ANTAGONISTIC EFFECTS OF HUMORAL ISOANTIBODIES ON THE IN VITRO CYTOTOXICITY OF IMMUNE LYMPHOID CELLS

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Immune lymphoid cells have been recently shown to be capable of inflicting cytotoxic damage on various normal and neoplastic target cells in tissue culture in different immunological systems (1–7). The effect was attributed to "cell-bound" antibodies, as contrasted to humoral antibodies, since it did not require complement (2, 5, 7), which is necessary for the cytotoxic action of the latter. The possibility that immune lymphoid cells secrete diffusible substances responsible for target cell destruction was made unlikely since their effect was abolished by separating the two cell types by cell impermeable filters in a diffusion chamber (8). Cell-free supernatants of cultivated immune lymphoid cells did not show any cytotoxic effects on sensitive target cells (8).

Different authors have pointed out that close cellular contact between immune lymphoid cells and target cells always precedes demonstrable cytotoxicity. Recent reports (9, 10) go one step further in demonstrating that agglutination of *normal*, non-immune lymphoid cells onto target cells *in vitro* brought about by inactivated heterologous antiserum (10), or by phytohemagglutinin (9, 10) leads to target cell destruction. The normal cells had no effect unless artificially aggregated on the targets. Target cell death was not due to aggregation as such, however, since isologous lymphoid cells had no effect (10).

This type of cytotoxicity could not be explained by a conventional immunological reaction since lymphoid cells of F_1 hybrid origin killed parental target cells after contact had been established by agglutination, in spite of the fact that hybrid cells are not believed to be genetically competent to react against parental target cells (10). These results suggested that contact between antigenically and/or structurally different cells may result in target cell destruction.

It has been previously shown that the growth-inhibiting effect of immune lymphoid cells on tumor cells *in vivo* can be partially or completely suppressed by the exposure *in vivo* to humoral antibodies directed against the neoplastic target cells (11, 12). The present study represents a similar investigation by the use of *in vitro* systems, with the specific aim of obtaining some knowledge about the kinetics of "cell-bound" and humoral immunity and their possible

interaction in the destruction of tumor target cells. The use of *in vitro* systems is motivated by the fact that the investigation can be performed in the complete absence of complement activity, thus avoiding complications of humoral immunity which might interfere with the action of the cells.

Materials and Methods

Mice.—Tumors and tissues were sampled from inbred mice of the strains C57BL/Kl ($H\text{-}2^b$ $H\text{-}2^b$), C3H/Kl ($H\text{-}2^k$ $H\text{-}2^k$), A.CA/Kl ($H\text{-}2^f$ $H\text{-}2^f$), A/Sn ($H\text{-}2^a$ $H\text{-}2^a$), and A.SW/Kl ($H\text{-}2^s$ $H\text{-}2^s$).

Tumors.—FHA is a sarcoma induced by the implantation of cellophane film into a C3H mouse and has been described previously (13). MC57S is a methylcholanthrene-induced sarcoma of the C57BL strain (14), and MACD a methylcholanthrene-induced sarcoma of (A $\times$ A.CA)F_1 hybrid origin (11).

Antiserum.—An A.CA anti-C57BL serum was used. It was collected by puncturing the retroorbital sinus of A.CA mice, preimmunized with 6 to 10 weekly injections of living C57BL spleen and lymph node cells. The last challenge was given 6 days prior to bleeding. The hemagglutinating titers varied between $1/256$ and $1/2048$. All sera were sterilized by ultrafiltration through 0.45 μ millipore filters prior to use.

Immune Lymphoid Cells.—A distinction will be made between hyperimmune and immune lymphoid cells. The former were obtained from animals immunized 6 to 10 times as described above. Spleen and lymph nodes were removed aseptically and pressed through a 60 mesh stainless steel screen into balanced salt solution (BSS), containing 100 IU of penicillin and 100 μg streptomycin/ml. "Immune" lymphoid cells were taken exclusively from the regional lymph nodes of mice treated with a single subcutaneous injection of allogeneic lymphoid cells 7 to 10 days earlier. Control lymphoid cells were collected in the same way from unimmunized animals.

Complement.—Fresh guinea pig serum was used as a source of complement. It was sterilized by ultrafiltration, and used in a final concentration of 10 per cent. In some cases the complement was inactivated by heating at 56°C for 30 minutes.

