and ADCC cells such as described for human cells[20]. On the other hand, it must be noted that immune interferon, which is also present in Con A-conditioned medium[21], stimulates ADCC activity of polymorphs[22]. In conclusion, it appeared that the complexity of K cell subpopulations was readily apparent with the mitogens used as probes. Each mitogen had its particular effect. In particular, ADCC activity and FcR expression did not seem to correlate, as is readily apparent from table 3, which summarizes results obtained with cultured cells. FcR expression is probably a necessary but not a sufficient condition for cells to perform ADCC activity.

Table 3. Summary of results with mitogen or CM activated cells

	Con A	PHA	LPS	BCG	CM
FcR	↓	±	↑	n.d.	↓
ADCC	↓	↓	↓	↓	↑

1 Acknowledgments. We would like to express our thanks to the excellent technical assistance of Mrs J. Higelin.
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Studies on chemically induced tumors in rats: I. Heterogeneity of tumor cells and establishment of syngeneic, tumor-specific cytotoxic T cell clones*

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Summary. Sarcoma P1 was induced in DA rats by DMBA. Anti-P1 antibodies were produced in DA rats, purified via fixed tumor cells and used to induce anti-idiotypic antibodies in syngeneic rats. The anti-idiotypic antibodies were used to generate cytotoxic, P1 specific DA T cells in vitro. These cytotoxic T cells and P1 tumor

cells were cloned by limiting dilution. Using the DA anti-P1 specific cytotoxic T cell clones, we were able to characterize 2 types of P1 tumor cell clones, namely those which were susceptible and those which were resistant to the P1 specific cytotoxic T cells. Cytotoxic T cell injected i.v. into syngeneic DA rats could not prevent the development of lethal P1 tumors.

Specific and nonspecific mechanisms such as cytotoxic T cells, macrophages and naturally occurring cytotoxic cells (NK) can eliminate tumor cells and may play a role as defense mechanisms during tumorgenesis[1,8,17-20,24-26]. Immunsurveillance against the development of tumors has been considered a possible way through which the frequency of tumors may be reduced[7]. In particular, this may be the case in tumor systems which express tumor-associated antigens that are frequently seen on chemically or oncogenic viruses induced tumors. This view is supported by the observation that immunosuppression and specially T cell deficiencies can result in an increase in the frequencies of certain tumor types[23].

In a variety of conventional systems effective and specific regulative mechanisms have been induced by anti-idiotypic immunity[2,4,6,11,15,16,21,22,29]. During the last few years attempts have also been made to demonstrate regulation of tumor growth by anti-idiotypic immune responses. Mice auto-immunized with tumor-specific T lymphoblasts developed autoimmune anti-idiotypic responses permitting a progressive growth of tumor cells expressing the corresponding antigens[13]. Anti-idiotypic killer or suppressor cells have been demonstrated in such auto-immunized animals[14] thus confirming earlier results in conventional transplantation systems[3]. These data indicate that specific T cell dependent mechanisms can influence, at least in certain systems, the in vivo growth of tumor cells.

We have previously shown[5] that DA rats, immunized with tumor-specific antibodies directed towards antigenic determinants on chemically induced syngeneic tumors, can develop auto-anti-idiotypic antibodies. Rats producing such antibodies permit an enhanced growth of the corresponding tumor cells, indicating that the auto-anti-idiotypic immune response had led to loss of the in vivo transplantation resistance. The anti-idiotypic antibodies could also be used to specifically restimulate tumor-primed syngeneic T cells in vitro, thus allowing the generation of tumorspecific cytotoxic T cells.

Material and methods

1. Rats. DA rats ($RT1^a$), BN rats ($RT1^n$) and L.BN rats ($RT1^n$) were bred and maintained in our own colony. Animals were 6–12 weeks of age when used for the experiments and they were sex-matched.

2. Tumors. P1 and P2 sarcomas were induced in DA rats by single s.c. injections of 1 mg of 7.12 dimethylbenz(a)anthracen (Fluka AG, Buchs, Switzerland) dissolved in olive oil. The tumors were maintained in vivo by injecting single cell suspensions s.c. into DA rats or, alternatively, were grown in vitro in RPMI 1640 complemented with 10% FCS. Both cell lines were kept in parallel in vitro and in vivo. The PM tumor represents a spontaneous sarcoma from L.BN rats kept in vitro and in vivo in syngeneic rats as described for P1 and P2.

Characteristics of tumor cell lines: P1 and P2 grow partly, PM sarcoma exclusively, adherent. The in vivo cell lines kill their syngeneic hosts within 1 month after the defined minimal dose of tumor cells is injected s.c. The minimal doses necessary are as follows: P1: 10^6 cells, P2: 10^5 cells, and PM: less than 50 cells. None of the tumor cell lines grow in allogeneic rats.

The human cell line K562 and mouse lymphoma YAC-1 ($H\text{-}2^a$) were kept in vitro using Iscove's medium complemented with 10% FCS. They represent standard target cell lines for NK-cells. After 20 passages in vitro YAC-1 cells were injected i.p. into A/J mice and K562 tumor cells into nude mice. In vitro cultures from harvested cells were established and kept in vitro for a further 20 passages.

