Tumor Cell Mediated Degradation In Vitro of Antitumor Antibodies

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Introduction

An in vivo interaction between tumor cells and humoral immune components was strongly supported by the findings that malignant tumor cells of human and animal origin are coated in vivo with Ig molecules. In some tumor host systems it was possible to show that at least some of the tumor bound Ig molecules expressed antibody activity against the tumor cells [13].

The type and expression of such an immune interaction between tumor cells and immune components may be the outcome of the mutual effects which both parties might exert on each other. Cellular factors which may play an important role in such an interaction on behalf of the tumor cells, are lysosomal enzymes which exhibit pronounced activity within [1, 10], and in the close vicinity of the tumor cells [12]. Such enzymes originating from mouse and human tumor tissue, have the capacity to degrade in vitro the corresponding Ig [6, 14].

In vitro studies on the interaction between antitumor immune factors and tumor cells [5] mainly emphasized its outcome on the viability and cellular functions of those cells. It is, thus, the interest of the present study to demonstrate how tumor cells and tumor cell components may affect the structure and function of antibodies upon such an interaction.

A. Degradation of Cytotoxic Antibodies by Tumor-Derived Lysosomal Extracts (LE)

I. The Effect on Antibody Structure and Function

The IgG fraction from rabbit antisera with cytotoxic activity against a mouse plasmacytoma (RaPCT-IgG) was incubated at pH 3,8 for 4–6 hr at 37°C, with lysosomal extracts (LE) from solid plasmacytomas of BALB/c mice (PCT) [7].

The complement-dependent cytotoxic activity of LE treated RaPCT-IgG was markedly reduced after the treatment when compared to RaPCT-IgG incubated under the same conditions but in the absence of LE (pH treated control) and native RaPCT-IgG (Table 1).

A kinetic study showed that with incubation time, increasing amounts of LE treated RaPCT-IgG were required to obtain 50% cytotoxicity, indicating

Table 1. The effect of plasmacytoma (PCT) derived lysosomal extracts on the complement-dependent cytotoxicity of rabbit IgG anti-PCT antibodies (RaPCT-IgG)

Exp. No.[a]	Reciprocal of RaPCT-IgG dilution giving 50% cytotoxicity		
	Lysosome treated[b]	pH treated control[c]	Native
1	2–4	128–256	256
2	16	64	64–128
3	2	128–256	256–512
4	2–4	256	128–256
5	<1	64–128	64–128
6	4–8	128–256	128–256
7	2–4	128–256	128–256
8	4–8	16	64
9	2–4	128–256	128–256

[a] Individual experiments were done with a different antiserum pool. IgG protein concentration was constant within each experimental group.
[b] Globulin incubated with lysosomal extracts (LE) at pH 3,8 for 6 hrs at 37°C. The reaction mixture contained an 8-fold excess of IgG protein over LE protein.
[c] Globulin incubated at pH 3,8 for 6 hrs at 37°C without LE

a time dependent process which generated non-cytotoxic products during the incubation.

Physicochemical changes of LE treated RaPCT-IgG were observed by following the precipitability at different ammonium sulfate (AS) concentrations, and the gel filtration pattern of ^{125}I labelled IgG.

A differential precipitation at 40% and 70% saturation of AS revealed that essentially all of the protein bound radioactivity in the pH treated control preparations precipitated at 40% saturation of AS, whereas only 60–80% of the LE treated IgG could be precipitated. Ten to 30% of the radioactive material in the LE treated preparations precipitated when the AS concentration was increased to 70% saturation.

^{125}I labelled LE treated and ^{131}I labelled pH treated control preparations of RaPCT-IgG were filtered through Sephadex G-100 columns. The filtration pattern of LE treated RaPCT-IgG revealed a shift towards the lower molecular weight range.

The incubation of RaPCT-IgG with LE was, thus, observed to render the antibodies less cytotoxic to the tumor cells and generated molecules, which compared to the untreated RaPCT-IgG showed a decreased precipitability at AS and slower gel filtration characteristics.

Attempts were made to spearate and study the nature and activity of those degradation products.