Tissue Culture.—Tumor cell suspensions were prepared for explantation by treating finely minced tumor tissue with 0.25 per cent trypsin solution for 1 hour at room temperature. Subsequently the cells were washed and 10^6 trypan blue–unstained cells suspended in 1 ml of lactalbumen in Earle's solution supplemented with 10 per cent calf serum (or, in later experiments, with the same concentration of fetal calf serum), were added to each culture tube. The medium contained mycostatin (30 IU/ml) and penicillin (100 IU/ml). After 24 hours it was replaced with Parker 199 medium supplemented with 10 per cent serum as above.

In experiments designed to study the cytotoxic effect of immune and non-immune lymphoid cells, the tubes were exposed to the lymphoid cells immediately after the first change of medium; *i.e.*, 24 hours after explantation. As a rule, 10^7 lymphoid cells were added to each tube and incubated for 48 hours. Analogous experiments were performed with humoral isoantibodies by adding 0.1 ml undiluted serum to each tube. In experiments designed to study the interaction of serum antibodies and immune lymphoid cells, the serum was added 30 minutes prior to the admixture of the cells.

After 48 hours' incubation the tubes were treated with 1 ml 0.25 per cent trypsin solution for 30 to 60 minutes as 37°C, interrupted by repeated shaking. They were centrifuged and part of the supernatant removed. The number of trypan blue–unstained tumor cells was counted and the volume of the supernatant was measured. Due to the pronounced size difference between tumor and lymphoid cells, the two cell types could be readily distinguished.

RESULTS

Cytotoxic Effects of Immune Lymphoid Cells on Target Tumor Cells in Vitro.—As a first step, the *in vitro* cytotoxicity of immune lymphoid cells was studied with the purpose of working out favorable experimental conditions. The target tumor cells were derived from sarcomas MC57S of C57BL origin, FHA of C3H origin, and MACD of (A × A.CA)F_1 hybrid origin.

The best cytotoxic effect was obtained with regional lymph node cells derived from an animal pretreated with a single immunizing injection 7 to 10 days earlier. Between 25.6 and 96.2 per cent target tumor cells were killed with maximum effect obtained after 48 hours' incubation. Some cytotoxicity was apparent after 24 hours. Complement was not required for cytotoxic action, in contrast to cytotoxic reactions involving humoral isoantibodies. Control cultures were either left untreated or were incubated with non-immune lymphoid cells derived from the same H-2 incompatible strain as the immune cells. In comparison with untreated controls, there was a detectable cytotoxicity in the cultures exposed to non-immune allogeneic lymph node cells, but this effect was always much weaker than the action of the immunized cells (Table I).

As shown previously the cytotoxic action of the non-immune cells could be attributed to the action of heterologous antibodies capable of reacting with the mouse tissues and present in the calf serum used for supplementation of the medium. This effect could be reproduced and amplified experimentally by treating the target cells with heat-inactivated rabbit anti–mouse tissue serum prior to the addition of normal allogeneic lymphoid cells (10).

In contrast to the strong effect of the lymph node cells 8 to 10 days after primary immunization, spleen cells of the same animals showed only a weak ability to kill the tumor cells (Table II). Furthermore, a mixture of spleen and lymph node cells of animals repeatedly immunized against the target cell genotype did not kill the target cells. The regional lymph node cells from hyperimmune mice were highly cytotoxic, however, while the spleen cells had a much lower effect (Table II).

Thus the source of the lymphoid cells did seem to affect the outcome of the experiments, whereas their primary or hyperimmune state did not. It seemed conceivable that the inability of the spleen cells to kill H-2 incompatible target cells was due to their production of humoral isoantibodies *in vitro*, since the spleen is known to be involved in humoral antibody production to a major extent (15). If so, the humoral antibodies in the medium would combine with the antigenic determinants of the target cells, thus protecting them from destruction by the immunologically competent lymph node cells. Such an "efferent inhibition" of transplantation immunity by humoral antibodies has been demonstrated repeatedly in various *in vivo* systems (11).