3. Preparation of lymphoid cell suspensions. Rats were killed over carbon dry ice. This method was chosen due to the much better lymphocyte responses in vitro when compared with the responses of lymphocytes of rats killed by ether. Spleens and lymph nodes were aseptically removed. Single cell suspensions were prepared in D-PBS (Ca^{++} and Mg^{++} free) using a stainless steel mesh. Large particles were removed by sedimentation and single cells were washed once in D-PBS. Red cells were lysed by hypotonic shock using 0.9 ml of distilled water for 2 sec followed by 0.1 ml of 10 times concentrated D-PBS. Cells were washed once more in D-PBS and resuspended in EHAA-medium (see below). The cell number was determined with a Biophysics 6300 A Cytograph (Biophysics Systems Inc., Mahopac, N.Y.).

4. Preparation of T lymphocytes. T lymphocytes were purified on 1.5×10 cm Ig anti-Ig columns[30] using sterile glass beads, 80–120 mesh (Serva, Heidelberg, FRG). Spleen cells at concentrations of less than 5×10^7 per ml were passed over the double-coated glass beads at a flow rate of 30 drops per min. Cells passing the column were collected and washed once in D-PBS. The passed preparations contained less than 1% of Ig-positive B cells when tested with a FITC-labeled rabbit anti-rat Ig.

5. Complement dependent cytotoxicity. The assay was performed in flexible flat bottomed polyvinyl chloride microtiter plates (M29, Dynatech Laboratories, Alexandria, Virg). 100 µl of Con A (0.5 mg/ml) in D-PBS were added per well and incubated for at least 2 h at room temperature. 2×10^5 P1 or P2 tumor cells in 50 µl D-MEM were incubated in the wells for 1 h at 37 °C. Plates were washed once with D-MEM. PM tumor cells were grown in flat bottomed microtiter plates (M29 ART, Greiner, Nuertingen, Germany) until a monolayer was observed. Attached cells were labeled with 0.2 µCi of ^{51}Cr (NEZ-030S, New England Nuclear, Boston, Mass.) per well in 50 µl of D-MEM for 30 min at 37 °C. Plates were washed 4 times very carefully using D-MEM. Cells were incubated with 50 µl of antiserum or control serum as indicated in the figures for 30 min at room temperature. 50 µl of guinea-pig complement was added at a final concentration of 1:6 and cells were incubated for an additional 30 min at 37 °C. Plates were centrifuged at $400 \times g$ for 5 min at 50 µl of the supernatant were counted in the gamma counter equipped with a 3′ well-type crystal (model CG-4000, Intertechnique SA, Plaisir, France). Maximal release was determined by adding 50 µl of 0.1% SDS instead of antiserum. Spontaneous release was determined by using 50 µl D-MEM with 10% FCS and complement. Percent cytotoxicity is expressed as

$$100 \times \frac{\text{experimental} - \text{sponateous } ^{51}\text{Cr release}}{\text{maximal} - \text{sponateous } ^{51}\text{Cr release}}$$

6. Stimulation of DA T lymphocytes with anti-idiotypic antibodies. T lymphocytes from primed DA rats (see below) were prepared by Ig anti-Ig columns (see above). T cell were stimulated with anti-idiotypic antibodies in 3013 Falcon tissue culture flasks (Falcon, Div. of Becton, Dickinson & Co., Oxnard, Calif.) using EHAA medium[9] complemented with 5×10^{-5} M 2-ME and 0.5% fresh normal BN serum as previously described. Anti-idiotypic antiserum was used at concentrations of 0.2%. Each flask contained 15 ml of a suspension with 1.25×10^6 cell per ml.

7. Preparation of T cell growth factor (TCGF; concanavalin A supernate). Rat lymphocytes were cultured in EHAA medium complemented with 0.5% fresh normal BN rat serum and stimulated with 5 µg/ml of Con A (Pharmacia Fine Chemicals, Uppsala, Sweden). 40 ml of a suspension containing 5×10^6 lymphocytes per ml were cultured in a 3024 Falcon tissue culture flask and the supernate harvested 48 h later, sterile filtered, and kept at −20 °C. Con A was neutralized by adding α-methylmannoside.

8. Expansion of anti-idiotype stimulated T cells with TCGF. Restimulated DA T lymphocytes (see above) were collected on day 6 of culture and purified on Ficoll-Paque[12]. Cells were expanded on DA macrophages and TCGF in the following way. Macrophages were harvested in D-PBS from the peritoneal cavity of DA rats using a 10-ml plastic syringe. Cells were washed once and irradiated with 2500 rad. Macrophages obtained from 1 rat were distributed among 3 3013-tissue-culture flasks and cultured in 5 ml EHAA medium complemented with 10% FCS and 20% TCGF for 24 h. 10 ml of a suspension containing 1.25×10^6 purified restimulated T cells (see above) in EHAA medium complemented with 10% fetal calf serum, 20% TCGF and 0.05% anti-idiotypic antiserum (see below) was added to each flask. Cells were harvested 1 week later and cultured on new macrophages under the same conditions, with the harvested cells from 1 flask divided among 3 new 3013 flasks. The procedure was repeated twice. Cells were finally harvested 3 days after the last transfer, purified on Ficoll-Paque and used for the experiments.

