Biochemistry (Moscow), Vol. 66, No. 1, 2001, pp. 27-33. Translated from Biokhimiya, Vol. 66, No. 1, 2001, pp. 37-45.

ACCELERATED PUBLICATION

Thermodynamic Stability and Functional Activity of Tumor-Associated Antibodies

O. P. Bliznukov[1], L. D. Kozmin[1], V. B. Klimovich[2], Y. I. Tsybovsky[3], Z. I. Kravchuk[3], and S. P. Martsev[3]*

[1]St. Petersburg Institute for Nuclear Physics, Russian Academy of Sciences, Orlova Roshcha, Gatchina, 188300 Russia; fax: (81271) 32-302; E-mail: leokon@yahoo.com
[2]Central Research Roentgeno-Radiological Institute, Poselok Pesochnoe, St. Petersburg, 188646 Russia
[3]Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, ul. Akademika Kuprevicha 5/2, Minsk, 220141 Belarus; fax: +375-17-2-63-72-74; E-mail: martsev@ns.iboch.ac.by

Received July 5, 2000

Abstract—Tumor-associated antibodies of human IgG1 subclass were eluted from cell-surface antigens of human carcinoma cells and studied by differential scanning calorimetry and binding to local conformational probes, protein A from *Staphylococcus aureus* and a monoclonal antibody targeted to the CH2 domain of the Fc fragment. At pH 2.0-7.0, we observed virtually identical enthalpies of thermal unfolding for IgG1 from normal human sera and tumor-associated IgG1. The exact values of calorimetric enthalpy (Δh) at pH 7.0 were 6.1 and 6.2-6.3 cal/g for IgG1 from normal serum and IgG1 from carcinoma cells, respectively. The affinity constants of protein A binding to the CH2–CH3 domain interface demonstrated differences between serum IgG1 and tumor associated IgG1 that did not exceed 3-8-fold. The binding affinity toward the anti-CH2 monoclonal antibody determined for serum IgG1 and IgG1 from carcinoma cells differed not more than 2.5-fold. The thermodynamic parameters of IgG1 from carcinoma cells strongly suggest that protein conformational stability was essentially unaltered and that the Fc fragment of the tumor-derived IgG1 preserved its structural integrity.

Key words: IgG1, carcinoma, antitumor antibodies, differential scanning calorimetry, conformational stability, protein A

The humoral immune response to cell membrane antigens and intracellular proteins of a tumor cell involves a limited number of effector mechanisms that generally result in partial degradation of the tumor and a decrease in tumor cell dissemination through the circulation. Antibody-mediated complement-dependent cellular cytotoxicity (ADCC) and complement-mediated cytolysis (CML) are the two major mechanisms involved in these processes. Previous studies have shown the presence of clinically significant levels of serum antibodies specific to cell surface antigens of colon [1, 2], lung [3, 4], and ovary [5] carcinomas. However, low efficiency of the cytotoxic mechanisms mediated by the specific antitumor antibodies was found both *in vitro* [6] and *in vivo* [7].

In our work, we analyzed structural changes in the Fc fragments of human IgG1 antibody that form complexes with tumor cell surface antigens as a possible reason for the impaired effector function of antitumor antibodies. The lack of a pronounced effector function of antitumor antibodies could result from significant (global or subglobal) changes in the structure of their Fc fragments or, alternatively, from local changes in the binding sites that recognize Fc receptors and the C1 component of the complement. To discriminate between these two possibilities, two approaches were used in the present work. The first approach involved direct measurement of the antibody structural energetics using differential scanning calorimetry, which provides a solid and quantitative thermodynamic estimate of the amount of structure that is lost in stabilizing tertiary interactions. The second approach employed monoclonal "secondary" antibody directed to the CH2 domain and protein A from *Staphylococcus aureus* as conformational probes for the local changes in the CH2 domain or in the interface between the CH2 and CH3 domains that is responsible for the binding to leukocyte Fc receptors [8]. Recent studies revealed that protein A, judging from the location of its binding site on the IgG molecule, is a functional analog of neonatal Fc receptors [9] and anti-isotypic autoantibodies (rheumatoid factors) [10, 11]. The calorimetric measurements of the stabilization enthalpy performed in the present study demonstrated that tumor-associated immunoglobulins retained the structural

* To whom correspondence should be addressed.

0006-2979/01/6601-0027$25.00 ©2001 MAIK "Nauka/Interperiodica"

integrity of the Fc fragment as a four-domain module of an IgG molecule. However, in some cases changes in the cooperativity of the tertiary structure of IgG were observed without changes in the overall transition enthalpy. When the maximal changes in the protein A binding constant were obtained, the calorimetrically revealed changes in the cooperative structure were also maximal, this confirming the reliability of the approaches employed in this study.