TABLE I

Cytotoxic Effect of Normal and Isoimmune Lymph Node Cells on Sarcoma Cells in Tissue Culture in the Prescence of 10 Per Cent Heat-Inactivated Calf Serum*

Exp. No.	Target tumors‡	Lymph node cells from	No. of lymph node cells $\times 10^6$	No. of living tumor cells§ $\times 10^3 \pm$ SE	Per cent living cells and cytotoxic index‖ as compared to controls
1	MC57S¶	A.CA Anti-C57BL	10.0	34 ± 3.3	41.0 (0.59)
		A.CA Untreated	10.0	83 ± 11.9	100.0
2	MC57S	A.CA Anti-C57BL	10.0	91 ± 16.8	41.5 (0.59)
		A.CA Untreated	10.0	219 ± 35.8	100.0
3	MC57S	A.CA Anti-C57BL	7.5	140 ± 21.9	50.3 (0.50)
		A.CA Untreated	7.5	279 ± 35.8	100.0
4	MC57S	A.CA Anti-C57BL	5.0	21 ± 5.7	13.6 (0.86)
		A.CA Untreated	5.0	154 ± 35.0	100.0
5	MC57S	A.CA Anti-C57BL	5.0	126 ± 30.3	39.5 (0.61)
		A.CA Untreated	5.0	319 ± 60.5	100.0
6	MC57S	A.CA Anti-C57BL	5.0	119 ± 6.7	50.4 (0.50)
		A.CA Untreated	5.0	236 ± 25.3	100.0
7	MC57S	A.CA Anti-C57BL	20.0	98 ± 25.1	30.7 (0.69)
		" "	10.0	110 ± 18.1	34.5 (0.66)
		" "	5.0	276 ± 46.5	86.5 (0.14)
		A.CA Untreated	20.0	319 ± 56.6	100.0
8	MC57S	A.SW Anti-C57BL	10.0	7 ± 3.4	3.8 (0.96)
		A.SW Untreated	10.0	106 ± 13.0	58.3 (0.42)
		—	—	183 ± 14.0	100.0
9	MACD**	A.CA Anti-A	10.0	35 ± 4.6	27.4 (0.73)
		A.CA Untreated	10.0	109 ± 11.5	85.1 (0.15)
		—	—	128 ± 22.4	100.0

* Isoimmune lymph node cells were taken 7 to 10 days after the first subcutaneous inoculation of living spleen and lymph node cells of the tumor genotype.

‡ 10^6 living trypsinized tumor cells were added to each culture tube 24 hours prior to the experiment. The medium consisted of lactalbumin in Earle's solution for the explantation and was thereafter replaced with Parker's Medium 199, both supplemented with 10 per cent calf serum.

§ 48 hours after the addition of the lymph node cells the tubes were treated with 1 ml 0.25 per cent trypsin solution for 30 to 60 minutes at 37°C and the number of trypan blue–unstained tumor cells counted in a haemocytometer. The figures given are the mean of 5 tubes or more.

‖ The cytotoxic index is defined as the difference between the percentage living cells in the control and experimental group divided by the former figure.

¶ Sarcoma MC57S is of C57BL origin.