9. Stimulation of rat lymphocytes with Con A. Rat lymphocytes were prepared as described above. 15 ml of a suspension containing 3×10^6 cell per ml EHAA medium complemented with 0.5% fresh normal BN serum were added per 3013 Falcon tissue culture flask. Con A was used 3 µg/ml. Cells were harvested on day 3 of culture and purified on Ficoll-Paque.

10. Cell mediated cytotoxicity assay (CML). T lymphocytes from P1 primed DA rats were restimulated in vitro with anti-idiotypic antibodies as described above. Blasts and viable cells were purified on Ficoll-Paque[12]. CML was performed in round bottomed microtiter plates (Cook M24 ART, Greiner, Nürtingen) in 200 µl of EHAA medium complemented with 5×10^{-5} 2-ME and 10% heat inactivated FCS. Assays were performed in triplicate using 10^4 ^{51}Cr labeled target cells with effector to target cell ratios as indicated in the figures. The latter were prepared and labeled as described for the complement-dependent cytotoxic assay. Plates were incubated for 6 or 8 h at 37 °C in 5% CO_2 in air. Plates were centrifuged for 5 min at $400 \times g$. 100 µl of the supernatant were counted in the gamma counter. The percentage of cytotoxicity is expressed as described above.

11. Immunization of DA rats with P1 tumor cells. DA rats were immunized with 2×10^7 12,500 rad-irradiated tumor cells obtained from in vitro cultures. Animals were injected at 2–3 week intervals s.c. under the skin of the belly. Each rat received a minimum of 8 injections. Rats were bled for the first time after the 6th injection and 10 days after each further injection. Sera from individual rats were pooled and absorved on FCS coupled to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) in order to remove anti-FCS antibodies. Sera were heat-inactivated (30 min at

56 °C), stored at −20 °C and tested for their anti-P1 activity using the complement-dependent cytotoxic assay or the protein A assay (previously described) for the present tumor system[5]. Attempts to induce similar antibodies against P2 in DA or PM in L · BN rats always failed (data not shown).

12. Purification of DA anti-P1 antibodies. P1 tumor cells from in vitro cultures were fixed with 4% paraformaldehyde in D-PBS for 1 h at 4 °C and washed 3 times thereafter. 1×10^9 tumor cells were incubated for 15 min with 5 ml of isotonic glycin-HCl buffer pH 2.8. Cells were washed again with D-PBS and incubated with 5 ml of positive DA anti-P1 antiserum for 30 min at 4 °C. Cells were washed 3 times with D-PBS and incubated for 15 min with 5 ml of isotonic glycin-HCl buffer pH 2.8. Cells were removed by centrifugation for 10 min at 2000 × g. The supernate was neutralized with 0.1 N NaOH and dialyzed against D-PBS. Each serum sample was absorbed 3 times and the eluted material pooled, concentrated in a dialysis bag using cane sugar and dialysed again against D-PBS. Concentrated DA anti-P1 antibodies were filtered through a G-200 Sephadex column in 1 N acetic acid. Proteins corresponding to molecular weights of 150,000 were neutralized, concentrated and dialysed against D-PBS as described above, sterile filtered and kept at 4 °C. Activity of the purified antibodies was tested in the protein A assay or in the complement-dependent cytotoxic assay.

13. Induction of anti-idiotypic antibodies in DA rats. 3 ml of purified DA anti-P1 antibodies corresponding to about 4 mg of Ig were mixed with 7 ml of normal DA serum and crosslinked with glutaraldehyde as described[10]. Cross-linked proteins were homogenized and washed 3 times with D-PBS by centrifugation for 10 min at 4000 × g. The material was emulsified in 10 ml D-PBS and mixed with 10 ml complete Freund's adjuvant. The emulsion was distributed among 10 DA rats s.c. and i.p. Animals were boosted 3 weeks later following the same procedure with the exception that incomplete Freund's adjuvant was used. Animals were bled 3 weeks after the 1st injection and 1 month after the 2nd. Sera from individual rats were heat-inactivated and stored at −20 °C. The content of anti-idiotypic antibodies was determined by a solid phase radioimmuno assay or by the restimulation assay using P1 primed DA T lymphocytes as described before[5].

