IMMUNOGLOBULINS AND IMMUNOGLOBULIN COMPLEXES ASSOCIATED WITH HUMAN MALIGNANT TUMORS

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ABSTRACT

Single cell suspensions of 14 malignant tumors were prepared from fresh tumors obtained in the operating room. The amounts of IgG were quantitated using a radioimmunoassay of tumor cell suspension. The level of elutable IgG varied from 8 to 300 ng/10^7 cells. Twenty-three malignant tumor tissues were eluted with 15% NaCl buffer and analyzed for Ag–Ab complexes. The cell-bound immunoglobulins were further assayed for complement binding using an indirect complement consumption assay. These studies show that IgG molecules are complexed with cell components. The complexed molecules can be eluted with IgM in a sephadex G–200 column. The high molecular weight IgG also consumes complement as opposed to normal tissue eluates.

INTRODUCTION

IT has been previously shown that various human and animal tumors are coated *in vivo* with immunoglobulins[1–5]. Immunoglobulins have been detected in acid eluates of animal tumors[6,7] and in human tumors[8,9]. It has also been shown that the eluates occasionally contained antibody activity against sarcomas[10] and melanoma[11,12]. Using polyoma virus induced SFYF tumor cells, Braslawski *et al.*[13] have shown that there exists a relationship between *in vivo* coating of tumor cells by potentially cytotoxic antitumor anti-bodies and the expression of immune complex receptors. In this paper, we summarize the quantitative studies in the area of human tumor associated immunoglobulins.

MATERIAL AND METHODS

A. *Tumor Cell Suspension*

Tumor tissue samples from malignant tumors were obtained during surgery. Tissue was thoroughly minced with iris scissors and suspended in Hank's balanced salt solution. To remove coarse particles the suspension was then filtered through sterile gauze. The cells in the filtrate were washed and used to determine the amount of IgG on tumor cells. Tissue, not used immediately, was embedded in phosphate buffered saline, pH 7·2 (PBS) and stored at − 70° C in plastic bags.

B. *Serum and Serum Fractionation*

Normal human serum was pooled from healthy donors. Immunoglobulin G (IgG) was isolated from serum by gel filtration as described by Flodin and Killander[14] and then treated twice with the chloride form of DEAE sephadex[16]. Antiserum to human IgG was produced by subcutaneously injecting rabbits with 200 μg of IgG in complete Freund's adjuvant at multiple sites every week for 10 weeks. The rabbits were bled after the last injection. The antiserum thus obtained established a single precipitin line of identity with antiserum specific to human IgG purchased from Hyland Laboratories (Costa Mesa, California, U.S.A.).

C. *Radioimmunoassay for Quantitation of IgG*

The assay is essentially the same as described in a previous communication[4]. Briefly, the optimum ratio of specific antiserum to labelled and unlabelled IgG was determined by testing a series of increasing dilutions of antiserum against a constant amount of labelled IgG. Diluent was normal rabbit serum in borate buffer, pH 8·4. To obtain a standard curve, 0 to 800 ng of unlabelled human IgG was preincubated with rabbit anti-human IgG of appropriate dilution as described above. After 24 hours of incubation at 4° C ^{125}I-labelled IgG was added, and the incubation was continued for another 24 hours. The binding of ^{125}I-labelled IgG in the presence of different amounts of unlabelled IgG was calculated and a standard curve constructed.

To determine the quantity of IgG on the tumor cell surface or in tumor cell eluate from a known number of cells, an amount of eluate was incubated in triplicate with the antiserum of the same dilution as used to obtain the standard inhibition curve. The amount of ^{125}I-labelled IgG that can be precipitated by this absorbed antiserum was found. By referring to the standard inhibition curve, the amount of IgG on the tumor cell surface or in the tumor eluate was determined.

D. Quantitative Complement Assay

Minced tissues of known weight were thoroughly washed and eluted with 15% NaCl in PBS, pH 7·3, for 1 hour at 37° C and centrifuged. A known amount of supernatant was dialyzed against 3 × 500 volume of PBS for 24 hours. A small amount of dialyzed eluate was used to determine its complement fixing capacity. Detailed technique is described elsewhere[16]. Briefly, known amounts of the eluate are preincubated with increasing concentrations of guinea pig complement. The complement fixing capacity of the eluate is

determined. This is extrapolated to find the complement fixing capacity per gram of tissue.