** Sarcoma MACD is of (A × A.CA) F_1 hybrid origin.

TABLE I—*Concluded*

Exp. No.	Target tumors‡	Lymph node cells from	No. of lymph node cells × 10^6	No. of living tumor cells§ × 10^3 ± SE	Per cent living cells and cytotoxic index‖ as compared to controls
10	MC57S	A.CA Anti-C57BL	5.0	156 ± 32.9	33.2 (0.67)
		A.CA Untreated	5.0	396 ± 16.7	80.5 (0.20)
		—	—	469 ± 51.4	100.0
11	MACD	A.SW Anti-A	6.0	170 ± 26.2	38.8 (0.61)
		A.SW Untreated	6.0	342 ± 37.2	78.0 (0.22)
		—	—	439 ± 67.2	100.0
12	MC57S	A.CA Anti-C57BL	8.5	102 ± 19.0	41.4 (0.59)
		A.CA Untreated	8.5	222 ± 27.8	89.9 (0.10)
		—	—	247 ± 39.4	100.0
13	MC57S	A.CA Anti-C57BL	10.0	183 ± 37.5	42.8 (0.57)
		A.CA Untreated	10.0	382 ± 34.3	89.2 (0.11)
		—	—	428 ± 28.7	100.0
14	MC57S	A.CA Anti-C57BL	10.0	237 ± 17.2	44.5 (0.56)
		A.CA Untreated	10.0	475 ± 48.1	89.1 (0.11)
		—	—	533 ± 75.1	100.0
15	MC57S	A.CA Anti-C57BL	10.0	156 ± 32.3	48.8 (0.51)
		A.CA Untreated	10.0	312 ± 50.1	97.5 (0.03)
		—	—	320 ± 39.4	100.0
16	MC57S	A.CA Anti-C57BL	20.0	162 ± 18.7	50.9 (0.49)
		" "	10.0	185 ± 17.4	58.2 (0.42)
		" "	5.0	193 ± 26.9	60.7 (0.39)
		A.CA Untreated	20.0	261 ± 11.0	82.1 (0.16)
		" "	10.0	266 ± 38.5	83.6 (0.16)
		" "	5.0	246 ± 22.8	77.9 (0.22)
		—	—	318 ± 15.9	100.0
17	MC57S	A.CA Anti-C57BL	10.0	176 ± 23.5	56.1 (0.44)
		A.CA Untreated	10.0	265 ± 24.0	84.5 (0.16)
		—	—	314 ± 20.7	100.0
18	MC57S	A.CA Anti-C57BL	10.0	258 ± 25.3	62.4 (0.38)
		A.CA Untreated	10.0	406 ± 24.2	98.0 (0.02)
		—	—	414 ± 39.4	100.0
19	MC57S	A.CA Anti-C57BL	7.0	271 ± 41.7	70.0 (0.30)
		A.CA Untreated	7.0	379 ± 24.0	98.5 (0.02)
		—	—	387 ± 35.0	100.0
20	MC57S	A.CA Anti-C57BL	10.0	343 ± 29.4	74.4 (0.26)
		A.CA Untreated	10.0	451 ± 8.5	98.0 (0.02)
		—	—	462 ± 37.3	100.0

Comparisons between the Cytotoxic Effect of Humoral Antibodies and Cell-Mediated Reactions in Vitro.—Pronounced differences were found between the kinetics of the cytotoxic effect on MC57S cells of humoral antibodies and cell-mediated reactions. Immune lymph node cells had no demonstrable effect

TABLE II

In Vitro Cytotoxicity of Immune and Hyperimmune Spleen and Lymph Node Cells on Allogeneic Target Tumor Cells

Exp. No.	Target tumor	A.CA lymphoid cells derived from	No. of living tumor cells $\times 10^3 \pm$ SE	Per cent living tumor cells as compared to controls
1	FHA*	Hyperimmune‡ spleen and lymph node	343 ± 33.0	91.0
		Normal spleen and lymph node	377 ± 24.7	100
2	MC57S§	Hyperimmune spleen and lymph node	396 ± 18.9	>100
		Normal spleen and lymph node	390 ± 29.9	100
3	MC57S	Hyperimmune lymph node	156 ± 32.9	33.3
		" spleen	291 ± 13.5	62.0
		Normal lymph node	396 ± 16.7	84.4
		" spleen	392 ± 32.2	83.6
		—	469 ± 51.4	100
4	MC57S	Immune lymph node‖	346 ± 39.0	67.1
		" spleen	442 ± 23.8	85.7
		Normal lymph node	488 ± 59.5	94.6
		" spleen	527 ± 49.5	>100
		—	516 ± 42.5	100
5	MC57S	Immune lymph node	38 ± 7.7	26.4
		" spleen	106 ± 16.5	73.6
		Normal lymph node	102 ± 16.2	70.8
		" spleen	152 ± 16.3	>100
		—	144 ± 18.0	100

* FHA is a sarcoma indigenous to the C3H strain.

‡ Hyperimmune cells were derived from A.CA mice repeatedly immunized against the corresponding tumor genotype.

§ MC57S is a sarcoma indigenous to the C57BL strain.

‖ Immune cells were derived from A.CA mice injected once 7 to 10 days before explantation.

prior to 24 hours of incubation; the number of killed target cells reached a maximum after 48 hours and did not increase subsequently. The cytotoxic sensitivity to humoral isoantibodies of tumor cells in suspension varies with the cell type. The MC57S tumor has an "intermediate" sensitivity to H-2

isoantibodies in the presence of complement, as judged by the cytotoxic assay by Gorer and O'Gorman (16). The titer was 1/20 to 1/40 in repeated experiments and the cytotoxic index varied between 0.33 and 0.76. Cytotoxic tests with humoral antibodies and MC57S target cells were also performed in tissue culture. When a 1/10 diluted A.CA anti-C57BL serum was added to the tumor cells in culture in the absence of complement, there was no detectable effect on the number of cells. If complement was added (diluted 1/10), there was a marked decrease in the number of living cells as early as 2 hours, and the effect increased during the subsequent 2 hours. After 24 or 48 hours' incubation the cell number was not significantly different from the controls, however.