Results

1. Tumorgrowth in vivo is not prevented by inoculation of polyclonal tumorspecific cytotoxic T lymphocytes. Anti-idiotypic antibodies have been shown in several systems to be potent mitogens for the relevant idiotype positive T lymphocytes in vitro[15,16,,20]. We have shown previously that anti-idiotypic antibodies can even induce cytotoxic T-lymphocytes specific for rat P1 tumor cells[5]. We now studied whether such T lymphocytes would prevent growth of otherwise lethal doses of tumor cells in syngeneic rats. Tumorspecific cytotoxic T cells were produced by stimulating P1 primed DA T lymphocytes in vitro using anti-idiotypic antibodies (see 'Material and methods'). On day 6 of stimulation T cells were expanded on syngeneic macrophages using anti-idiotypic antibodies and TCGF (IL 2) (see 'Material and methods'). Such polyclonal activated T lymphocytes were tested for their cytotoxic effect on P1, P2 and PM tumor cells. Figure 1 shows the results of such an experiment. Cytotoxic effects could be demonstraded only for P1 tumor cells. The fact that syngeneic P2 tumor cells were not killed by the cytotoxic cells eliminates the possibility that the observed results were due to xenogeneic serum effects[27].

DA rats were injected i.v. with 2×10^8 tumorspecific, syngeneic and expanded T-lymphocytes (see 'Material and methods') or with 2×10^8 Con-A stimulated T lymphoblasts. One day later rats were injected with 2×10^6 P1 tumor cells corresponding to 2 times the required tumor cell dose to induce lethal tumors. As seen in table 1, all rats developed lethal tumors within 30 days. Similar results were obtained in a 2nd experiment.

Table 1. Tumor specific cytotoxic T cells (CTL) and Con A blasts were prepared as described under 'Material and methods'. Cells were injected i.v. and 2×10^6 P1 tumor cells were injected s.c. 1 day later

Experiment No.	Rats	Injected with	Development of tumors within 30 days
1	3	2×10^8 CTL*	All 3
	3	2×10^8 Con A*	All 3
2	3	2×10^8 CTL*	All 3
	3	2×10^8 Con A*	All 3

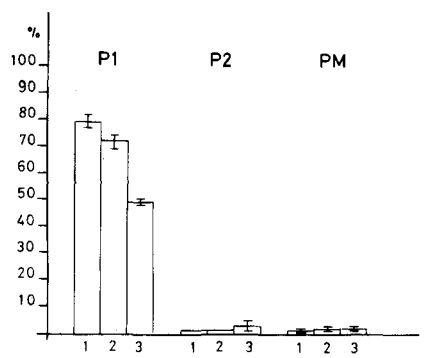

Figure 1. P1 primed DA T lymphocytes were stimulated for 6 days with anti-idiotypic antibodies and expanded on syngeneic macrophages as described under 'Material and methods'. Viable cells were purified on Ficoll-Paque and incubated for 8 h with ^{51}Cr labeled P1, P2 and PM target cells. Effector to target cell ratios: 1 = 100:1, 2 = 75:1, 3 = 50:1.

2. Cloning of DA anti-P1 cytotoxic T cells and establishment of P1 tumor cell clones. As we were not able to prevent the development of lethal tumors in DA rats using polyclonal P1 specific cytotoxic T cells, we tried to establish the extent to which P1 specific cytotoxic T cell clones are more efficient in the killing of the P1 tumor cells. In addition, we cloned P1 tumor cells in order to analyze the antigenic variability and to obtain a more homogenous population of tumor cells. P1 primed DA T lymphocytes were restimulated with anti-idiotypic antibodies and expanded with TCGF as described under 'Material and methods'. T cell clones were established in microtiter plates on syngeneic macrophages using limiting dilution as described. P1 tumor cell clones were established from in vitro cultures using limiting dilution. Tumor cell clones and cytotoxic T cell clones were expanded from microtiter plates first via 24-hole Costar plates and then produced in 3013 Falcon tissue culture flasks.

In a 1st series of experiments different cytotoxic T cell clones were tested for cytotoxic activity against several P1 tumor cell clones (data not shown). Two sets of information were obtained. First, several different T cell clones tested showed no cytotoxic activity against any of the P1 tumor cell clones examined. Second, about half of the P1 clones were not affected by any of the established cytotoxic T cell clones. We selected those T cell clones which showed cytotoxic capacity towards P1 tumor cells and P1 tumor cell lines and clones. Such T cell clones were then used as effector cells against different P1 tumor cell clones. Figure 2 shows the results of such an experiment. 6 anti-P1 cytotoxic T cell clones (1-6) lysed very efficiently P1 tumor cell clones 4, 9 and 11. P1 clones 2, 6, 8, 10 and 15 were not affected at all by the killer T cell clones. Specificity was indicated by the fact that the cytotoxic T cells failed to affect P2 or YAC-1 target cells. The latter cells, however, were lysed by DA spleen cells used for the NK control test (A). It was thus possible to establish highly efficient P1 specific T cell clones. The cytotoxicity of these T cell clones was subsequently tested at about 2-week intervals. Figure 3 shows such a killer assay. This experiment confirmed in general the results obtained earlier (fig. 2), namely that cytotoxic T cell clones 1-6 lysed P1 tumor cell clones 4, 9 and 11 and not P1 clones 2, 6, 8, 10 and 15. Pooled P1 tumor cells again were affected by the cytotoxic T cell clones. A new