E. Immunodiffusion

Immunodiffusion was performed as described by Ouchterlony[17]. All experiments were carried out in 1% agarose in phosphate buffered saline, pH 7·2. Plates were read after 24 to 72 hours.

Results

Tumor Bound Immunoglobulin Characterisation

Cell suspensions from each of the tumors were tested for the presence of IgG. In some instances we measured IgG using tumor cell suspensions after four washes in medium 1640. The amounts of IgG per 10^5 cells varied from 68 ng to 800 ng. In 17 cases we were able to eluate IgG using low pH buffer. The results of these experiments, given in Table I, showed that elutable IgG varied from 8 to 300 ng of IgG/10^5 cells. In addition, IgG, IgA, IgM and albumin were also recovered in eluates in some cases. In 15 instances tumor and normal tissues were used for elution with 15% NaCl in phosphate buffer and used for complement consuming test (results shown in Table II).

Table I

Amounts of IgG found in tumor eluates

Tumor Identification	Tumor Type	Ng of IgG/ Million Cells
101	Adenocarcinoma	40
103	Breast Carcinoma	70
B16	Lung Carcinoma	300
D2	Melanoma	35
D14	Bladder Carcinoma	30
E11 (*a*)	Thyroid Ca primary	100
E11 (G)	Thyroid Ca metastatic	80
G15	Breast Carcinoma	8
SE2	Melanoma	45
H17	Breast Carcinoma	70
RLS1	Melanoma	60
ES2	Pancreatic Tumor	12
RP2	Melanoma	105
LF2	Ovarian Carcinoma	28

All the tumor eluates fixed the complement, their capacities ranging from 188 to 960 units per gram equivalent of tumor. On the other hand, normal uterus, normal stomach and normal mucosal eluates consumed 15 to 56 units of the complement. These complement consuming molecules were IgG molecules but were somewhat altered. They were elutable from G 200 column along with IgM and were partly excluded from the column (Figs. 1 *a* and 1 *b*). When these eluates were digested with pepsin, the complement fixing ability was lost, evidencing the destruction of complement binding sites on immunoglobulin molecules.

Table II

Complement consumption by tumor and normal tissue eluates

Tumor Identification	Type of Tissue	Unit of Complement Consumption/g Tissue
CTE 2	Melanoma	250
CTE 4	Lung Carcinoma	222
CTE 5	Melanoma	400
CTE 6	Bronchogenic Ca	600
CTE 7	Sarcoma	546
CTE 8	Br. Ca/Met. Liver	960
CTE 9	Melanoma	445
CTE 10	Melanoma	428
CTE 11	Ca of Uterus	188
CTE 12	Ca of Colon	284
CTE 13	Melanoma	212
NTE 1	Normal Uterus	31
NTE 2	Normal Stomach	56
NTE 3	Normal Stomach	15
NTE 4	Normal Mucosa	16

Discussion

Reports of Pilch and Riggins[18] and Morton *et al.*[3] show that the titer of circulating antibodies increases after primary tumor has been removed. Jewell and Hunter[19] in an animal model have shown that the turnover of circulating IgG is higher in tumor hosts as compared to normal animals. These findings suggest that either the antibody is absorbed *in vivo* by tumor cells or that the antigen released by tumor neutralizes the antibody by forming antigen-antibody complexes. After removal of the tumor, excess antibody would be present, as the antigen has been removed. From our work and from the reports of other investigators, there is evidence that tumor cells are coated with immunoglobulins *in vivo*. Whether these globular molecules are in fact antibodies, specifically directed against tumor cells or are antibodies attached to tumor cells by nonimmunological mechanisms is not clear. The alternatives are not mutually exclusive. The fact that certain populations of tumor cells have a higher affinity for IgG(Fc) of complexed antibodies[20] strengthens the view that IgG molecules may be attached to tumors nonimmunologically. We have