TABLE III

Effect of Complement on the Cytotoxic Action of Immune Lymphoid Cells on MC57S Tumor Cells in Tissue Culture

Exp. No.	Type of A. CA lymphoid cells	Complement*	No. of living tumor cells $\times 10^3 \pm$ SE	Per cent living tumor cells as compared to controls
1	Hyperimmune spleen and lymph node	Active	408 ± 25.8	68.9
	"	Inactive	442 ± 35.0	74.7
	Normal spleen and lymph node	Active	592 ± 22.9	100
	"	Inactive	550 ± 44.8	92.9
2	Immune lymph node	Active	95 ± 16.9	31.9
	"	Inactive	144 ± 8.2	48.3
	Normal lymph node	Active	298 ± 31.5	100
	"	Inactive	246 ± 21.6	82.6

* Complement was inactivated by heat at 56°C for 30 minutes.

This might be related to an exhaustion of complement and subsequent growth of surviving cells, or to a decrease of cell number in the cultures exposed to normal serum during the first days of incubation, leading to a diminution and eventual disappearance of the difference between the two groups. Complement alone did not have any significant effect on the cells. Thus, the humoral and the cell-mediated reaction culminated at very different times. It has been shown previously that immune lymphoid cells do not require complement for their cytotoxic action, in contrast to humoral antibodies. This was confirmed in the present study and is illustrated in Table III.

Inhibition of the Cytotoxic Effect of Immune Lymphoid Cells by Humoral Antibodies.—Since immune lymphoid cells and humoral antibodies show marked differences with regard to their requirements for cytotoxic efficiency, an investigation on the possible interaction between these types of immune

mechanisms appears of interest. As stated above, *in vivo* investigations of this problem have demonstrated synergistic and antagonistic interactions as well, depending on the experimental conditions (11, 12). The present *in vitro* system allows the study of such interactions in the absence of complement, in order to avoid the complications resulting from cytotoxicity of humoral antibodies.

TABLE IV

Antagonistic Effects of Humoral Isoantibodies on the in Vitro Cytotoxicity of Immune Lymph Node Cells on MC57S Target Tumor Cells

Exp. No.	Type of A. CA lymph node cells	Type of serum*	No. of living tumor cells × 10 ± SE	Per cent living tumor cells as compared to controls
1	Immune	Immune	228 ± 39.4	73.1
	"	Normal	156 ± 31.2	50.0
	Normal	Immune	239 ± 46.6	76.6
	"	Normal	312 ± 51.0	100
2	Immune	—	140 ± 22.5	50.2
	Normal	—	255 ± 37.1	91.4
	—	Immune	361 ± 45.0	>100
	—	Normal	298 ± 72.0	>100
	Immune	Immune	265 ± 39.3	95.0
	"	Normal	135 ± 11.9	48.4
	Normal	Immune	338 ± 33.8	>100
	"	Normal	279 ± 22.3	>100
3	Immune	—	34 ± 3.3	46.6
	Normal	—	83 ± 11.9	>100
	—	Immune	79 ± 7.4	>100
	—	Normal	79 ± 7.2	>100
	Immune	Immune	80 ± 9.6	>100
	"	Normal	57 ± 5.1	78.1
	Normal	Immune	72 ± 7.8	98.6
	"	Normal	73 ± 6.8	100

* The immune serum employed was an A.CA anti-C57BL serum with a hemagglutinating titer of 1/256 to 1/2048. Normal serum was from untreated A.CA mice.

Humoral antibodies (A.CA anti-C57BL) taken from repeatedly immunized mice were added to the cultures of MC57S sarcoma cells in a final dilution of $\frac{1}{10}$. One hour later 10^7 lymphoid cells from A.CA mice immunized 8 days previously were added to the tumor cells. Various controls were run in parallel. Thus, the MC57S target cells were exposed to humoral antibodies, to immune lymph node cells, to normal lymph node cells, to normal serum, and to various combinations of these. All experiments were performed with tissue culture medium supplemented with fetal calf serum instead of adult serum, since

previous experiments demonstrated that normal, non-immune lymphoid cells brought about a certain target cell destruction when calf serum was added to the medium, whereas fetal calf serum did not cause significant cytotoxicity (10).