MATERIALS AND METHODS

Reagents. The following reagents were used in this work: Tris, glycine, protein molecular weigh standards (Serva, Germany); BrCN-Sepharose 4SL (Pharmacia Biotech, Sweden); DEAE-Toyopearl 650 (Toyosoda, Japan); ammonium sulfate (Fluka, Switzerland); biotinamidocaproate N-hydroxysuccinimide ester, bovine serum albumin, streptavidin conjugated to horseradish peroxidase, protein A, protein A conjugated to horseradish peroxidase, *o*-phenylenediamine (Amersham, USA); antibodies to human IgG conjugated to horseradish peroxidase (Central Roentgeno-Radiological Research Institute, Russia). Other reagents were domestic products of chemical purity grade.

Immunoglobulins. Preliminary fractionation of antibodies directed to human carcinoma cells was previously described by Bliznukov and coauthors [12]. Human immunoglobulin of IgG1 subclass was isolated using monoclonal antibodies directed against human IgG heavy chain of γ1 isotype [13] that were immobilized on BrCN-Sepharose 4SL according to the manufacturer's instructions. The isolated IgG1 was at least 97% pure judging from electrophoresis in the presence of SDS, only two bands (23 and 51 kD) being observed. The absence of molecules of other IgG subclasses was monitored by enzyme immunoassay with the use of a panel of monoclonal antibodies against heavy chain isotypes of human immunoglobulin [13].

Biotinylated IgG1 was prepared by mixing 0.36 mg of biotinamidocaproate N-hydroxysuccinimide ester diluted in 0.12 ml of dimethylsulfoxide with 1 ml of protein solution containing 1.0 mg IgG1 in 0.1 M Na-borate buffer, pH 8.5, at a modifying agent-to-protein molar ratio 120 : 1. After 4 h of incubation at room temperature, the excess of the reagent was removed by dialysis against 0.1 M Na-phosphate buffer, pH 7.4.

The CH2 domain-specific monoclonal antibody was obtained as described by Klimovich and co-workers [13].

Ligand-binding assay. All ligand-binding experiments were performed at room temperature in triplicate; mean values were used for graphic presentation of results. The binding constants were determined from double reciprocal plots. The variation of independent measurements of the binding constant was within 16%.

In competitive assay for the binding of protein A to IgG, polystyrene tubes (12 × 75 mm) were incubated overnight with a solution containing 3 μg of protein A in 0.25 ml of 50 mM Na-borate buffer, pH 8.5. After three washings with the same buffer, the remaining absorption sites of the tube surface were blocked by addition of 0.25 ml of PBS-BSA (1% bovine serum albumin in 0.1 M Na-phosphate buffer, pH 7.4) followed by incubation for 40 min. After washing with distilled water, tubes were filled with 0.25 ml of PBS-BSA containing 25 ng of biotinylated IgG1 together with increasing amounts of IgG1. After 1.5 h of incubation followed by two washings with distilled water, 250 ng of streptavidin conjugated to horseradish peroxidase in 0.25 ml of PBS-BSA were placed into each tube. After 1 h of incubation and washing with distilled water, 0.6 ml of 0.02 M of *o*-phenylenediamine and 0.02 M H_2O_2 in 0.1 M Na-citrate buffer, pH 5.0, was added to each tube and incubation was continued for 10 min with shaking. After addition of 0.2 ml of 10% H_2SO_4 to stop the reaction, optical absorption at 492 nm was measured.

Two-site immunoassay for the binding of protein A to IgG1 was performed as follows. Protein A (3 μg) was immobilized as described above for the competitive immunoassay. After addition of PBS-BSA to block the remaining absorption sites followed by washing, increasing concentrations of IgG1 in 0.25 ml of PBS-BSA were added and incubated for 1.5 h. Tubes were washed and filled with solution containing 250 ng of protein A conjugated to horseradish peroxidase. After 1.5 h of incubation, peroxidase activity was measured as described above.

To study the interaction of IgG1 with the antibody directed against the CH2 domain, polystyrene tubes were filled with solution containing 3 μg of CH2-directed antibody in 0.25 ml of 50 mM Na-borate buffer, pH 8.5, and left overnight. After addition of PBS-BSA followed by washing as described above, increasing amounts of IgG1 were added and incubated for 2 h. Tubes were washed twice with distilled water, and antibodies to human IgG conjugated to horseradish peroxidase were added. After 2 h of incubation followed by washing with distilled water, the solid phase bound peroxidase activity was measured as described above.

Differential scanning calorimetry. Calorimetric measurements were made with a DASM-4 differential scanning microcalorimeter (Biopribor, Pushchino, Russia) in the temperature range 10-100°C at heating rate 60°C/h. The reference cell was filled with the buffer used for the preceding dialysis of the sample. The following buffer solutions were used: 50 mM sodium phosphate, pH 7.0, 50 mM sodium citrate-phosphate or 50 mM sodium phosphate with HCl at lower pH. The instrumental base line that was obtained by filling both cells with the buffer was subtracted from the original calorimetric curves. Protein concentration varied within the range

1.0-1.5 mg/ml. At least two scans were recorded for every sample, the deviation of individual measurements being within 6%. The calorimetric enthalpy was calculated according to Privalov and Khechinachvili [14]. Calorimetric curves were further processed and deconvoluted using TERMCALC software (Biopribor).

