06.86.16

COMUNICACIONES BIOLOGICAS

Vol 4 N°4 (1986)
p 387-394

IN VIVO ASSESSMENT OF ANTIGEN-MONOCLONAL ANTIBODY BY RADIOIMMUNO SCINTIGRAPHY

HM Glait, AL Horestein, Á Koss, E Sliak, E D'Orio, AJ Olivari

Oncologic Center of Nuclear Medicine.CNEA, UBA, Facultad de Medicina. Instituto de Oncología "Angel H Roffo"

Key words: Carcinoembryonic antigen. Gamma Camera radioimmunoscintigraphy. Hybridoma tumor. Monoclonal antibody

INTRODUCTION

For many years radiolabelled polyclonal antibodies have been used for tumor imaging (1,2), but the use of these antibodies has been limited, since the preparations are not specific for tumor tissues alone. The hybridoma technology has eliminated many of these problems, and it is now possible to produce monoclonal antibodies that are highly specific for tumor associated antigens like CEA (3). Recently, there have been many reports describing the use of radiolabelled monoclonal antibodies in tumor imaging (3,4), but there is a number of difficulties such as those associated to the availability of an specific human tumor xenograft model for evaluating in vivo the antibody biological activity (5).

In this paper computerized studies were performed with the gamma camera to assess the immunological activity of a

ACKNOWLEDGEMENTS: The authors thank Miss Claudia Simon for typing the manuscript.

ABBREVIATIONS: CEA: Carcinoembryonic antigen, PBS: Phosphate Buffer Saline, MAb: Monoclonal antibodies.

monoclonal antibody by means of radioimmuno-imaging a subcutaneous hybridoma tumor producing monoclonal anti-CEA antibodies, obtained in our laboratory and implanted in mice after several months of in vitro stabilization.

MATERIALS AND METHODS

HYBRIDOMA PRODUCTION

The methodology used for cell fusion of spleen cells, obtained from mice immunized with purified CEA, with mouse myeloma P3-X63-Ag 8.653 as well as the selection and screening of specific antibody secreting clones have been described previously (6,7). The anti-CEA antibody secreting hybridomas were cloned at least twice by limiting dilution. One such cell line, named A_4, taken from a vigorously growing culture, was centrifuged and resuspended to a cell density of about 10^7/ml in PBS and derived as solid tumor in Balb/c mice.

MONOCLONAL ANTIBODY SPECIFICITY

A cross-over immunoelectrophoresis carried out on agar gel in Veronal buffer, pH 8.2 was applied to assess the specificity of monoclonal antibodies secreted by hybridoma A_4 (7).

RADIOIODINATION OF CEA

Antigen CEA was labelled by iodination with $Na^{125}I$, using the Iodogen reagent (1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril)(8). The radioactive protein, decanted from free ^{125}I onto a DOWEX 1-X8 resin (Baker Chem.)-5ml syringe column was evaluated for incorporation of iodide by paper chromatography in 10% trichloroacetic acid. The reactivity of CEA before and after iodination was tested in a radioimmunoassay using the MAb A_4 as target for the antigen (7).

RADIOIMMUNO IMAGING

A Balb/c mouse bearing a non-palpable subcutaneously growing graft of anti-CEA producing hybridoma A_4 was given 50uCi ^{125}I-CEA intravenously in 0.1ml PBS. The total body

images were obtained at 1,24,48,72 and 144hs with a Technicare Corp. Gamma Camera, using a high resolution collimator. Images of 20,000 counts or 20min, whichever ocurred first, were obtained and processed by a MCS 560 computer system (9). Another mouse, with a good size A_4 hybridoma tumor of 4.8g, orally pretreated for 4 days with Lugol's solution to minimize non-specific uptake of unincorporated radioiodine, was injected intravenously with ^{125}I-CEA and the images obtained in the same conditions . After correction for radioactive tracer decay and differences in counting time, results were expressed as number of counts per pixel (minimal information unit of the computer image screen) of a 128x128 matrix display.

RESULTS

Once conditions for optimal incorporation of iodine ^{125}I were found, the effect of iodination on the immunological reactivity of the labelled CEA was carried out by cross-over immunoelectrophoresis (Fig.1). ^{125}I-CEA was immunoprecipitated by MAb-A_4 as well as by mouse anti-CEA serum, but not by a MAb* used as control.

A Balb/c mouse, bearing a non-palpable A_4 hybridoma tumor, was injected with ^{125}I-CEA and imaged at daily intervals (Fig.2B). As can be seen, the radioimmuno-localisation was clearly visible by camera scintigraphy, 24hs after administration of the labelled probe and became increasingly clear throughout the study. Only tumor and thyroid tissue remained visible by day 7. Table 1 lists computer-integrated counts from pixels over the total body, tumor, liver and thyroid tissue. Whole-body activity remains nearly constant during the analyze time. Tumor radioactivity uptake showed progressive antigen accumulation during the first 3 days and no loss thereafter, suggesting that the antigen is retained in the tumor during the observation period. As expected, there was an important uptake of free radioiodine by the thyroid gland, whereas for the liver it declined after a non-specific initial binding.