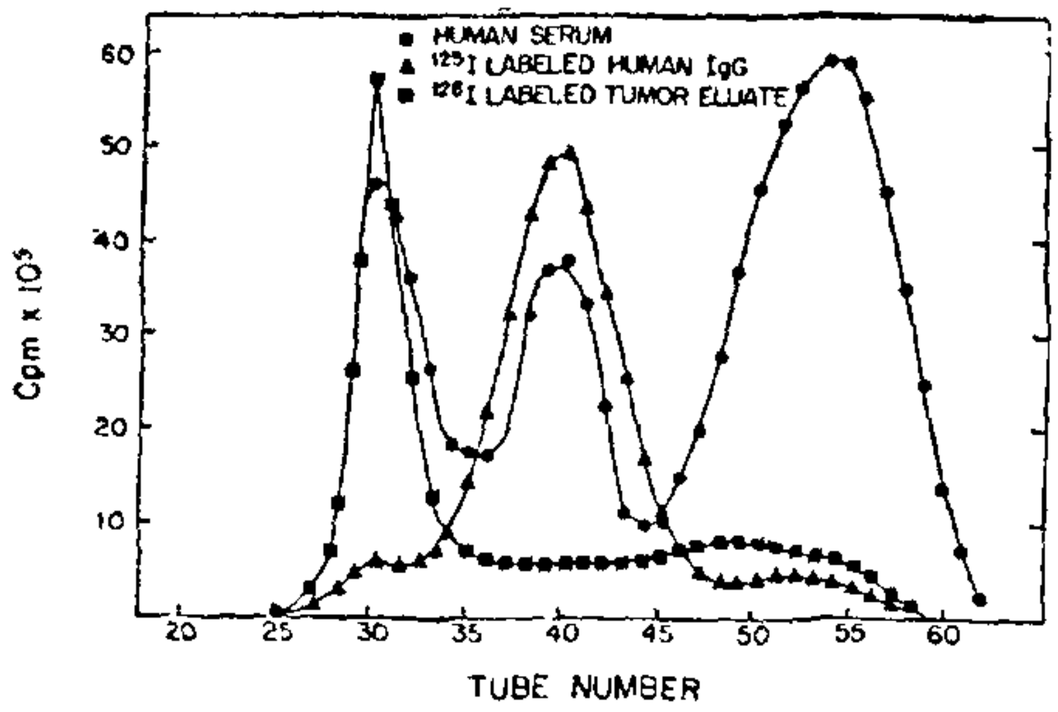

FIG. 1 (*a*)