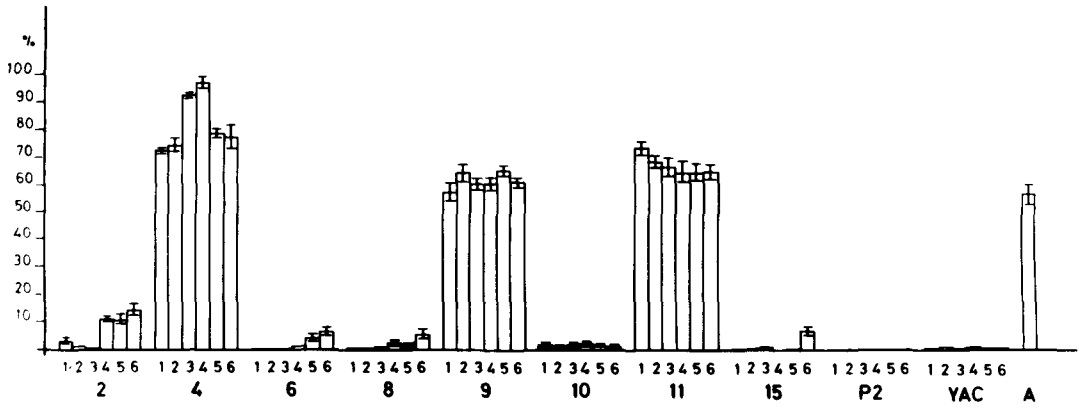

Figure 2. P1 tumor cells specific cytotoxic T cell clones (numbered as 1-6) were generated from restimulated T cells as described under 'Material and methods'. P1 tumor cell clones 2, 4, 6, 8, 9, 10, 11 and 15 were established from pooled P1 tumor cells. Effector T cell clones were incubated with the different P1 tumor cell clones at a ratio of 5:1 for 8 h. P2 and YAC-1 were used as control target cells at the same ratios. A = NK-Test with DA spleen cells as effectors used at an effector to target cell ratio of 100:1.

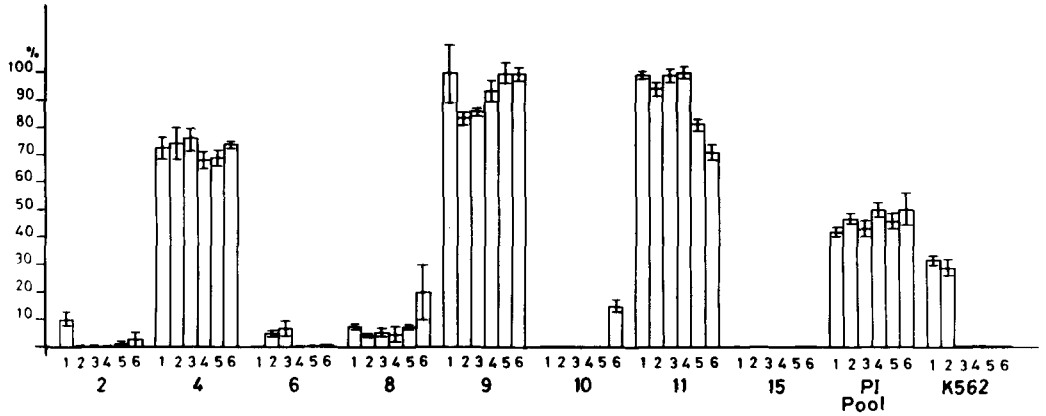

Figure 3. P1 specific cytotoxic DA T cell clones (1-6) were incubated with P1 tumor cell clones 2, 4, 6, 8, 9, 10, 11 and 15 as described under legend to figure 2. P1 pool = uncloned P1 tumor cells used as targets. K562 = control targets for the cytotoxic T cell clones.

observation revealed that T cell clones 1 and 2 did lyse K562 target cells, a standard target for NK activity. This effect was not observed in experiments carried out earlier using the 'younger' T cell clones.

We then posed the question as to whether the cytotoxic effect of T cell clones can be inhibited by cold target cells (fig. 4). This experiment was carried out using T cell clones 1-6 and P1 tumor cell clones No. 10 (A) and 11 (B). The DA anti-P1 effector cells which were used at an effector to target cell ratio of 5:1 were confronted with cold target P1 tumor cell at a ratio of cold to hot target of 100:1 (C), 50:1 (D), 25:1 (E), and 1:1 (F). Effector cells were also confronted with K562 (L) and YAC-1 (M) targets. All 6 DA anti-P1 T cell clones could be inhibited by P1 tumor cells used as cold targets. The specificity of the reaction is demonstrated by the fact that cold targets from clone 10 used at ratios of 100:1 (G), 50:1 (H) and 25:1 (I) did not inhibit the cytotoxic T cell clones. The cytotoxic effect of T cell clones 1 and 2 towards K562 target cells was observed once again, but this time it was much more pronounced than in earlier experiments.

