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In Vitro Evaluation of Radiolabeled Antibodies for Radioimmunolocalization of Cancer

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SUMMARY

Over 200 iodinations of anti AFP and of anti CEA antibodies were performed according to the chloramine T technique. The appropriate experimental conditions for obtaining predetermined specific activity of iodinated IgG are suggested. Immunological reactivity of radiolabeled antibodies containing up to 5 atoms of iodine per IgG molecule remained unchanged. On the other hand antibodies of higher specific activity were less satisfactory in reaction with antigen and their electrophoretic mobility was changed for beta-alpha. A nomograph for determination of the specific activity of antibodies is presented. In addition a technique of radio-rocket-immunoelectrophoresis suitable for assessment of radiolabeled antibodies prior to their use in vivo is described.

Radioimmunolocalization of human tumours was achieved by workers from few centers (3, 5) who used ^{131}I-antibodies (^{131}Ig) directed to various tumour markers. While procedure of labeling antibodies suitable for radioimmunolocalization is of primary importance (2) we do not know any systematic studies focused on this subject. Techniques of protein iodination are well developed including those suitable for antibodies (especially for radioimmunometric assays) but it must be realized that desired standard of ^{131}Ig for radioimmunolocalization of cancer might be difficult to meet: very high specific activity and at the same time unchanged affinity of Ig to the antigen are required. In addition although the successful labeling of tumour is a final prove of appropriate labeling of Ig it is of primary importance to develop simple and quick in vitro technique for assessment of the labeled Ig.

In this paper we attempt to determine the experimental conditions suitable for labeling of Ig to demanded specific activity. In addition we try to set up a simple and quick in vitro method for evaluation of the reaction of ^{131}Ig with the appropriate antigen.

Key words: Radioimmunolocalization, Radiolabeled antibodies, AFP, CEA

MATERIAL AND METHODS

Horse gamma globulins anti human alphafetoprotein (a-AFP) were kindly donated by Prof. Hirai (Sapporo, Japan) while goat gamma globulins anti -carcinoembryonic antigen (a-CEA) were a gift of Prof. Balbierz (Wrooaw, Poland). One day prior to iodination specific antibodies (sp-a-CEA or sp-a-AFP) were obtained by eluation from immunosorbents (Sepharose 4B-CEA or Sepharose 4B-AFP). 1 M glycine-HCl pH 2.2 was used for dissociation. Protein concentration in the eluted fractions was determined by spectrophotometry and fractions were concentrated by ultrafiltration if necessary.

$Na^{131}I$, $Na^{125}I$ and $Na^{127}I$ (cold iodine supplemented with traces of ^{131}I) were used for iodination. The first two were purchased either from Swierk, Poland or from Amersham, England. Iodination was performed in 0℃ using the chloramine T technique according to the following protocol based on the technical details described by Hunter (4):

1. 1 mCi ^{125}I, or 1 mCi ^{131}I, or 8-15,000 ng ^{127}I	10-30 μl
2. 0.1-50 μg Ig(0.07-1 $\mu g/\mu l$)	20-50 μl
3. 1-100 μg of chloramine T (0.07-2 $\mu g/\mu l$)	2-10 μl
4. After 0.5 to 2,000 min iodination was stopped with 1-100 μg of sodium metabisulfate (0.07-2 $\mu g/\mu l$)	2-10 μl
5. 3 mg of human albumin (3 mg/ml)	1,000 μl

Labeled antibodies were separated from unbound iodine on Sephadex G 25 column. Labeling efficiency was estimated by protein precipitation with trichloroacetic acid. Number of iodine atoms introduced into one molecule of immunoglobulin was calculated according to nomograph (Fig. 1). The nomograph was prepared assuming that 1 mCi of ^{131}I contains 8 ng of iodine and 1 mCi of ^{125}I contains 55 ng of iodine. It was also assumed that immunoglobulin G of approximate molecular weight 160,000 daltons predominates in Ig preparations. Mobility of labeled Ig was determined in agarose electrophoresis and reaction of I-Ig with antiserum anti goat serum proteins and with either CEA or AFP were studied in radio-rocket-immuno -electrophoresis.