II. The Properties of the Degradation Products

Separation of the degradation products from LE treated RaPCT-IgG was carried out by an initial step of differential AS precipitation at 40% and 70% saturated AS solutions followed by gel filtration on Sephadex G-100 of the resulted precipitates.

The fraction precipitated by 40% saturated AS solution (LE treated RaPCT-IgG-40) emerged as a single distinct peak following filtration. The IgG in this fraction retained its antigenic characteristics and cytotoxic potential.

The fraction which could be precipitated by a 70% (but not a 40%) saturated AS solution (LE treated RaPCT-IgG-70) was separated upon filtration into two major dispersed peaks, which were divided into six subfractions (I–VI). Subfractions I–IV, which emerged first, contained antigenic IgG but lacked any expression of antigenic Fc. All of the above subfractions were incapable of mediating complement-dependent lysis of PCT cells.

The LE treated RaPCT-IgG subfractions precipitated at 70% saturated AS solutions seem, therefore, to contain IgG fragments devoid of Fc expression in terms of both antigenicity and biological activity.

Some of these subfractions could, however, compete with native, cytotoxic RaPCT-IgG for cellular antigens. Preincubation of viable cells with 50 μg of the LE treated RaPCT-IgG-70 subfractions which emerged first from the column and designated I and II, protected the treated cells from lysis when subsequently subjected to native, cytotoxic RaPCT-IgG and complement (Table 2). These subfractions seem, therefore to maintain their antigen binding capacity.

Table 2. The specific blocking activity of rabbit IgG anti-plasmacytoma (RaPCT-IgG) treated with lysosomal extracts (LE)

IgG source[b]	% Blocking of cytotoxic RaPCT-IgG[a]			
	LE treated IgG subfraction added[c]			
	I	II	III	IV
RaPCT	62	87	0	6
RaDNP-BSA	0	0	0	0

[a] Blocking was achieved by preincubation of target cells with 50 μg of the tested subfractions prior to the addition of native RaPCT-IgG which lysed 80% of the cells in the presence of complement.

[b] LE treated IgG was obtained from rabbit antisera directed against plasmacytoma cells (RaPCT) and a conjugate of DNP-bovine serum albumin (RaDNP-BSA).

[c] LE treated IgG subfractions were obtained by precipitation by 70% saturation of ammonium sulfate followed by filtration through a Sephadex G-100 column

B. Degradation of Antibodies by Viable Tumor Cells in Culture

The results presented here indicate that tumor cells contain proteolytic enzymes capable of degrading antitumor antibodies as well as other IgG molecules in cell free systems. We tested next whether or not such a proteolytic potential is expressed by the viable cell under physiological conditions.

Viable or formalin fixed EL-4 lymphosarcoma cells from C57B1/6 mice were sensitized with ^{125}I-labelled IgG isolated from rabbit antisera against EL-4 cells (RaEL-4-IgG) and washed of excess unbound IgG. The sensitized cells were incubated at 37°C or 4°C in a Marbrook type culture apparatus [8], composed of a large external chamber which housed an internal one. The chambers which contained culture media were separated by a dialysis membrane and the sensitized cells were placed in the small inner chamber. At the end of the incubation we monitored the radioactivity level bound to the cells, in the cell culture medium (supernatant), and in the external chamber (dia-

lysate). Degradation of IgG into dialysable low molecular weight fragments should be detected by the appearance of radioactive materials in the external chamber.

A time-dependent study of the degradation of IgG by viable and formalin fixed cells showed a progressive generation of radioactive materials of low molecular weight. Up to 40% of the labelled proteins initially bound to viable cells were degraded at 37°C into dialysable fragments, concomitant with a gradual loss of radiolabelled antibody from the cells (Table 3).

Table 3. Degradation of IgG by viable cells in culture

	IgG present in culture compartments (% of initially cell bound radioactivity)[a]			
	Viable EL-4 cells		Formalin fixed EL-4 cells	
	2 hrs[b]	24 hrs	2 hrs	24 hrs
Cell bound[c]	79	30	78	75
Cell supernatant[c]	17	27	21	24
Dialysate[c]	4	43	1,0	1,0

[a] Viable and formalin fixed EL-4 cells were sensitized with ^{125}I-labelled rabbit IgG anti-EL-4 cells. The cells were washed of excess IgG and incubated in a Marbrook type culture at 37°C.