In conclusion we could establish highly specific syngeneic cytotoxic T cell clones capable of eliminating, at a high rate, P1 tumor cells in vitro. Two such clones (No. 1 and No. 2) also progressively acquired the ability to lyse NK-sensitive target cells. In addition, we found among P1 tumor cells clones which are susceptible to the cytotoxic T cells and clones which are resistant.

3. DA anti-P1 antibodies do not react with P1 tumor cell clones resistant to cytotoxic T cell lysis. Having the information that certain P1 tumor cell clones cannot be lysed by anti-P1 cytotoxic T cell clones, we tested whether the same clones would be recognized by DA anti-P1 antibodies. P1 tumor cell clones No. 10 and 11 as well as uncloned P1 and P2 tumor cells were labelled with ^{51}Cr. Their susceptibilty for DA anti-P1 antibodies was tested in a complement-dependent cytotoxic assay. The results are shown in figure 5. P1 tumor cell clone No. 10 was not lysed by DA anti-P1 antibodies, whereas P1 clone No. 11 was eliminated at high rate. Uncloned P1 tumor cells were only partly affected by the same antibodies. DA normal serum did not touch any of the targets tested.

Similar results were obtained in the protein A assay. DA anti-P1 antibodies were found to bind only to P1 tumor cell clone No. 11 and not to clone No. 10 (data not shown). This eliminates the possibility that P1

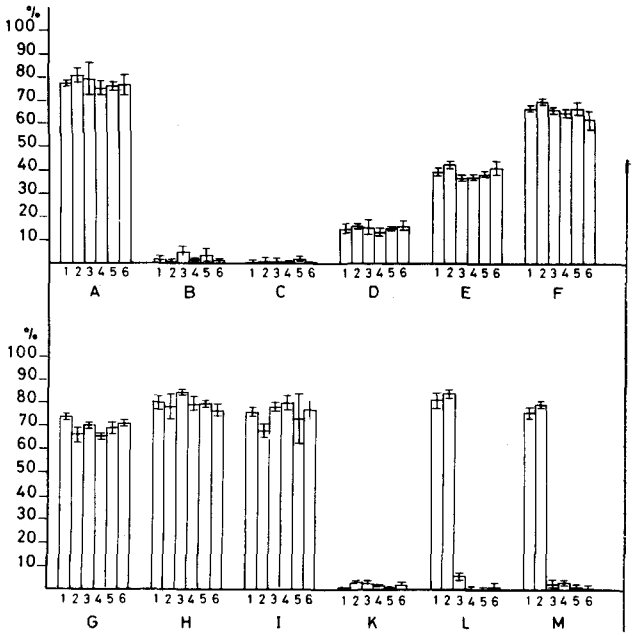

Figure 4. Cytotoxic P1 specific DA T cell clones (1-6) were generated as described in legend to figure 2. They were tested at an effector to target cell ratio of 5:1 against target cells from P1 tumor cell clone 10 (A) and clone 11 (B). Cytotoxic T cell clones incubated with tumor cells from clone 11 were confronted with cold targets at ratios of cold to hot targets of 100:1 (C), 50:1 (D), 25:1 (E), and 1:1 (F). Cold targets from P1 clone No. 10 were used at ratios of 100:1 (G), 50:1 (H) and 25:1 (I). Effector cells were also exposed to control targets P2 (K), K562 (L) and YAC-1 (M).

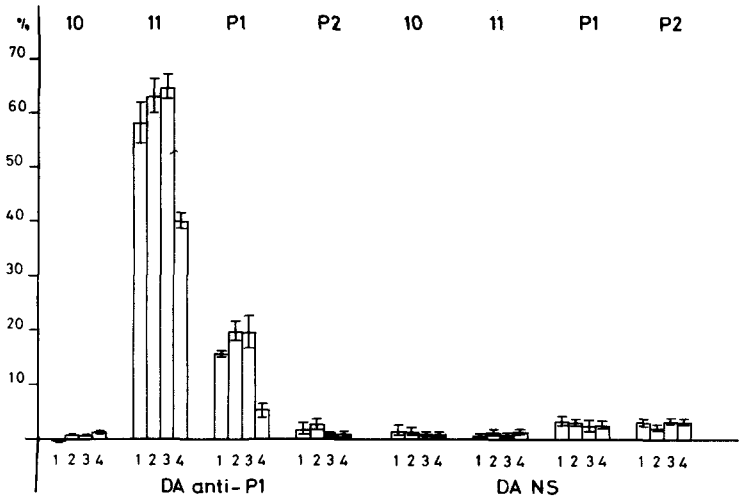

Figure 5. The complement-dependent cytotoxic assay was carried out as described under 'Material and methods', using tumor cells from P1 clones 10 and 11, uncloned P1 tumor cells and P2 tumor cells as targets. DA anti-P1 and DA normal serum (DA NS) were used at concentration of 1:2 (1), 1:4 (2), 1:8 (3), 1:16 (4).

tumor cell clone No. 10 is merely resistant to lysis and still carries the specific antigen.