Other methods. Protein purity was assessed by electrophoresis in polyacrylamide gel in the presence of SDS using 5% stacking gel and 12.5-15% running gel according to Laemmli [15].

IgG1 concentration was measured spectrophotometrically using extinction coefficient $A^{1\%}_{1cm,280} = 14.7$.

RESULTS

In this work, we considered the thermodynamic stability of tumor-associated antibodies of human IgG1 subclass eluted from the cell surface of human carcinomas. The ability of tumor-associated IgG1 to bind to protein A and the anti-CH2-domain antibody was analyzed in comparison with "normal" serum immunoglobulins that were treated in the same way as the carcinoma-derived antibodies.

The following eight samples of human IgG1 were analyzed: an individual control sample obtained from the serum of a healthy patient, pooled control IgG1 isolated from the sera obtained from 50 healthy patients 18-30 years of age, and six samples of tumor-associated antibodies eluted from cell surface antigens of lung (n = 2, patients B and E), ovary (n = 2, patients A and C), and colon (n = 2, patients D and F) carcinoma tissues. For all carcinoma samples, diagnoses were confirmed histologically. The sample of patient F was not analyzed because of the heat-induced proteolysis observed for this sample.

Thermodynamic stability. Calorimetric studies were performed in the pH range from 2.0 to 7.0 (Fig. 1). Lowering the pH decreased the specific transition enthalpy (Δh) from 6.1 cal/g at pH 7.0 to 2.1 cal/g at pH 3.0 (table). The control IgG1 antibodies demonstrated the same conformational stability in terms of enthalpy of thermal unfolding (Δh) over the whole pH range. At pH 4.6, de-cooperation of the IgG1 structure occurred as judged from the broadening of the melting curve and the appearance of well-resolved thermal transition(s) that often preceded the unfolding of the most stable structural blocks of the molecule (Fig. 1b). Lowering the pH to 3.0 resulted in a dramatic structural rearrangement of IgG1 as demonstrated by a 3-fold decrease in the transition enthalpy and the disappearance of two of five two-state transitions revealed by deconvolution of the transition curve at neutral pH (Fig. 1, a-c). At pH 2.0, the calorimetric profile did not demonstrate any transition peak, this being indicative of the loss of cooperative structure. The same results were obtained for IgG1 from patient A (ovary carcinoma) (Fig. 2). The specific transition enthalpy was 6.4 cal/g at pH 7.0, 5.1 cal/g at pH 4.6, and 1.8 cal/g at pH 3.0. Tumor-associated immunoglobulins demonstrated significant de-cooperation of the structure at pH 4.6 (Fig. 2b) and strong destabilization at pH 3.0 (Fig. 2c, table). Given the above calorimetric data, the pH values of 7.0 and 4.6 were chosen for further study in order to analyze IgG1 stability at neutral pH and under the moderately acidic pH that provided the maximal de-cooperation of the antibody structure with minimal contribution of inter-domain interactions. At pH 7.0, both the control and the tumor-associated immunoglobulins demonstrated nearly the same transition enthalpy of 6.1-6.4 cal/g (table). The corresponding values at pH 4.6 were 4.9-5.1 cal/g, the difference