RESULTS

Over 200 iodinations were performed. In every experiment iodine atoms were introduced into Ig molecules but labeling efficiency differed from few to up to 95%. Basing on the performed iodinations five protocols of Ig iodination are suggested (Table 1). The efficiency of labeling experiments performed in similar conditions but using various bathes of iodine and of a-CEA or a-AFP were similar but not identical. The efficiency of labeling depended on concentrations of iodine, Ig,

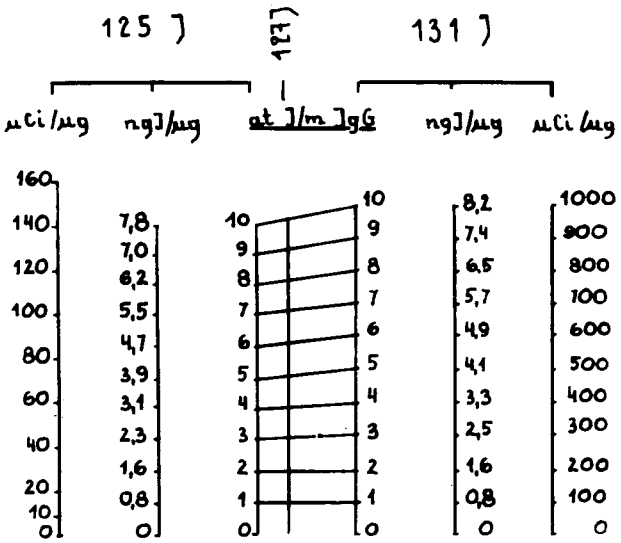

Fig. 1 Nomograph for the determination of specific activity in atoms of iodine per one molecule of IgG. Efficiency of labeling in μCi/μg of IgG must be first determined e. g. with trichloroacetic acid precipitation.

Table 1 *Protocols of iodination of IgG with ^{125}I, ^{127}I and ^{131}I Time of iodination—2 min. temperature O°C.*

No.	Iodine Preparation	ng	(μl)	IgG μg	(μl)	Chloramine T μg	(μl)	Efficiency of iodination: % of iodine incorporated into IgG	Efficiency of iodination: No. of iodine atoms incorporated into one IgG molecule	Specific activity of IgG μCi/μg	Electrophoretic mobility of I-Ig
1	1 mCi ^{125}I	55	(10)	25	(25)	2	(10)	60	1.5	24	gamma
2	1 mCi ^{131}I	8	(10)	15	(10)	2	(10)	65	0.4	43	gamma
3	1 mCi ^{131}I	8	(10)	3	(2)	2	(2)	35	1.2	120	gamma
4	1 mCi ^{131}I	8	(10)	0.3	(2)	2	(2)	15	5	500	gamma–beta
5	^{127}I	3,000	(10)	25	(25)	100	(10)	20	30	–	beta-alfa

chloramine T and on time of the labeling (Fig. 2).

The electrophoretic mobility of I-Ig containing less than 2 I atoms per molecule remained unchanged, while the mobility of I-Ig containing 2 to 10 I atoms per Ig molecule changed for beta-gamma. (Fig. 3). I-Ig containing 10 to 30 I atoms per molecule migrated like alpha-beta globulins. All preparations of I-sp-a-CEA were recognized by antiserum anti goat serum proteins (Fig. 4). In case of one preparation of ^{127}I-a-CEA containing about 30 I atoms per Ig molecule the morphology of IgG rocket was changed.