We thus conclude that the same P1 tumor cell clones are recognized or not by syngeneic cytotoxic T cell clones or tumorspecific antibodies as demonstrated by T cell-dependent cytotoxicity or complement-dependent cytotoxicity.

4. P1 specific cytotoxic T cells do not prevent the development of lethal cytotoxic T cell susceptible P1 tumor cell clones in vivo. In earlier experiments we found that P1 specific cytotoxic T cells cannot prevent the outgrowth of lethal P1 tumors in vivo (see above). With the information that 2 different subpopulations of P1 tumor cells can be defined by their susceptibility or resistancce to cytotoxic T cell clones or P1 specific syngeneic antibodies, we tested whether P1 specific cytotoxic T cells could inhibit the development of lethal tumors using the T cell susceptible tumor cell clone No. 11 as the test tumor. P1 specific cytotoxic T cells were generated by restimulating P1 primed DA T lymphocytes with anti-idiotypic antibodies as before. Cells were expanded with TCGF (see 'Material and methods'). The cytotoxic effect of these cells was tested against P1, P2 and K562 targets and the cells could be shown to be specific. From the cells killed, P1 tumor cells were pooled with an efficiency of about 35%. P1 tumor cells of clone 10 were not touched and cells from clone 11 were almost completely eliminated (more than 90% were destroyed). P2 tumor cells and K562 targets were not lysed (data not shown). 15 DA rats were injected with 8×10^7 cytotoxic T cells i.v. 1 day later, 5 DA rats were injected s.c. with 10^7 tumor cells from P1 tumor cell clone 10 (group I). 5 DA rats were injected with 10^7 tumor cells from clone 11 (group II) and DA rats were injected with 10^7 uncloned P1 tumor cells (group III). Rats were monitored for the development of lethal tumors.

The results are summarized in table 2. By day 20 the 5 DA rats injected with uncloned P1 tumor cells had developed tumors of about 1.5 cm in diameter. Surprisingly, the 5 DA rats injected with P1 tumor cells from clone 11 all had tumors of about 2-3 cm in diameter. Rats injected with tumor cells from clone 10 had tumors of about 1.5-2 cm in diameter. On day 30 all rats of group II were dead. Rats from groups I and III were still alive but carried huge tumors and were therefore sacrificed. We conclude from these data that the P1 specific cytotoxic T cell clones could not prevent the outgrowth of P1 tumor cells of P1 clone 11 in vivo although the very same targets could efficiently be eliminated in in vitro tests. The in vivo study showed somewhat opposite effects, namely, there was enhanced growth of tumor cells of clone 11 as compared with the tumor cells from clone 10.

Table 2. Each rat received 8×10^7 P1 specific cytotoxic T cells i.v. One day later rats were injected subcutaneously with 10^7 tumor cells as indicated

Group No.	No. of rats	Injected tumor cells	Size of in vivo tumors on Day 20	Day 30
I	5	Clone 10	1.5-2 cm	> 5 cm
II	5	Clone 11	2-3 cm	> 5 cm, all rats dead
III	5	Uncloned P1	1.5	> 5 cm

Discussion

Tumor cells induced by chemical means have long been known to frequently express tumor-specific antigens allowing the production of T cell dependent, tumor specific immune reactions[1,17-20,24]. Our present data confirm these findings and add some new information with respect to the DMBA-induced P1 sarcoma system[5]. We knew, already earlier, that it is possible to substitute immunogens capable of stimulating T cells with anti-idiotypic antibodies under certain defined conditions[5,15,29]. In the present syngeneic DA anti-P1 tumor system the use of anti-idiotypic antibodies in the presence of TCGF has been found a most efficient way to expand in a selective manner anti-P1 reactive, idiotype-positive T cells[5]. Some of these T cells are cytolytic cells.

In the present study we were successful in cloning several such anti-P1 specific killer T cells using the above described approach. We do not know the pharmacological activity of the other T cell clones which were selected by the same procedure but which did not display cytolytic properties. When analyzing the ability of cloned cytolytic anti-P1 T cell clones for their ability to kill different P1 clones, we found a high frequency of tumor cell clones resistant to the lytic activity of any out of several tested cytolytic T cell clones.

We had initially found that cytolytic T cells directed against P1 tumor cells failed to eliminate in vivo the tumor cells in the same efficient manner as they could in vitro. This could be due to a use of a mixture of T cell susceptible and resistant P1 sarcoma cells being present in the tumor cell inoculum.

We thus attempted to use cloned, T cell sensitive P1 tumor cells in vivo as targets for in vivo transferred killer T cells instead of using the 'mixed' P1 tumor cell line. However, we again found no significant inhibitory impact on P1 tumor growth by the inoculated immune T cells. Rather, if anything, the growth behavior of the tumor cells was enhanced. We do not know as yet whether this was due to the presence among the anti-P1 T cells of suppressor or other types of T cells that could counteract the lytic activity of the killer T cells present in the same cell population. This question must be resolved by using cloned killer T cells in a similar transfer system coupled with an analysis of the T cell susceptibility of the P1 tumor

cells that continued to grow in vivo in the presence of transferred killer T cells. Other workers have shown that the impact of in vivo transfer of immune T cells in similar tumor systems may have inhibitory, not stimulatory effects on the grafted tumor cells[13,14,28]. In fact, it is by no means clear whether killer T cells in vivo constitute the most efficient graft-rejecting mechanism.

