumor/nontumor ratios for all of the tissues tested except the spleen, as compared to those of animals not given the second antibody. Although tumor/nontumor ratios continued to improve after injection of the second antibody, the amount of  $^{131}\text{I}$ -labeled anti-CEA antibody in the tumor remained the same until 24 hr. However, 24 hr after the injection of the second antibody, there was significantly less ( $P < 0.005$ )  $^{131}\text{I}$ -labeled anti-CEA antibody in the tumors from animals that received the second antibody as compared to control group tumors (Fig. 2).

Comparison of the localization ratios (ratio of  $^{131}\text{I}$ -labeled anti-CEA antibody to  $^{125}\text{I}$ -labeled normal goat IgG) showed that the tumors from the animals given the second antibody had significantly higher ( $P < 0.025$ ) ratios than those of tumors from the control group at 24 and 51 hr after injection of the second antibody (Table 2). The enhancement of the localization ratios did not result from a more rapid clearance of the  $^{125}\text{I}$ -labeled normal goat IgG from the circulation because the blood clearance of both  $^{131}\text{I}$ -labeled anti-CEA antibody

and  $^{125}\text{I}$ -labeled normal IgG were similar up to 24 hr after injection of the second antibody (Table 3). Furthermore, localization ratios in the other tissues, such as the liver, spleen, and kidneys, remained constant throughout the study. These data suggest that the accumulation of normal IgG in tumors is related to its persistence in the blood, whereas the anti-CEA antibody is selectively bound and retained in the tumor by antigen.

## DISCUSSION

The injection of a second antibody directed against a primary radiolabeled anti-CEA antibody has been shown to enhance the blood clearance of radiolabeled antibody without subsequent accretion of the antibodies in the liver or other major organs. These results are in agreement with the preliminary findings of Bradwell *et al.* (15), who showed a loss in total body radioactivity by injecting a second antibody alone, and are in contrast to the enhanced liver and spleen uptake of radiolabeled primary antibody when LESA is used, as reported by Ryman and Barratt (9). The rate of blood clearance of radiolabeled primary antibody by the second antibody alone had similar clearance rates to those shown for LESA (9). Thus, it appears that entrapment of a second antibody in liposomes is not essential for accelerating the clearance of a primary radiolabeled antibody. Because LESA has inherent problems associated with the efficient incorporation of functionally active antibody into liposomes and uncertainty about the *in vivo* stability of the liposome-IgG complex, the use of second antibody alone would appear to be preferred over the use of liposomally entrapped antibody.

The mechanism of primary antibody clearance by a free second antibody may be similar to the proposed mechanism

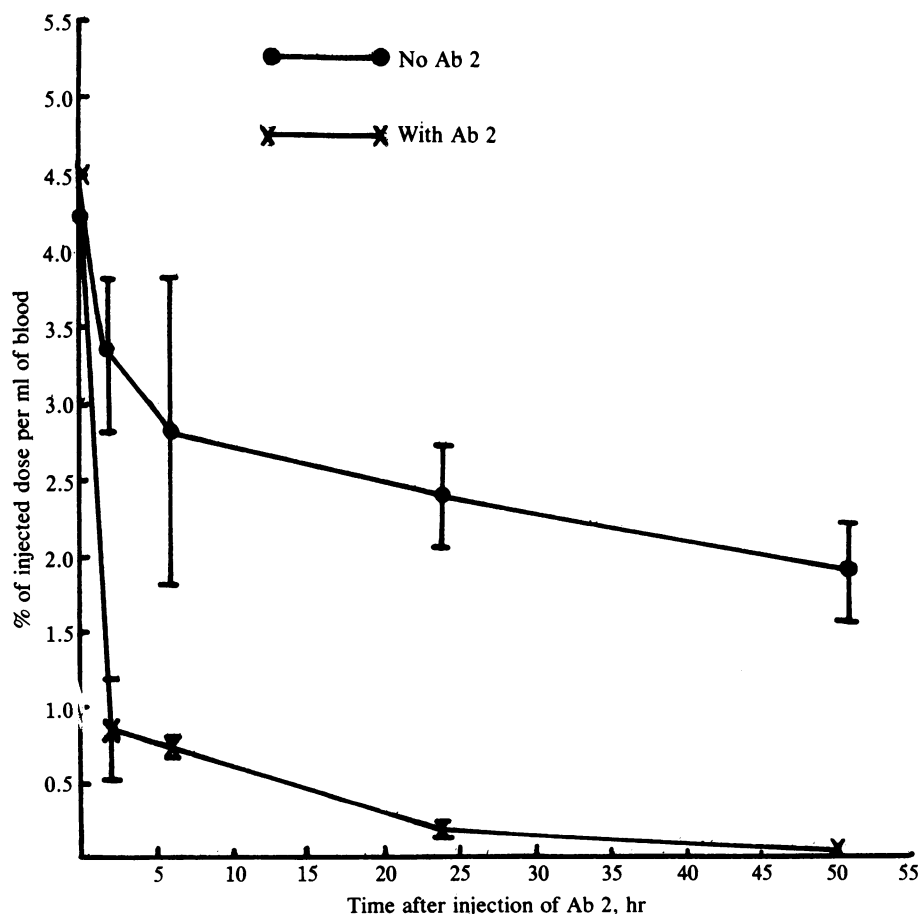


FIG. 1. Blood clearance of  $^{131}\text{I}$ -labeled goat anti-CEA antibody by donkey anti-goat IgG (second antibody). Bars represent means  $\pm$  SEM.

