ABSTRACT

The simultaneous distribution of monoclonal 125I-labeled anti-carcinoembryonic antigen (CEA) immunoglobulin (IgG) (NP-2) or 131I-labeled irrelevant myeloma IgG (Ag8) and 3H]thymidine was studied in hamsters bearing transplants of the GW-39 human colon carcinoma by qualitative double-tracer whole-body autoradiography. Autoradiography showed that large solid GW-39 tumors are characterized by heterogeneity of double-tracer whole-body autoradiography. Autoradiography showed that both 131I-labeled monoclonal antibody and control 125I-labeled IgG targeted nonproliferating tumor zones, suggesting a mechanism of nonspecific tumor uptake of radioantibodies in these areas. Absence of tumor center labeling with 3H]thymidine, associated with cellular necrosis, was confirmed by histology and microautoradiography in separate animal studies. In confirmation of earlier reports, 125I-labeled anti-CEA monoclonal antibody gave higher tumor-to-non-tumor labeling patterns than did control 125I-labeled IgG, at both 3 and 7 days following treatment. Immunohistochemical localization of CEA in GW-39 tumors with necrotic centers showed the presence of CEA in viable cells, but CEA antigen concentrations were diminished as compared to cells located in the tumor's periphery. The results indicate that double-tracer whole-body autoradiography is well suited for studying the kinetics of radioantibody localization in relation to regional tumor cell viability.

INTRODUCTION

Radioisotope-labeled antibodies have been successfully used to image tumors in both animals (1-4) and humans (5-8). However, a growing body of evidence supports the view that only small, well-vascularized tumors show a dose-related incremental uptake of tumor-directed radioantibodies (9, 10). Moreover, a differential viability associated with solid tumors has been established in studies which indicate that the core regions of solid tumors greater than several millimeters diameter are characterized by central necrosis (11) and reduced vascularity (12, 13).

We have previously utilized the CEA-secreting GW-39 human tumor model to demonstrate that quantitative WBAR has sufficient spatial resolution to provide detailed histological imaging of affinity-purified anti-CEA radioantibody in hamsters bearing tumors on the dorsum (14) or in GW-39 lung tumors (16) in their hind leg musculature or s.c. in the region of the nape of the neck were given injections i.p. of 0.15 mCi of either 125I-labeled mouse monoclonal anti-CEA antibody designated NP-2 (17) or an irrelevant murine IgG (Ag-8). The chloramine-T method (18) was used to radioiodinate the IgGs to a specific activity of 12-18 mCi/mg. Both radioiodinated IgGs had less than 2% free iodine and were free of contamination between nuclide pairs, the WBAR images derived from single nuclide-treated hamsters bearing GW-39 tumors were studied. These separated control autoradiographic images showed that 3H]thymidine, at 10 µCi/g, did not darken LKB Ultrofilm after an initial period of 81 days of storage decay and subsequent exposure at -20°C. Exposed films were made by standard film developing procedure. As a control for possible cross-contamination between nuclide pairs, the WBAR images derived from single nuclide-treated hamsters bearing GW-39 tumors were studied. These separated control autoradiographic images showed that 3H]thymidine uptake was not appreciably blacken Dupont Low-Dose Mammographic Film with exposures of 3-5 days' duration. Similarly, 131I-labeled antibody, at 0.75 µCi/g, did not darken LKB Ultrofilm after an initial period of 81 days of storage decay and subsequent exposure on this film for 10 weeks. The interposition of 2 mil thick Kapton plastic films between sections and x-ray films, as a filter to absorb β emissions from 3H]thymidine, was found to have no appreciable effect on the completed double-tracer autoradiographic images at the exposures used. Moreover, there were no chemographic artifacts observed with both positive and negative chemographic controls processed by the same WBAR procedures.
fully cut out of the enlarged projections. The cut out portions, corresponding to areas of $^{131}$I and $^{3}$H radioactivity uptake in tumors, were then weighed on a microbalance. The fractional areas of $^{131}$I or $^{3}$H radioactivity localization, relative to the entire tumor area, were then expressed on a weight basis.