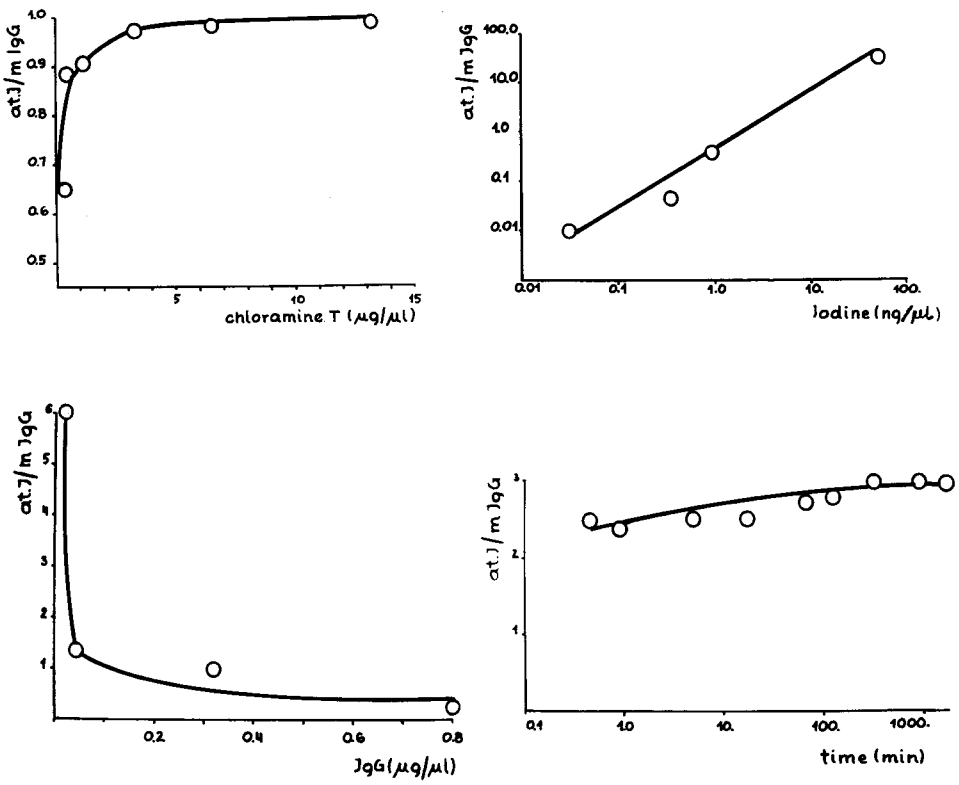

Fig. 2 Dependence of efficiency of iodination (measured in atoms of iodine incorporated in one IgG molecule) on concentration of chloramine T, iodine and IgG and on the time of iodination.

All iodinated preparations of sp-a-CEA and of sp-a-AFP remained active to the appropriate antigen, however this reaction decreased in case of preparations containing more than 10 atoms of I per Ig molecule (Fig. 5). When Gel Bound Film was used as a support instead of a glass plate rockets could be cut off and their radioactivity determined in the gamma scintilation counter (Polon, Poland). In these experiments up to 60% of radioactivity applied to the sample could be demonstrated in the rocket. 100% precipitation of I-Ig could not be detected in the rocket because as seen on Fig. 3 some Ig molecules migrated to cathode.

Five samples of I-Ig were separated on Sephadex G 150 column. In case of I-a -CEA containing more than 10 I atoms per Ig molecule the high molecular fraction probably corresponding to aggregated Ig was largely increased as comparing to unlabeled sp-a-CEA.

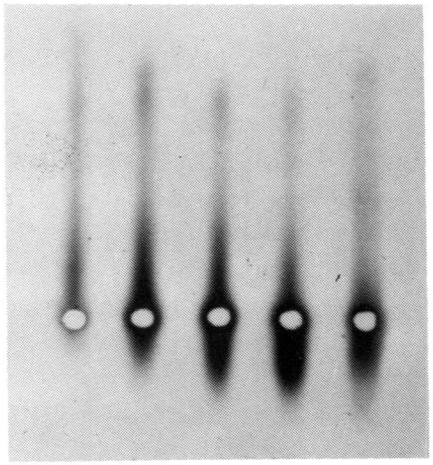

Fig. 3 Agarose electrophoresis (anode to the top). Wells contain various ^{131}I-Ig in buffer containing albumin. From left: ^{131}I-a-AFP (10 at. I per m. IgG), ^{131}I-a-AFP (5 at. I per m. IgG), ^{131}I-a-AFP (1 at. I per m. IgG), ^{131}I-a-AFP (0.1 at, I per m. IgG) and ^{131}I-a-CEA (1 at. I per m. IgG).