A mouse bearing a well developed A_4 hybridoma tumor of 4.8g was imaged in the manner described above but previously treated with IK to block the thyroid gland (Fig.2C). Two days after i.v. injection of the labelled preparation, the tumor was only visible after computarised substraction of the 1h background image. The high blood level of circulating MAb-A_4 released by

Scintigraphyc monoclonal antibody detection

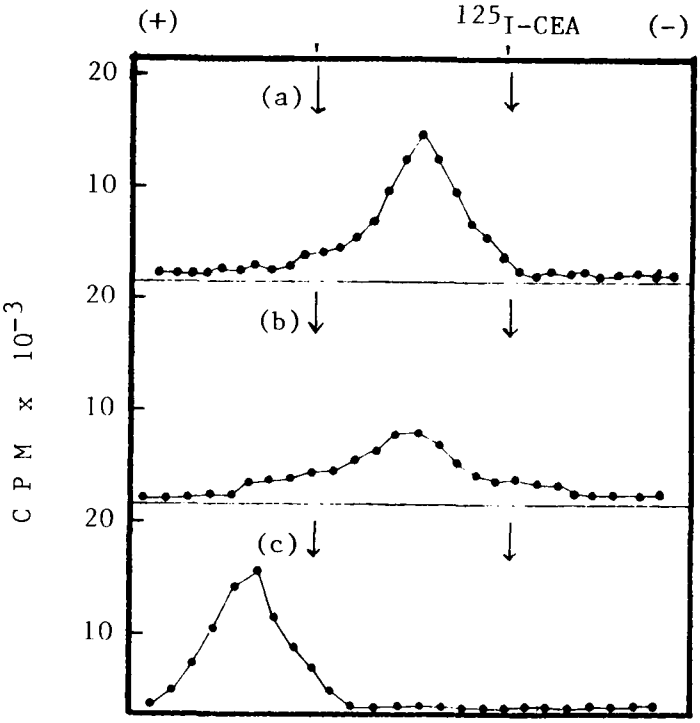

Fig.1 Cross-over immunoelectrophoresis. The profile curve of radioactivity in agar-gel strips is shown for: (a)anti-CEA MAb A4; (b)mouse anti-CEA serum and (c)control mouse serum.

the hybridoma tumor might decrease the amount of ^{125}I-CEA capable of reaching the tumor mass.

When a control mouse with an implanted myeloma X63-Ag 8.653-subcutaneous graft, was also given 50uCi of ^{125}I-CEA i.v., the tumor was not visible at any time after injection of the

* Horenstein, A.L. et al. (Unpublished results).

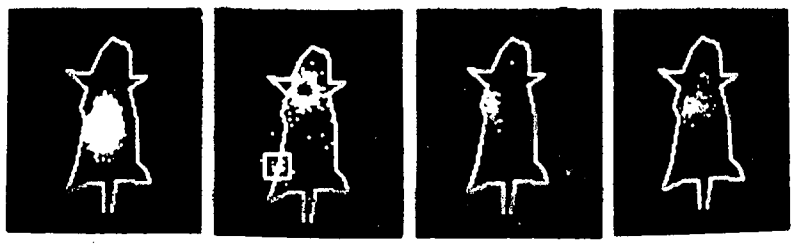

Fig.2 Gamma Camera scintigraphy for tumor localisation using 125I-CEA in mice bearing: a non-palpable A4 hybridoma tumor (b); a 4.8g-subcutaneous A4 hybridoma tumor (c) and a myeloma tumor (d). The initial image obtained 1h-post injection of 125I-CEA for mice (b),(c) and (d) is shown in (a).

labelled CEA and no specific organ localisation was observed (Fig.2D).

DISCUSSION

An antigenic determinant associated with CEA has been recognised in vitro by a MAb obtained in our laboratory. The immunoprecipitation of ^{125}I-CEA by MAb-A4 and the lack of cross-reaction with a control-MAb* confirmed that the mild Iodogen technique caused no reduction in immunoreactivity and specificity of the labelled antigen.