Microautoradiography and Immunohistochemistry. Light microscopic autoradiography of $[^3H]$thymidine distribution in GW-39 tumors was performed on separate groups of animals. Details of the microautoradiographic method used has been published previously (20, 21). Briefly, 5-µm tumor sections were dewaxed and hydrated to water. Sections were then dipped in an aqueous (1:1) dilution of Kodak NTB-2 emulsion in the darkroom. Coated slides were air-dried for 45 min in the darkroom with relative humidity maintained at 75–90%. Exposures were made for 10–14 days in light-tight boxes. Exposed slides were developed in D-19 developer and GBX fixer, washed, and counterstained with cresyl violet.

A peroxidase-antiperoxidase procedure as described by Primus and Goldenberg (22) was used for the immunohistochemical localization of CEA. Briefly, 1- to 2-cm hind leg GW-39 tumors were removed from untreated animals and fixed for 48 h in acetic acid:95% ethanol. After embedding in paraffin, 5-µm sections were dewaxed and pretreated with methanol and hydrogen peroxide to inhibit endogenous peroxidase activity. The sections were overlaid with either affinity-purified goat anti-CEA or normal goat IgG as a control for 24 h at 4°C. After 2 washes, the sections were sequentially incubated in donkey anti-goat IgG and finally goat peroxidase-antiperoxidase complexes. After exposure to diaminobenzidine, the sections were counterstained with hematoxylin.

RESULTS

Double-Tracer Whole-Body Autoradiography. The macroautoradiographic results showed that imaging patterns derived from $^3$H and $^{131}$I with the same GW-39 tumor could be obtained by exploiting the differences in the physical half-life and radiation energy between the radioisotopes (Figs. 1–8). The fine spatial detail provided by WBAR permitted the superimposition and alignment of two autoradiographs made with the same tumor section (e.g., compare Fig. 1, a to b). In this manner, it was possible to correlate intratumor localization of $^{131}$I-labeled antibody with the pattern of $[^3H]$thymidine registration, the latter providing an index of rapid tumor cell proliferation.

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**Fig. 1.** Double-tracer WBAR localization of GW-39 tumors (arrows) by monoclonal $^{131}$I-anti-CEA antibody (a) or $[^3H]$thymidine (b) in the same section 3 days after administration of radioantibody. Note the pattern of radioantibody retention corresponding to actively proliferating tumor perimeter (TP) and high radioantibody uptake in the necrotic tumor center (NC). B, bone tissue.

**Fig. 2.** Double-tracer WBAR localization of GW-39 tumors (a) after injection of $^{131}$I-labeled irrelevant IgG (a) or $[^3H]$thymidine (b) in the same section. Note that actively growing sites in the tumor periphery show little concentration of $^{131}$I-labeled IgG radioactivity. Accumulation of control $^{131}$I-labeled IgG is seen in necrotic tumor cores.
At 3 and 7 days after treatment, the $[^3]H$thymidine labeling pattern of GW-39 was observed to be highly variable, with evidence of the highest $[^3]H$ radioactivity accumulation in the outer perimeter (Fig. 3b) or in the leading edge of tumor growth (Fig. 8b). Even in adjacent sections of the same tumor, heterogeneity of $[^3]H$thymidine uptake was evident, indicative of widely varying physiological environments within cortical and medullary tumor zones. Confirmation of growth in outer and inner GW-39 tumor zones was made with paraffin block microautoradiography conducted with hamsters bearing 2- to 3-cm tumors transplanted to the hind limb musculature and receiving injections of $[^3]H$thymidine. These specimens revealed a consistent pattern of absence of $[^3]H$thymidine labeling in tumor cell nuclei of the medulla (results not shown). The geometric centers of large GW-39 tumors were found to be largely acellular and gave evidence of widespread cytolysis. A sparse microvasculature was observed in the surviving stroma of medullary and juxtamedullary tumor zones. The histopathological condition of condensation of nuclear chromatin in apparently nutritionally starved central tumor cells suggested that these cells had lost their physiological ability to incorporate available $[^3]H$thymidine into replicating DNA. In marked contrast, clusters of cortically located GW-39 cells in pseudoacinic structures were observed to be of normal histological appearance and were also labeled intensely with $[^3]H$thymidine in their nuclei (results not shown).