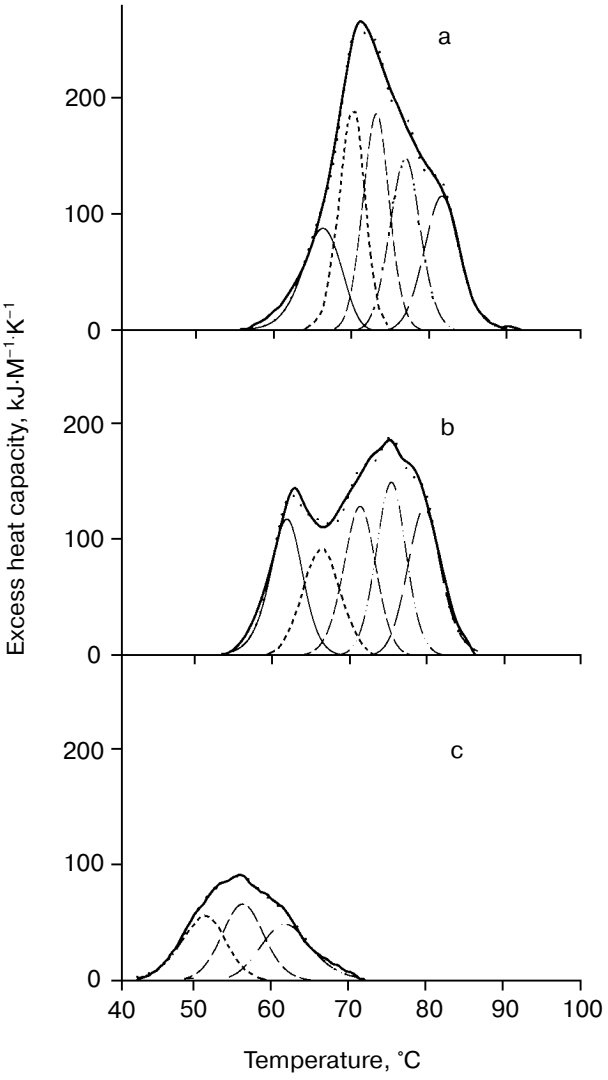

Fig. 1. Heat capacity curves for control IgG1 at pH 7.0 (a), 4.6 (b), and 3.0 (c) in 0.05 M sodium phosphate (pH 7) or sodium citrate-phosphate (pH 4.6 and 3.0) buffers. Dotted lines represent two-state transitions obtained by deconvolution of the experimental heat capacity curves.

Binding constants (K_a) and transition enthalpies (Δh) of tumor-associated IgG1

Patient	Binding constant for IgG1 binding to the anti-CH2-domain antibody ($K_a \times 10^8$, M^{-1})	Binding of protein A		Transition enthalpy, cal/g		
		competitive assay ($K_a \times 10^8$, M^{-1})	two-site assay ($K_a \times 10^7$, M^{-1})	pH 7	pH 4.6	pH 3
Control	1.5	0.95	1.75	6.1	5.3	2.1
A	1	2.5	6	6.3	5.1	1.8
B	—	0.35	0.6	—	—	—
C	1.2	0.8	1.7	6.2	5.1	—
D	0.7	0.25	0.6	—	5.1	—
E	0.6	0.12	0.2	—	4.9	—

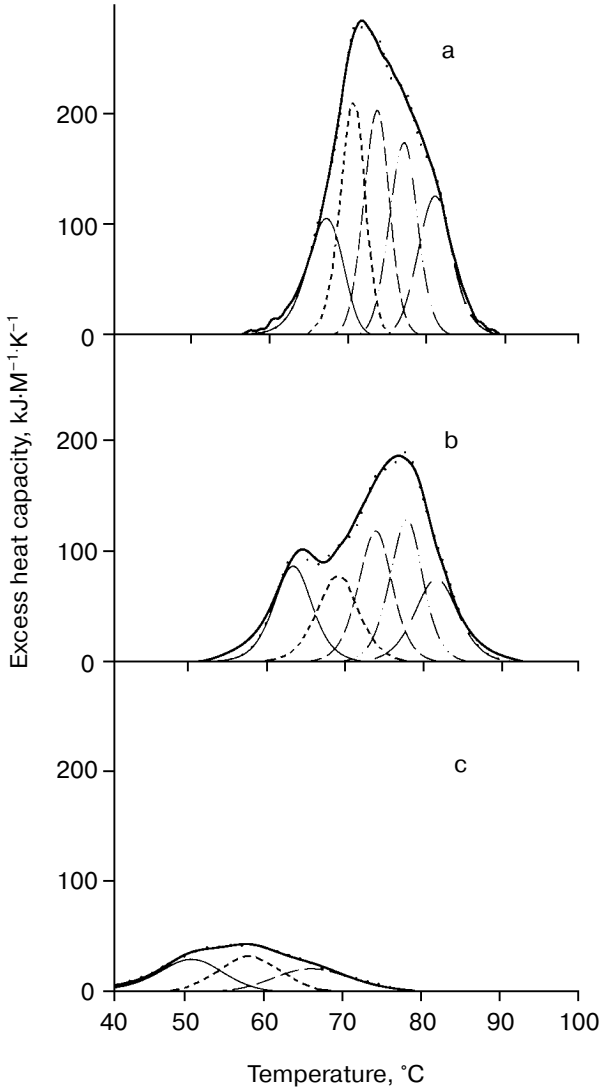

Fig. 2. Heat capacity curves for tumor-associated IgG1 at pH 7.0 (a), 4.6 (b), and 3.0 (c). IgG1 was isolated from carcinoma cells of patient A. See Fig. 1 for details.

between control and tumor-associated IgG1 remaining insignificant (table). Only one IgG1 sample demonstrated a significant change in the cooperativity of the structure (Fig. 3). The cooperativity of a structure is frequently characterized by the ratio of the maximal heat capacity of a heat absorption peak (ΔCp_{max}) to the temperature interval of denaturation. Analysis of the thermal transition curves obtained at pH 4.6 revealed the value of 19-21°C for the half width of the transition for all antibody