Table 1. Comparison of tumor/nontumor ratios in hamsters bearing colonic tumor xenografts in the presence or absence of a second antibody (Ab 2)

| Tissue | Time after injection of Ab 2 | Tumor/nontumor ratio* |            |
|--------|------------------------------|-----------------------|------------|
|        |                              | Without Ab 2          | With Ab 2  |
| Liver  | 2                            | 1.7 ± 0.2             | 0.8 ± 0.2  |
|        | 6                            | 1.4 ± 0.2             | 2.4 ± 0.4  |
|        | 24                           | 2.9 ± 0.3             | 9.0 ± 2.5  |
|        | 51                           | 4.3 ± 0.3             | 15.5 ± 1.6 |
| Spleen | 2                            | 2.0 ± 0.3             | 1.3 ± 0.4  |
|        | 6                            | 1.5 ± 0.2             | 2.1 ± 0.5  |
|        | 24                           | 3.7 ± 0.6             | 3.7 ± 0.9  |
|        | 51                           | 4.3 ± 0.3             | 3.7 ± 0.6  |
| Kidney | 2                            | 1.4 ± 0.2             | 1.8 ± 0.5  |
|        | 6                            | 0.9 ± 0.1             | 2.8 ± 0.5  |
|        | 24                           | 1.8 ± 0.2             | 4.7 ± 1.0  |
|        | 51                           | 2.6 ± 0.2             | 7.4 ± 1.7  |
| Lungs  | 2                            | 0.7 ± 0.1             | 1.3 ± 0.5  |
|        | 6                            | 0.7 ± 0.1             | 3.0 ± 0.5  |
|        | 24                           | 1.6 ± 0.2             | 6.4 ± 1.7  |
|        | 51                           | 2.1 ± 0.2             | 12.0 ± 0.5 |
| Blood  | 2                            | 0.5 ± 0.1             | 1.4 ± 0.04 |
|        | 6                            | 0.3 ± 0.04            | 2.1 ± 0.4  |
|        | 24                           | 0.8 ± 0.1             | 3.8 ± 0.7  |
|        | 51                           | 1.1 ± 0.08            | 12.4 ± 3.7 |

\*Values are the means ± SEM (*n* = six tumors; *n* = three animals) of:

$$\frac{\text{tumor (cpm/g)}}{\text{nontumor (cpm/g or cpm/ml)}}$$

of clearance by LESA. Although there was no apparent accretion of radioactivity in the liver and spleen as early as 2 hr after injection of the free second antibody, there may have been an earlier accumulation of activity in these organs that was rapidly cleared. At 2 hr, the tumor/liver and spleen ratios were slightly lower than the control group, while other tumor/nontumor ratios were similar or slightly higher than the control group. We also observed a 4-fold increase after 6

Table 2. Influence of second antibody (Ab 2) clearance on the ratio of <sup>131</sup>I-labeled anti-CEA antibody to <sup>125</sup>I-labeled goat IgG in tumor-bearing hamsters

| Tissue | Time after injection of Ab 2, hr | Localization ratio* |            |
|--------|----------------------------------|---------------------|------------|
|        |                                  | Without Ab 2        | With Ab 2  |
| Tumor  | 2                                | 1.2 ± 0.10          | 1.1 ± 0.07 |
|        | 6                                | 1.1 ± 0.10          | 1.4 ± 0.09 |
|        | 24                               | 1.7 ± 0.50          | 3.6 ± 0.52 |
|        | 51                               | 2.1 ± 0.07          | 5.0 ± 1.10 |
| Liver  | 2                                | 1.0 ± 0.01          | 1.0 ± 0.03 |
|        | 6                                | 1.0 ± 0.02          | 1.0 ± 0.05 |
|        | 24                               | 1.0 ± 0.00          | 1.3 ± 0.03 |
|        | 51                               | 0.9 ± 0.01          | 1.5 ± 0.07 |
| Kidney | 2                                | 1.0 ± 0.02          | 1.0 ± 0.04 |
|        | 6                                | 0.9 ± 0.01          | 1.0 ± 0.02 |
|        | 24                               | 0.9 ± 0.01          | 1.1 ± 0.03 |
|        | 51                               | 0.9 ± 0.00          | 1.2 ± 0.03 |
| Spleen | 2                                | 1.0 ± 0.02          | 1.0 ± 0.00 |
|        | 6                                | 1.0 ± 0.05          | 0.9 ± 0.04 |
|        | 24                               | 0.9 ± 0.00          | 1.0 ± 0.03 |
|        | 51                               | 0.9 ± 0.04          | 1.0 ± 0.05 |