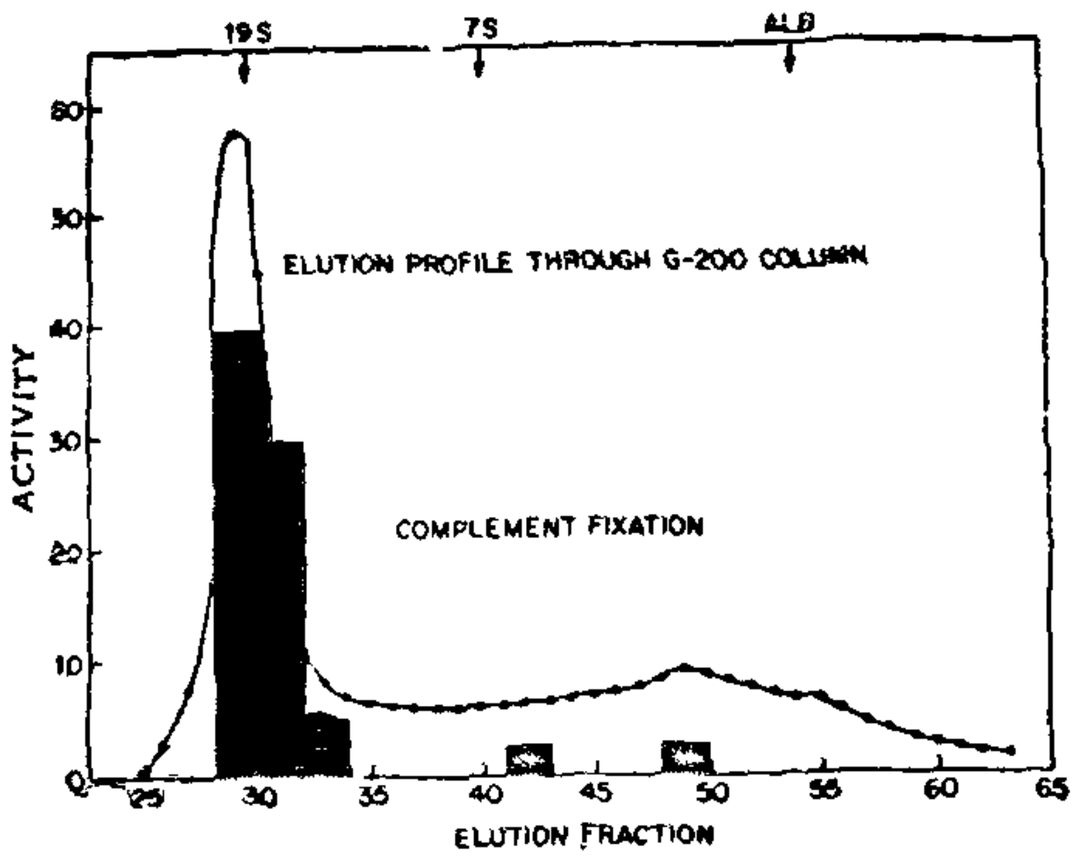

FIG. 1 (*b*)

FIGS. 1 (*a*) AND 1 (*b*). Profile through a sephadex G–200 column eluted with 0·1 M Tris-HCl in 0·2 M NaCl (pH 8·0). Fig. 1 (*a*). Tumor eluted immunoglobulins compared with normal serum and normal IgG-Fig. 1 (*b*). Profile of complement fixing immunoglobulin G in tumor eluate.

observed fragments of $IgG(Fab)_2$ and IgG(Fc) along with aggregated IgG in tumor eluates. In support of our finding, Fish *et al.*[21] reported that eluted fragments of degraded IgG had a higher affinity for tumor cells than native IgG molecules.

The quantitative estimation of IgG on each tumor cell is 10^4 to 10^5 molecules. Consequently, the tumor cells should have been lysed if the classical Ag–Ab reaction were to occur against tumor cells.

The presence of complexes and/or degraded immunoglobulins in the tumor mass complicates the issue, since these molecules can inhibit host reactivity at the target level as well as at the effector level. Recently, Rao and Mitchell[22] have shown that Ag–Ab complexes can suppress *in vivo* macrophage mediated immunity. Our results indicate: (*a*) that tumor associated IgG is complexed with cellular components, and (*b*) that tumor associated IgG fixes the complement[20].

In this regard it has been suggested that the effectors abrogating lymphocyte-mediated destruction of tumor cells, *in vitro* are complexes of tumor antigen and specific antibody[23,24]. It is possible that metabolic turnover of tumor cell membrane may release antigens from the tumor cell surface. The released antigen molecule may form antigen-antibody complexes in the vicinity of the tumor mass. These antigen-antibody complexes could subsequently bind nonspecifically to the tumor surface. Furthermore, they might also bind to other Fc receptor-positive lymphoid cells. These immunoglobulins may also undergo degradation by tumor associated lysosomal enzymes. It has been reported that xenogenic anti-tumor antibodies lost their ability to mediate complement-dependent cytotoxicity following a treatment with lysosomal enzymes extracted from corresponding tumor tissues[6]. Romsdahl and Cox[5] have demonstrated that in human sarcoma eluates, free $IgG(Fab)_2$ was found in excess of Fc fragment and suggested that $IgG(Fab)_2$ lacking the Fc end could make lymphoid cells incapable of mediating lymphotoxicity.

The overall emphasis of our study focuses on the very nature of the escape mechanism of antigenic tumor cells from immune response. Factors that circumvent immune surveillance include the masking of tumor antigens by immunoglobulins and the inactivation of effector cells.

Further investigation has to be carried out to ascertain whether these complexed immunoglobulins, in fact, interfere with the reactivity of host cells as such or if some other biological reaction modifies the characteristics of antibodies, which, in turn are manifested as modified immunoglobulins that fix complement.