The specificity of killer T cells is normally to 'see' foreign antigens, including tumor-specific ones, in association with major histocompatibility complex antigens of class I[31]. Antibodies to the foreign antigens react to a major extent, with other antigenic determinants on the same immunogen. However, the fact that anti-idiotypic antibodies raised against idiotypic, antigenspecific antibodies can be used to stimulate or kill cytolytic T cells[5,6,11,15,29] clearly argues for the existence of some antibodies with similar specificity as killer T cells although the frequency of such antibodies may be low. In the present system we have added further data supporting such a notion.

Anti-idiotypic antibodies could thus be used to selectively stimulate killer T cells of the relevant specificity of TCGF was present at the same time. Furthermore, presence or absence of the relevant, tumor specific antigens on cloned P1 tumor cells showed a parallel variation whether analyzed by killer T cells or anti-P1 humoral antibodies. The P1 tumor system should therefore allow a detailed study at the tumor cell level of T or B cell specific reactions.

Finally, the present study contains a preliminary yet intriguing finding with respect to NK cells. Natural killer cells have some features in common with killer T cells whereas others are quite different[17]. The possibility that NK cell specificity may represent not a unique receptor structure on these cells but, rather, a more general binding ability of certain bone marrow derived cells being allowed to express itself as a NK cell if that cell also has lytic capacity, is suggested by certain binding studies in vitro (R. Kiessling, personal communication). The present findings that some, but not all, anti- P1 specific killer T cell clones may also start, with time, to develop a NK-like specificity in addition to the anti-P1 specificity are quite intriguing. Such clones, when analyzed in detail, may thus demonstrate the existence of killer T cells capable of lysing cells via 2 different mechanisms, either via binding through idiotypic, antigen-specific receptors or via 'NK-like' recognition structures. We could in fact demonstrate that T cells from clone No. 1 or No. 2 lyse P1 tumor cells and NK targets by 2 independent 'receptors'[32]. Anti-idiotypic antibody blocked reactivity towards P1 target cells but not towards YAC-1 or K562[32].

* Acknowledgments. This work was supported by the Swiss Cancer Foundation grants FOR.135.AK.79 and FOR.203.AK.81 and the Swedish Cancer Society.

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0014-4754/83/010039-09$1.50 + 0.20/0

Minireviews

The biological aging process

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In any particular field of research it is advisable, from time to time, to take stock and reconsider the 'state of the art', and to re-formulate, in the light of scientific developments and one's personal reflections, the aims and targets for future work. It seems to me that such a time has come for experimental research on aging, and I want to say, quite briefly, where, in my view, we stand today and suggest which directions research into the process of aging might take in the future. In the short space available here it will of course not be possible to deal exhaustively with such a complex problem. It is my intention to discuss a few selected points which presently appear to me of highest interest.

Perhaps the only fact that all those engaged in aging research will agree on is that the individual lifespan of animals (or at least of mammals) – and of man – is limited by biological factors. It has not required modern experimental science to uncover this fact; it is, for example, stated *expressis verbis* in Christoph Wilhelm Hufeland's admirable and still quite contemporary treatise 'Macrobiotics: The art of Prolonging Life'[1], first published in Jena, 1798. The great physician states clearly and as accepted fact that for each animal species and for mankind there is an upper limit to lifespan, that this limit is fixed, is different and specific for each species, and that for man it has not changed since the earliest written records of human history. Recent careful study by phylogenetic analysis, with the help of an empirical equation using brain- and body weight estimations from fossils, has led to the conclusion that for *Homo sapiens* the maximum potential lifespan (Hufeland's 'absolute' lifespan as opposed to 'relative' or average lifespan of a particular selected population) has remained unchanged at around 95 years for the past 100,000 years[2].

This is certainly the basic tenet of gerontology which has stood firm ever since the hapless search for the Fountain of Youth and the Philosopher's Stone of the Middle Ages. As long as we remain *Homo sapiens* we must face the fact that we are mortal, that our days are numbered, and that nothing can be done about it. There is a fixed, immutable upper limit to our length of life. I am quite aware that there are people who, for personal or ideological reasons, refuse to accept such a deterministic view, and who think that lifespan can be lengthened by a factor of 2–3 merely through the proper biochemical manipulation of metabolism. I am stating here my own views and beliefs.

Nobody has put this into clearer and more convincing terms than Fries and Crapo in their recent booklet, 'Vitality and Aging'[3]. I believe the time has come for all of us engaged in one way or another in aging research to accept their concept that attaining the 'rectangular society' is the real and practical aim of our work. The 'rectangular society' means that for any human (or mammalian) population, individual lifespans come as close to the species-specific lifespan potential as possible: then the shape of the survival