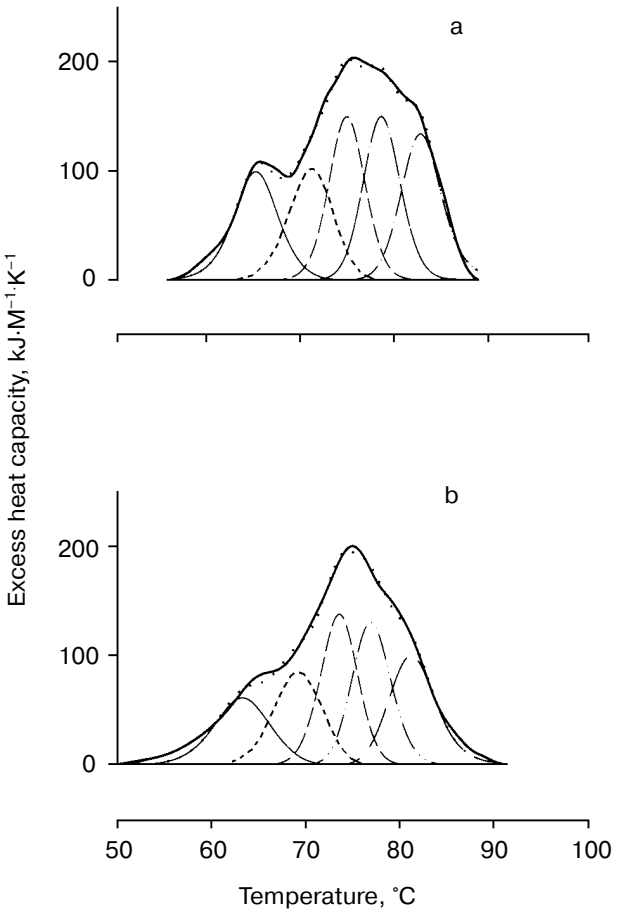

Fig. 3. Heat capacity curves of tumor-associated IgG1 of patients C (a) and E (b) in 0.05 M citrate-phosphate buffer, pH 4.6.

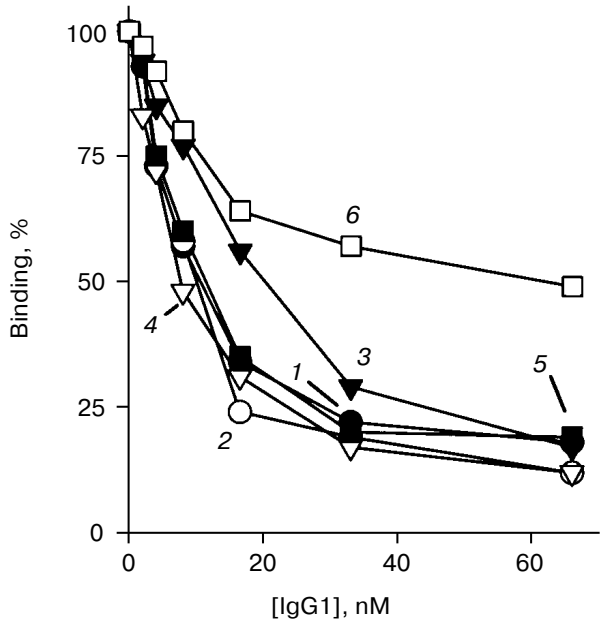

Fig. 4. Competitive immunoassay for binding of protein A to IgG1: *1*) control; *2*) patient A; *3*) patient B; *4*) patient C; *5*) patient D; *6*) patient E. Human IgG1 in increasing concentrations competed with biotinylated IgG1 for the binding to protein A which was immobilized on polystyrene tubes. Binding was monitored by measuring peroxidase activity.

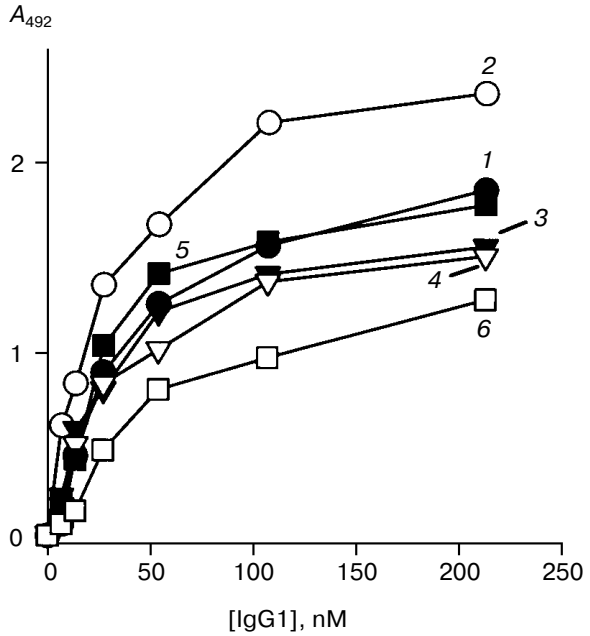

Fig. 5. Two-site immunoassay for the binding of protein A to IgG1. Human IgG1 in increasing concentrations were allowed to bind to protein A immobilized on polystyrene tubes. Binding was monitored by measuring peroxidase activity. See Fig. 4 for details.

samples (Figs. 1b, 2b, 3a). However, tumor-associated IgG1 eluted from the carcinoma cell surface antigens of patient E (squamous lung carcinoma) demonstrated at pH 4.6 a lower ΔCp_{max} value for the low temperature transition. The half width of the corresponding integral transition was small, 12.9°C (Fig. 3b). These data suggest a rearrangement of the domain interaction in the carcinoma-derived immunoglobulins without changes in overall stability of the IgG1 molecule.