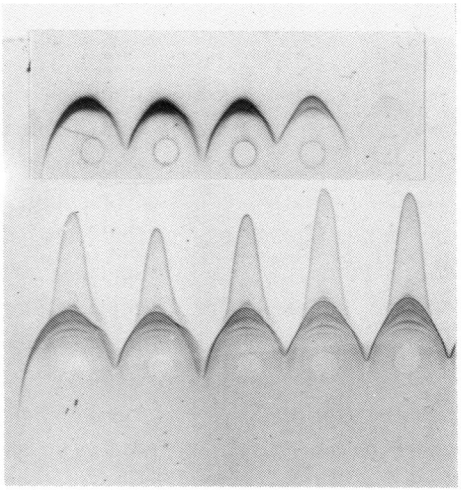

Fig. 4 Rocket immunoelectrophoresis: bottom — plate; top — autoradiograph. Upper gel contains horse antiserum anti goat serum proteins. Wells contain 2 μl of goat a-CEA and various ^{131}I-a-CEA preparations.

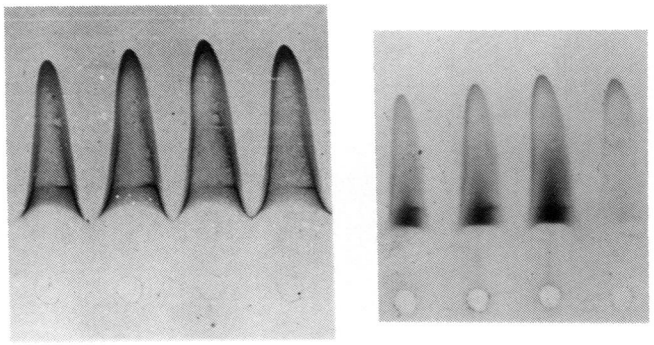

Fig. 5 Rocket immunoelectrophoresis: left — plate, right — autoradiograph. Upper gel contains a-AFP antiserum. Wells contain purified AFP and various ^{131}I-a-AFP preparations. Slightly radioactive rocket corresponds to ^{131}I-a-AFP of specific activity over 5 atoms of I per one IgG molecule. Activity of the remaining preparations: 0.1−5 atoms of I per one IgG molecule.

DISCUSSION

We demonstrated that ^{131}I–Ig of predetermined specific activity (up to 30 atoms of I per Ig molecule) can be obtained. Special nomograph for determination of the number of iodine atoms incorporated into one IgG molecule is presented. Judging on data given by Goldenberg and (3) concerning the specific activity of their antibodies we believe that they used ^{131}I–a–CEA of much lower specific activity than 1 atom of I per Ig molecule. It remains to be demonstrated whether ^{131}I–a –CEA of higher specific activity, preferably one atom of I per IgG molecule would be superior for the radioimmunolocalization of cancer. It cannot be excluded that antibodies of even higher specific activities might be still better even if their reactivity to the antigens would be decreased. It is important to notice that the improvement in radioimmunolocalization of cancer can be achieved not only by the increase of the specificity of antibodies, e. g. by use of monoclonal antibodies (5) but also by optimalization of the labeling procedure.

In addition we believe that rocket immunoelectrophoresis might be a suitable technique for quick evaluation of ^{131}I–Ig before it is injected to patients. Alternatively tube technique like immunoprecipitation which we used before in studies of ^{14}C–AFP, might be useful (1). These methods can eliminate injection of patients with ^{131}I–Ig which was either destroyed during dissociation from affinity column and/or during iodination, or which was not iodinated to the appropriate specific activity.

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