\*Values are the means ± SEM (*n* = 6 tumors; *n* = 3 animals) of:

$$\frac{\text{cpm of } ^{131}\text{I-labeled anti-CEA antibody per gram of tissue}}{\text{cpm of } ^{125}\text{I-labeled normal goat IgG per gram of tissue}}$$

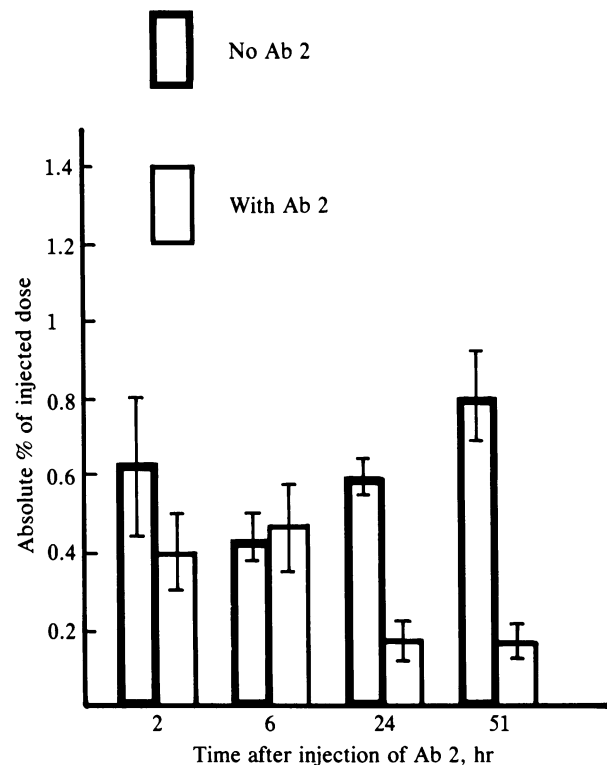


FIG. 2. Influence of clearance by a second antibody (Ab 2) on the anti-CEA antibody concentration in colonic tumor xenografts. Values are given as the percentage of the injected dose per gram tumor times the total tumor weight. Bars represent means ± SEM.

hr in urine radioactivity of animals receiving the second antibody, as compared to the control group (data not shown). These results suggest that there may have been an enhanced liver and spleen uptake of radioactive antibody immediately after injection of the second antibody, but by 2 hr, most of the radioactivity may have been catabolized and rapidly excreted in the urine. Presently, the relative role of the liver and spleen, as well as the kidneys and complement, in the removal of radiolabeled antibody by a second antibody is unknown and will require further investigation. The accumulation of radiolabeled antibody in the liver and spleen after injection of LESA (9) may have been due to a slow release of the second antibody from the liposomes, similar to that observed for other compounds incorporated into liposomes (16), or there may be a slower rate of catabolism of radiolabeled antibody bound to LESA. Thus, the mechanism of clearance of a radiolabeled antibody by either a free, second antibody or LESA may be similar, only differing in the rate of clearance.

The clearance of radiolabeled anti-CEA antibody from the blood did not alter the amount of activity in the tumor until 24 hr after injection of the second antibody. The decreased

Table 3. Comparison of the blood clearance of <sup>131</sup>I-labeled anti-CEA antibody to <sup>125</sup>I-labeled normal goat IgG

| Time after injection of Ab 2, hr | % of injected dose per ml of blood* |                                  |
|----------------------------------|-------------------------------------|----------------------------------|
|                                  | <sup>131</sup> I-goat anti-CEA IgG  | <sup>125</sup> I-normal goat IgG |
| 2                                | 0.92 ± 0.35                         | 0.77 ± 0.27                      |
| 6                                | 0.83 ± 0.03                         | 0.72 ± 0.05                      |
| 24                               | 0.17 ± 0.03                         | 0.12 ± 0.05                      |
| 51                               | 0.04 ± 0.01                         | 0.011 ± 0.003                    |

Ab 2, second antibody; <sup>131</sup>I-goat anti-CEA IgG, <sup>131</sup>I-labeled goat anti-CEA IgG; <sup>125</sup>I-normal goat IgG, <sup>125</sup>I-labeled normal goat IgG. \*Values are the means ± SD (*n* = 3 animals).

antibody concentration in the tumor after 24 hr may have been partially due to the lower antibody concentration in the tumor's vascular bed. However, a lower concentration of antibody in the blood may have reduced also the amount of antibody complexed with antigen within the tumor. Thus, the recirculation of antibody through the tumor may permit additional binding of antibody to newly formed or previously unbound antigenic sites and also may maintain the concentration of antibody initially bound to antigen within the tumor. The fact that the localization ratio of anti-CEA antibody/normal goat IgG in the tumor increased indicates that the specific antibody was firmly bound to the tumor. Further experiments are required to optimize clearance of blood radioactivity by a second antibody while activity in the tumor is maintained. The current study already suggests that this method ultimately may be useful for improved radioimmuno-detection of cancer and even may have applicability for antibody-mediated therapy.

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