Binding to protein A. To study the conformation of the CH2–CH3 domain interface in tumor-associated immunoglobulins, the ability to bind protein A was analyzed by means of competitive (Fig. 4) and two-site (Fig. 5) immunoassays. The competitive assay provided a probe for the local conformation of protein A binding sites, while the two-site assay probed the relative spatial location of the two identical sites through analyzing the ability of an antibody to bind two molecules of protein A.

In most cases, protein A binding assay for samples of control and tumor-associated immunoglobulins did not demonstrate a dramatic difference in binding capacity. The protein A binding constant for tumor-associated IgG1 was 2.8-4-fold lower than that for control IgG1 (two patients) or 2.8-fold higher (1 patient); in one patient the K_a value was the same as for control IgG1. Only IgG1 of patient E (squamous lung carcinoma) demonstrated a significant (8-fold) decrease in the protein A binding constant. It is worth noting that the maximal change in the protein A binding constant (Figs. 4 and 5) for patient E was consistent with the change in the calorimetrically revealed cooperativity of the structure (Fig. 3).

Competitive immunoassay revealed that the protein A binding constant obtained for the control immunoglobulins of ($0.95 \cdot 10^8$ M^{-1}) was 5-fold higher than the K_a value obtained in the two-site immunoassay, which strongly suggests limited accessibility of the second protein A binding site on the IgG1 molecule. A strong correlation was observed between the binding constants that were obtained in the competitive and two-site immunoassays (table). This provided evidence that the structural changes in the tumor-associated IgG1 molecules did not result from rearrangement of domains.

Together, these results suggest that no significant changes occurred in the conformation of the CH2–CH3 domain interface in the tumor-associated immunoglobulins.

Interaction with the anti-CH2-domain antibody. The monoclonal antibody directed against the CH2 domain of human IgG1 [13] was used as a probe for the conformation of the CH2 domain. We did not observe a significant difference between the control and the tumor-associated immunoglobulins (Fig. 6). The decrease in constants of the anti-CH2 antibody binding to the tumor-associated immunoglobulins did not exceed 2-fold versus control IgG1 (table). Only IgG1 from patient E demonstrated a 2.5-fold decrease in the binding constant.

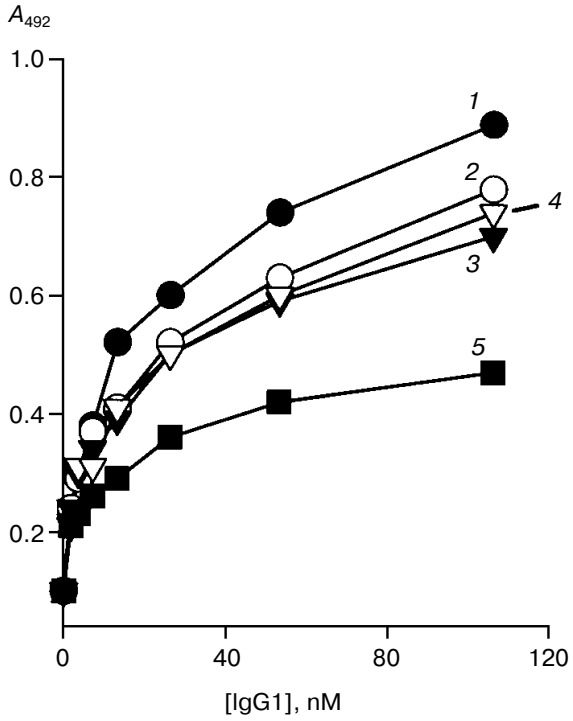

Fig. 6. Interaction between the anti-CH2-domain monoclonal antibody and human IgG1: *1*) control; *2*) patient A; *3*) patient C; *4*) patient D; *5*) patient E. Human IgG1 was allowed to bind to the anti-CH2 antibody that was immobilized on polystyrene tubes. Binding was monitored by measuring the activity of the horseradish peroxidase that was conjugated to the anti-CH2 antibody.

Thus, our data did not reveal significant conformational changes in the CH2 domain that would result dramatic changes in the epitope on tumor-associated IgG1.