Gamma Camera scintigraphy was applied to analize the in vivo reaction of ^{125}I-CEA with MAb-A4 produced by an hybridoma allograft implanted on mice. Our data showed a preferential localization, quickly achieved, of the labelled probe in the subcutaneous hybridoma tumor compared to other organs, as function of time. The tumor to whole-body ratio continually rose, indicating no dissociation of antibody-antigen complexes attached to the tumor cells, which contribute to improve imaging as seen by scintigraphy. Moreover, the radioimmuno-localization of a non-palpable tumor, suggest the possibility of an early detection of a tumoral mass. In addition, since the majority of uptake of ^{125}I-CEA was completed by 72hs, the use of higher γ-emitting shorter-half life radionuclides (ex:^{123}I, ^{131}I) is

TABLE I: Computer-integrated counts from pixels over the Total body, tumor, thyroids and liver.

counts x pixel / hours	Total body (%)	Tumor (%)	Thyroids (%)	Liver (%)
1	17.4 (100)	1.0 (5.7)	11.2 (64.3)	32.8 (188)
24	16.6 (100)	3.6 (21.6)	31.5 (189)	31.8 (191)
48	19.0 (100)	4.9 (25.8)	46.0 (242)	25.3 (133)
72	19.4 (100)	9.3 (47.9)	68.5 (353)	24.0 (124)
144	17.5 (100)	9.0 (51.4)	72.2 (412)	17.9 (102)

feasible. The increase of counts over thyroid gland was probably due to in vivo dehalogenation of ^{125}I-CEA, metabolised by the liver, and sequestration of free radioiodide in the thyroid. Accordingly, there was no accumulation of radioactivity when the thyroid uptake was blocked with IK solution. The diffuse distribution of the radioactivity observed in the control mouse suggests that ^{125}I-CEA reacted specifically with the MAb A_4 molecule in situ.

Radioimmuno-scintigraphy with ^{125}I-MAb has been performed in nude mice bearing human tumors producing CEA (3). However, when xenografts of antigen producer tumors are difficult to obtain, the use of our animal model provides a tool for in vivo visualization of the antigen-antibody reaction.

RESUMEN

Con el objeto de estudiar la actividad inmunológica "in vivo" de un anticuerpo monoclonal (A_4) dirigido contra el antígeno carcinoembrionario (CEA) se inyectó por vía endovenosa 50uCi de CEA, marcado con el radioisótopo 125I, a ratones portadores de implantes subcutáneos del hibridoma productor del anticuerpo monoclonal A_4.

Utilizando una Camara Gamma se obtuvieron imágenes, de 20K cuentas ó 20min indistintamente, de cuerpo entero a las 1,24,48, 72 y 144 horas luego de la administración de la sonda radioactiva. El tumor fue radioinmunolocalizado a las 24h, aumentado la incorporación específica de ^{125}I-CEA a lo largo del estudio. Resultados similares fueron obtenidos en ratones portadores de hibridoma A_4 palpable o no palpable mostrando la posibilidad de una observación precoz. Estos resultados permiten la aplicación de este modelo animal para la observación in vivo de una reacción antígeno-anticuerpo.

SUMMARY

To assess the immunological "in vivo" activity of monoclonal antibody A_4, specific for carcinoembryonic antigen (CEA), mice bearing subcutaneous growing grafts of hybridoma A_4, producing anti-CEA antibodies, were given 50uCi of ^{125}I-CEA intravenously. Total body images were obtained at 1,24,48,72 and 144h with a Gamma Camera. Images of 20K counts or 20min, whichever ocurred

first, were obtained and processed by a computer system. Radioimmunolocalisation was clearly visible by camera scintigraphy 24h after administration of the labelled probe and became increasingly clear throughout the study. Images were similar in mice bearing palpable or non-palpable hybridoma tumors.

We conclude that our animal model provides a tool for in vivo visualization of the antigen-antibody reaction.

REFERENCES

1- Pressman, D., (1980). Cancer Res. 40:2960-2964.
2- Mach, J.P., Carrel, S., Forni, M. et al., (1980). N.Engl. J. Med. 303:5-10.
3- Mach, J.P., Buchegger, F., Forin, M. et al., (1981). Immunol. Today 2:239-249.
4- Buraggi, G.L., Callegaro, L., Mariani, G. et al (1985). Cancer Res. 45:3378-3387.
5- Otsuka, F.L., Welch, M.J., Mc Elvany, K.D., Nicolotti, R.A. and Fleischman, J.B., (1984). J. Nucl. Med. 25:1343-1349.
6- Horenstein, A.L., Glait, H.M., Koss, A., Olivari, A.J., (1985) 28° Winter Meeting of Sociedad Argentina de Inmunología (Rosario) p.9
7- Horenstein, A.L., Glait, H.M., Koss, A., Capalbo, E., D'Orio,E., Olivari, A.J., (1985), Medicina 45:423.
8- Fracker, P.J., Speck, J.C., (1978). Biochem. Biophys. Res. Commun. 80:849.
9- Slipak, E., Glait, H.M., Olivari, A.J. (1984). IX Congreso de ALASBIMN. Montevideo - Uruguay.

RECEIVED: 25/5/86

Correspondencia dirigida a:

Dr. Horacio M. Glait
Centro Oncológico de Medicina Nuclear
Instituto de Oncología
"Angel H. Roffo"
Av. San Martín 5481
1417 Buenos Aires
ARGENTINA