When the distribution patterns of monoclonal $^{131}I$-labeled anti-CEA IgG and $[^3]H$thymidine uptake patterns were compared in the same tumor, good correlation between the images was found only with reference to the actively dividing outer tumor perimeter (Figs. 3 and 5). During these time intervals, the leading edge of GW-39 tumors and the subcapsular cortex were characterized by the highest levels of $[^3]H$thymidine incorporation (Figs. 3b and 6b). A better correlation was found between active sites of cellular proliferation in outer tumor perimeters and radioantibody localization in GW-39 tumors which grew in a noncystic configuration (Fig. 3) than tumors with a cystic growth pattern (Fig. 7). Radioimmunolocalization carried out with $^{131}I$-labeled irrelevant IgG, however, revealed a clear difference from $^{131}I$-labeled anti-CEA IgG with respect to correlation to tumor regions identified with $[^3]H$thymidine as actively dividing. Thus, double-tracer autoradiographic profiles showed that control $^{131}I$-labeled IgG uptake was only partially related to $[^3]H$thymidine registration in tumors at 3 days posttreatment and completely unrelated to $[^3]H$thymidine retention in tumors at 7 days after treatment (compare Fig. 4 with Fig. 6). This condition of differential and preferential targeting by proliferating tumor cells of $^{131}I$-labeled anti-CEA IgG establishes the basis for the observed high tumor-to-non-tumor uptake patterns identified with this radioantibody (Figs. 1a and 5a).
Both labeled anti-CEA antibody and control IgG were found to share a common strong affinity for nonproliferating, centrally located GW-39 tumor cells, when qualitatively evaluated by WBAR at 3 and 7 days after treatment (compare Figs. 3a and 4a with Figs. 7a and 8a). These autoradiographic data suggest a similar nonspecific mechanism responsible for labeled anti-CEA antibody and irrelevant antibody retention in necrotic GW-39 tumor centers.

When determinations were made with projections of the separated [3H]thymidine radioactivity images shown in Figs. 3b and 7b, it was found that on days 3 and 7 after injection an average of 40% of the tumor could be qualified as actively dividing cells. Area measurements carried out on day 7 after treatment showed that the radioactivity retention of 131I-labeled anti-CEA IgG was 34% of the total tumor area.

Immunohistochemistry. Immunoperoxidase localization studies of CEA in alcohol-fixed sections of xenografted hind leg GW-39 tumors demonstrated the presence of relatively large concentrations of CEA in tumor perimeter regions populated by GW-39 tumor cells of normal histological appearance. When the medullary regions of these same GW-39 tumors were stained for CEA, the antigen was found to be present in nonviable cells, but the intensity of staining was much less than that of the cortically located tumor cell fraction (Fig. 9).

DISCUSSION

The results of the present study show that application of double-tracer WBAR, with GW-39 tumors measuring 1–3 cm diameter, facilitates the analysis of the kinetics of radioantibody localization in these tumors which are characterized by rapid cellular proliferation in the peripheral regions and central necrosis. In the xenografted GW-39 human tumor model used here, 131I-labeled anti-CEA monoclonal antibody was combined with [3H]thymidine to simultaneously monitor the uptake of radioantibody and assess zonal viability within the same tumor. The feasibility of using double-label macroautoradiography for evaluation of physiological processes in laboratory animals has been previously reported for measurement of local cerebral blood flow and local metabolic rate for glucose (23), coronary...
circulation (24), and methionine metabolism (25). However, double-tracer WBAR has not yet been applied to the study of regional distribution of radioantibodies in tumors coupled with zonal viability assay. Our finding that a correlation exists between local accretion of radioantibody in tumor perimeters and rapid cell division confirms earlier studies linking linearity of radioantibody uptake with small viable tumors (1, 9).