DISCUSSION

Although IgG can bind to antigens exposed on a tumor cell membrane, the complement-dependent cellular cytotoxity and complement-mediated cytolysis, which are mediated by tumor-specific antibodies, remain generally low. Several hypotheses recently appeared in attempts to explain the above phenomenon in terms of inhibition by anti-idiotypic antibodies [16], shedding of antigen–antibody complexes from the tumor cell surface [17], poor immunogenicity, and low expression of tumor glycoprotein and glycolipid antigens [18, 19]. Another possible explanation would implicate a structural modification of an antibody Fc fragment involved in immune complexes that would occur in a pre-membrane milieu of a tumor cell after binding the antigen. Previous studies of Horejsi and coworkers [20] revealed changes in the number of disulfide bonds in pooled IgG that was isolated from the sera of lung carcinoma patients, which is consistent with the "Fc structural alteration" hypothesis.

To analyze the low efficiency of humoral immune response to tumor cells in terms of the above "Fc structural alteration" hypothesis, we considered the conformational stability and local structure of the Fc fragment of IgG1 eluted from the surface of human carcinoma cells. Conformational stability of the IgG1 was measured using differential scanning calorimetry, which provides a direct and solid estimate of protein structural stability in terms of enthalpy of thermal unfolding. The thermodynamic study was supplemented by probing local conformation using the monoclonal anti-CH2-domain antibody and protein A from *Staphylococcus aureus* as conformational probes that recognize their specific binding sites in the Fc fragment. Protein A binds to the interface between the CH2 and CH3 domains, the site that is also involved in the specific recognition of cellular Fc receptors and rheumatoid factors. In most cases, calorimetric studies did not reveal a significant difference between control and tumor-associated immunoglobulins in stability of the multi-domain structure or cooperativity of domain interactions (Figs. 1 and 2; table). The only exception is IgG1 isolated from patient E with squamous lung carcinoma (Fig. 3). This IgG1 displayed a significant decrease in the binding affinity to protein A and the anti-CH2-domain monoclonal antibody (table). The correlation between the calorimetric data and ligand binding assays confirms the utility of these methods as reliable probes for establishing the functionally significant conformational changes in IgG1. Other samples of tumor-associated antibodies demonstrated slight changes in the affinity towards protein A (Figs. 4 and 5) and the anti-CH2-domain antibody (Fig. 6) without any significant changes in thermodynamic stability (Figs. 1-3, table). Thus, we conclude that tumor-associated IgG1 antibodies display relatively small changes in ligand binding affinity together with lack of dramatic changes in thermodynamic stability, the latter demonstration being the structural integrity of the Fc fragment. Given the complicated relationships between changes in the ligand binding affinity and the effector activity of IgG [21, 22], development of adequate model systems for direct measurement of the effector activity for tumor-associated IgG1 is required to establish the physiological implications of the described combination of thermodynamic and ligand binding properties. To give an example of the complicated relationships mentioned above, as little as 3-fold difference in the constants for C1q binding to monomeric IgG1 versus IgG4 previously determined from independent biophysical and immunological studies [23-25] was accompanied by a dramatic distinction in the ability of these IgG subclasses to mediate complement-dependent cytolysis *in vitro* [26, 27]. Previous studies revealed that, although the existence of allotypes of heavy γ1 chain and its N-glycosylation-induced heterogeneity do not influence thermodynamic stability of the IgG1 Fc fragment [28, 29], the allotypes [30] and glycoforms [31, 32] of IgG1 demon-

strated significant differences in antibody-mediated cellular cytotoxity and complement-mediated cytolysis. Moreover, it is generally known that antigens mediate most effector functions of antibodies. It was recently shown that signal transmission from the antigen-binding site to the effector sites of immunoglobulins could be significantly altered without pronounced changes in thermodynamic stability [33]. In this context, one cannot rule out the possibility of altered domain interaction in tumor-associated IgG that would lead to changes of the signal transmission from the antigen binding site to effector sites, as was previously described for "normal" immunoglobulins [34].

From these results, we conclude, first, that the lack of a pronounced effector function of tumor-associated IgG *in vivo* cannot be attributed to unfolding of significant structural blocks in the Fc fragment. As a second conclusion, local structural changes in the interface between the CH2 and CH3 domains were found in tumor-associated IgG that resulted in 3-8-fold changes in the ligand binding affinity. The present work is a first attempt at thermodynamic description of the properties of tumor-associated IgG. Further detailed studies are required to reveal the structural mechanisms responsible for the lack of the effector functions of the immunoglobulins.

We thank P. I. Bliznukov for helpful discussions of the results.