[b] 2 and 24 hrs after the onset of the experiment samples were taken and monitored.

[c] At the end of each incubation period the radioactivity bound to the cells (cell bound), present in the cell culture medium (cell supernatant) and in the external culture chamber (dialysate) was monitored and expressed as percent of initial cell bound radioactivity

The disappearance of functional antibodies from the surface of sensitized cells was confirmed by measuring the residual cytotoxicity of RaEL-4-IgG upon incubation with cells in culture.

EL-4 cells were sensitized with a cytotoxic preparation of RaEL-4-IgG and incubated at 37°C and 4°C for 24 hr. At the end of the incubation cell cytotoxicity was determined following the addition of guinea pig complement. The cytotoxicity level of the IgG preparation which initially yielded 90% killing of the cells dropped to 25% killing after incubation at 37°C and 70% at 4°C.

Attempts to locate the site for antibody degradation indicated that the process occurred at the cell level. A pair labelled mixture composed of ^{125}I-RaEL-4-IgG and ^{131}I-RaDNP-BSA-IgG was added to viable cells in a short term culture and incubated for 24 hrs at 37°C. The data presented in Table 4 indicated that only a fraction of the specific RaEL-4 antibody was degraded by the viable EL-4 cells as revealed by the appearance of low molecular weight fragments in the dialysate. The non-related anti-DNP-BSA incapable of binding to the cells remained intact in the cell supernatant.

Whether degradation occurred on the cell surface or within the cells was examined next. It was found that a certain proportion of the ^{125}I-labelled RaEL-4-IgG remained bound to the sensitized cells after 24 hrs of incubation (see Table 3). These bound antibodies could be eluted from the cells by a low

Table 4. Preferential degradation of rabbit anti-EL-4 antibodies by cells in culture

	IgG present in culture compartments[b] (% of radioactivity added)	
	^{125}I-RaEL-4-IgG[a]	^{131}I-RaDNP-BSA-IgG[a]
Cell bound	6,5 ± 0,3	1,5 ± 0,8
Cell supernatant	82,5 ± 1,2	97,0 ± 0,2
Dialysate	11,0 ± 1,3	1,5 ± 0,5

[a] Viable EL-4 cells were incubated in culture in the presence of an IgG mixture which contained IgG from rabbit antisera against EL-4 cells labelled with ^{125}I (^{125}I-RaEL-4-IgG) and against DNP-bovine serum albumin labelled with ^{131}I (^{131}I-RaDNP-BSA-IgG). Cultures were incubated for 24 hrs at 37°C.

[b] At the end of the incubation, radioactivity levels on the cells (cell bound) in the cell culture medium (cell supernatant) and in the external culture chamber (dialysate) were monitored and expressed as percent of radioactivity added

pH buffer treatment. A comparison of the amounts of elutable antibody with the amount of non-elutable antibody revealed a relative increase in the amount of non-elutable antibody as a function of incubation time. It was possible to reveal the existence of the intracellular radiolabelled material by its release upon cell lysis, which indicated that endocytosis of the antibody was responsible for the non-elutable antibody fraction. Most of the released intracellular material could not be precipitated by AS solutions of 50% saturation, which suggested an intracellular location of degraded IgG fragments. Such fragments are released thereafter and detected as dialysable materials in the external culture chamber.

Discussion

Proteolytic enzymes of tumor cell origin play a very important role in the invasiveness and metastatic spread of malignant cells [9]. It was important, therefore, to study the effect they may have on humoral immune components.

In the model system used in this study, it was shown that in a cell free system tumor derived lysosomal enzymes deprived antitumor antibodies of their capacity to mediate complement dependent cytotoxicity, probably by degrading their Fc fragment. These antibodies, however, could specifically block humoral cytotoxicity at the level of the target cell. In other studies it had been shown that alloantibodies rendered non-cytotoxic by lysosomal extracts could specifically protect target cells from lysis by alloimmune lymphocytes [3].