Consistent with earlier findings (1, 14), our present WBAR data show that maximum discrimination between tumor and nontumor tissues was observed at 7 days after treatment with radiolabeled monoclonal antibody. Under these conditions, labeled anti-CEA monoclonal antibody (Fig. 5a), but not control immunoglobulin (Fig. 6a), was localized to the outermost tumor growth region, these latter sites also were identified as the zones of rapid cell proliferation (Figs. 5b and 6b). Similar observations of perimeter localization of radioantibody have been reported with other xenografted tumor models, including the 791T osteosarcoma system (26), malignant teratoma (27), and human colon cancer (4). Thus, as suggested elsewhere (1, 28), radiolabeled monoclonal anti-CEA IgG can best be confirmed in smaller tumors with well-developed vasculature, where tumor cell viability is maximized and nonspecific retention of radioantibody is diminished. Previous studies have shown that rapid mixing of injected $^{51}$Cr-labeled RBCs occurs between transplanted tumor vasculature and the general circulation of the host over a time period of 10–15 min after injection (29, 30). Furthermore, the computed ratio of the amount of RBCs in rat Walker carcinoma 256 and in the host blood was found to be unchanged when measured over the interval of 15–120 min (30). Based on these findings, it is reasonable to infer that GW-39 tumor cells were given access to injected $[^3]$H]thymidine within the 2-h utilization period used in this study, even in those medullary and juxtamedullary tumor zones where the surviving microvasculature was sparse.

The previously established growth rate heterogeneity among subclones of cancer cell populations (31, 32) and the present demonstration of zonal growth rate variation in GW-39 tumors measuring 1–3 cm emphasized the significance of tumor size-radioantibody relationships in radioimmunodetection. The consensus of data from the literature supports the existence of an inverse relationship between tumor size and uptake of antitumor radioantibodies in human tumor models (9, 10, 33, 34). In a recent investigation of radioantibody incorporation in nude mice bearing human colon tumors and human melanoma, it was concluded that there is a systematic decrease in uptake of radioantibodies in these tumors as they enlarged beyond a total weight of 0.5 g (10). These data were expressed as a percentage of uptake per g tumor weight and percentage of uptake in the whole tumor. Our present double-tracer WBAR imaging method adds the valuable dimension of historadiological spatial
judged to be nonviable. However, no direct assay for viability actively dividing at 3 and 1 days after injection of radioantibody of "I-labeled antibody mainly into areas of proliferating resolution and provides direct evidence for the negative relationship observed between incremental tumor growth and tumor-specific radioantibody accretion. Our studies showed uptake of 131I-labeled antibody mainly into areas of proliferating tumor cells. Area analyses demonstrated the relatively small fraction of tumor (i.e., 40% of total tumor area) which was actively dividing at 3 and 7 days after injection of radioantibodies, the same areas also showing selective accretion of radioantibody. These viability determinations for large GW-39 tumors are in excellent agreement with previously reported viability indexes, using the GW-39 tumor model. This approach will allow us to assess the WBAR patterns of radioantibody localization in tumors of varying size as a function of viability and presence or absence of the Fc segments in the radiotracers.

In summary, the current data suggest that the application of double-tracer WBAR will facilitate the analysis of radioantibody localization, particularly in solid neoplasms characterized by differential proliferation rates and central necrosis. This high-resolution imaging method should also find potential use in therapeutic applications, where assessment of tumor mass viability is of paramount significance.

REFERENCES


Relationship of Radioantibody Localization and Cell Viability in a Xenografted Human Cancer Model as Measured by Whole-Body Autoradiography

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