REFERENCES

1. Konstandoulakis, M. M., Syrigos, K. N., Leandros, M., Charalabopoulos, A., Manouras, A., and Golematis, B. C. (1998) *Hybridoma*, **17**, 431-435.
2. Scanlan, M. J., Chen, Y. T., Williamson, B., Gure, A. O., Stockert, E., Gordan, J. D., Tureci, O., Sahin, U., and Old, L. (1998) *Int. J. Cancer*, **76**, 652-658.
3. Gorsky, Y., Vanky, F., and Sulitzeanu, D. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 2101-2105.
4. Ronai, Z., and Sulitzeanu, D. (1986) *J. Natl. Cancer Inst.*, **77**, 1203-1209.
5. Kotera, Y., Fontenot, J. D., Pecher, G., Metzgar, R. S., and Finn, O. J. (1994) *Cancer Res.*, **54**, 2856-2860.
6. Koda, K., Nakajima, N., Saito, N., Yasutomi, J., McKnight, M. E., and Glassy, M. C. (1998) *Br. J. Cancer*, **78**, 1313-1320.
7. Dunn, W. (1999) *J. Immunol.*, **162**, 3663-3671.
8. Deisenhofer, J. (1981) *Biochemistry*, **20**, 2361-2370.
9. Burmeister, W. P., Huber, A. H., and Bjorkman, P. J. (1994) *Nature*, **372**, 379-383.
10. Artanidi, S. E., Calame, E. L., Morrison, S. L., and Bonagura, V. R. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 94-106.
11. Corper, A. L., Sohi, M. K., Bonagura, V. R., Ansel, B. M., Roitt, I. M., and Dwek, B. J. (1997) *Nat. Struct. Biol.*, **4**, 374-376.
12. Bliznukov, O. P., Klimovich, V. B., Kozmin, L. D., Tarasov, V. A., and Filatov, M. V. (2000) *Immunologiya*, **1**, 18-22.
13. Samoilovich, M. P., Klimovich, V. B., and Pashkova, S. F. (1996) *Immunologiya*, **2**, 38-43.
14. Privalov, P. L., and Khechinashvili, N. N. (1974) *J. Mol. Biol.*, **86**, 665-684.
15. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
16. Gilliland, L. K., Walsh, L. A., Frewin, M. R., Wise, M. P., Tone, M., Hale, G., Kioussis, D., and Waldmann, H. (1999) *J. Immunol.*, **162**, 3663-3671.
17. Vangsted, A. J., Clausen, H., Kjeldsen, T. B., White, T., Sweeney, B., Hakomori, S., Drivsholm, L., and Zeuthen, J. (1991) *Cancer Res.*, **51**, 2879-2884.
18. Livingston, P., Zhang, S., and Lloyd, K. (1997) *Cancer Immunol. Immunother.*, **45**, 1-9.
19. Ragupathi, G. (1996) *Cancer Immunol. Immunother.*, **43**, 152-157.
20. Horejsi, R., Kollenz, G., Dachs, F., Tillian, H. M., Schauenstein, K., Schauenstein, E., and Steinschifter, W. (1997) *J. Biochem. Biophys. Meth.*, **34**, 227-236.
21. Burton, D. (1985) *Mol. Immunol.*, **22**, 161-206.
22. Hughes-Jones, N. C. (1977) *Immunology*, **32**, 191-198.
23. Isenman, D. E., Dorrington, K. J., and Painter, P. H. (1975) *J. Immunol.*, **114**, 1726-1729.
24. Sledge, C. R., and Bind, D. (1973) *J. Biol. Chem.*, **248**, 2818-2832.
25. Schumaker, V. H., Calcott, M. A., and Spiegelberg, H. L. (1976) *Biochemistry*, **16**, 5175-5181.
26. Brekke, O. H., Michaelsen, T. E., Aase, A., Sandin, R. H., and Sandlie, I. (1994) *Eur. J. Immunol.*, **24**, 2542-2547.
27. Tao, M. H., Smith, R., and Morrison, S. (1993) *J. Exp. Med.*, **178**, 661-667.
28. Ghirlando, R., Lund, J., Goodall, M., and Jefferis, R. (1999) *Immunol. Lett.*, **68**, 47-52.
29. Tishchenko, V., Lund, J., Goodall, M., and Jefferis, R. (1998) in *Biocalorimetry: Applications of Calorimetry in Biological Sciences* (Ladbury, J. E., and Chowdhry, B. Z., eds.) John Wiley & Sons, London, pp. 267-275.
30. Greenwood, J., Clark, M., and Waldman, H. (1993) *Eur. J. Immunol.*, **23**, 1098-1104.
31. Boyd, P. N., Lines, A. C., and Patel, A. K. (1995) *Mol. Immunol.*, **32**, 1311-1318.
32. Jefferis, R., Lund, J., and Pound, J. D. (1998) *Immunol. Rev.*, **163**, 59-76.
33. Chumanevich, A. A., Kravchuk, Z. I., Vlasov, A. P., Zhorov, O. V., and Martsev, S. P. (1998) *Biochemistry (Moscow)*, **63**, 476-484.
34. Kravchuk, Z. I., Chumanevich, A. A., Vlasov, A. P., and Martsev, S. P. (1998) *J. Immunol. Meth.*, **217**, 131-141.