The study was extended to investigate the capacity of viable tumor cells in short term culture to express their proteolytic activity and degrade antitumor antibodies under physiological conditions.

The results show that the elimination of antibody from antibody-coated viable cells under metabolism permissive physiological conditions in culture,

is associated with antibody degradation. As a consequence of this process low molecular weight degradation products were generated, but no blocking factors capable of abrogating humoral cytotoxicity could be detected.

The tumor site serves as an interaction ground for immune effector mechanisms, which infiltrate the tumor site [4, 13], and tumor-derived proteases. If indeed the described process occurs in vivo, as suggested by the findings of partially degraded IgG molecules on tumor cells and in their close vicinity [2, 11], then the consequence of such an interaction between proteases and antitumor components will be a continuous consumption of the latter, resulting in their selective depletion.

References

1. Bosmann, H. B., Hall, T. C.: Enzyme activity in invasive tumors of human breast and colon. Proc. Nat. Acad. Sci. USA **71**, 1833–1837 (1974)
2. Cotropia, J. P., Gutterman, J. V., Hersh, E. M., Granatek, C. H., Mavligit, G. M.: Antigenic expression and cell surface properties of human leukemic blasts. Ann. N.Y. Acad. Sci. **276**, 146–164 (1976)
3. Dauphinee, M. J., Talal, N., Witz, I. P.: Generation of non-complement-fixing blocking factors by lysosomal extract treatment of cytotoxic anti-tumor antibodies. J. Immunol. **113**, 948–953 (1974)
4. Haskill, J. S., Yamamura, Y., Radov, L. A.: Host responses within solid tumors: Non-thymus derived specific cytotoxic cells within a murine mammary adenocarcinoma. Int. J. Cancer **16**, 798–809 (1975)
5. Hellstrom, K. E., Hellstrom, I.: Lymphocyte mediated cytotoxicity and blocking serum activity to tumor antigens. Adv. Immunol. **18**, 209–277 (1974)
6. Keisari, Y., Witz, I. P.: Degradation of immunoglobulins by lysosomal enzymes of tumors. I. Demonstration of the phenomenon using mouse tumors. Immunochemistry **10**, 565–570 (1973)
7. Keisari, Y., Witz, I. P.: The specific blocking of humoral immune cytolysis mediated by antitumor antibodies degraded by lysosomal enzymes of tumor origin. Eur. J. Immunol. **5**, 790 to 795 (1975)
8. Marbrook, J.: Primary immune response in cultures of spleen cells. Lancet **1967 II**, 1279 to 1281
9. Roblin, R., Chou, I. N., Black, P. H.: Proteolytic enzymes, cell-surface changes and viral transformation. Adv. Cancer Res. **22**, 203–252 (1975)
10. Shamberger, R. J., Hozumi, M., Morris, H. P.: Lysosomal and non-lysosomal enzyme activities of Morris hepatomas. Cancer Res. **31**, 1632–1639 (1971)
11. Sobczak, R., De Vaux St. Cyr, Ch. H.: Study of the in vivo fixation of antibodies on tumor provoked in hamsters by injection of SV-40 transformed cells (TSV_5Cl_2). Int. J. Cancer **8**, 47–52 (1971)
12. Sylven, B.: Lysosomal enzyme activity in the interstitial fluid of solid mouse tumor transplants. Eur. J. Cancer **4**, 463–474 (1968)
13. Witz, I. P.: Tumor bound immunoglobulins. In situ expressions of humoral immunity. Adv. Cancer Res. **25**, 95–148 (1977)
14. Witz, I. P., Levy, H. J., Keisari, Y., Izsak, F. Ch.: Tumor bound immunoglobulins: Their possible role in circumventing antitumor immunity. In: The role of immunological factors in viral and oncogenic processes. Beers, R. S., Jr., Tilghman, R. C., Bassett, E. G. (eds.). pp. 289 to 300. Baltimore, Maryland: Johns Hopkins Univ. Press 1974