In the presence of normal mouse serum, immune lymphoid cells exerted a marked cytotoxic effect on the tumor cells as found before. Normal lymph node cells had no effect, neither in the presence nor in the absence of isoantiserum (Table IV). The addition of immune serum to the cultures prior to the immune lymph node cells inhibited the cytotoxic effect partially or completely in repeat experiments.

The results clearly demonstrate that humoral antibodies can antagonize the cytotoxic effect of immune lymphoid cells. This is in line with the previously reported finding that humoral antibodies can counteract the growth-inhibiting action of "cell-bound" antibodies and thereby cause immunological enhancement. As a consequence, the low cytotoxic efficiency of immune spleen cells may be attributed to their high production of humoral antibodies, reacting the antigenic determinants and blocking the attachment site for immune lymphoid cells.

DISCUSSION

Much interest has been centered on the mechanism by which humoral and cell-bound antibodies kill target cells in the course of the homograft reaction (see references 17–19). Humoral antibodies are cytotoxic for some types of nucleated tissue cells (usually of lymphoid origin) *in vitro* in the presence of complement. Suspended cells are killed very rapidly (15 to 30 minutes). Other cells, such as certain tumor cells, are more resistant but can nevertheless be killed under appropriate circumstances (13, 20). The critical variable has been identified as the concentration of antigenic determinants (13, 21, 22). The characteristics of the cell-bound immunity are quite different, as shown in this and other studies. The effect takes longer time (24 to 48 hours), and does not require complement. Maximum cytotoxic activity of immune lymphoid cells is reached 7 to 10 days after immunization and decreases thereafter, in contrast to the production of humoral antibodies (at least those of the 7S type, which are mostly responsible for *in vitro* cytotoxicity) (23). Serum titers increase during the first 2 to 3 weeks after immunization and reach high levels only after hyperimmunization. Lymph node cells show a much higher cytotoxic activity *in vitro* than spleen cells. Probably, humoral antibodies synthesized in the spleen interfere with the cytotoxic effect.

A suggestion was put forward recently to explain the mechanism of cell-bound immunity (10). It was shown that normal allogeneic but not isologous lymphoid cells were fully competent to kill tumor target cells in tissue culture subsequent to their aggregation around the target cells, which was accom-

plished by treatment with heterologous antibodies or phytohemagglutinin. Cytotoxicity could not be explained by conventional immunological reactions (such as antibody production), since F_1 hybrid lymphoid cells killed parental target cells, in spite of the fact that the hybrid cells are not genetically competent to react immunologically against the target cells. It was also found that lymphoid cells pretreated with 1500 r were effective in this system (24). It seemed more likely that close contact between antigenically and/or structurally incompatible cells results in target cell destruction. However, *in vitro* cytotoxicity also occurred when the lymphoid cells did not contain foreign isoantigens (F_1 targets and parental lymphoid cells) (24). Two alternative explanations may account for these findings: (*a*) target cell destruction is the consequence of close contact between cells carrying foreign histocompatibility antigens; or (*b*) cell death occurs after aggregation with structurally incompatible cells which need not necessarily be antigenically incompatible. According to the first hypothesis, destruction of parental target cells subsequent to contact with semi-isologous hybrid cells would be a direct effect on the targets, whereas in the reverse situation cytotoxicity must be explained by an indirect effect; only the parental lymphoid cells can be killed in this mixture, but their death would secondarily lead to cytotoxic effects on the aggregating cellular partner. The second alternative implies that structural differences which are not necessarily related to antigenicity are responsible for cytotoxicity. An F_1 target cell aggregated to a parental lymphoid cell is not confronted with foreign isoantigens, but probably with a structurally different cell surface. If this structural discrepancy is sufficient to cause cell death, cytotoxicity would be a direct effect on the target cells.

It follows from the above results, that *in vitro* cytotoxicity of *immune* lymphoid cells on various target cells is caused by their capacity to aggregate specifically with antigenically incompatible target cells, most likely because they possess a specific receptor reacting with the antigens of the target cells. Cytotoxicity would be the consequence of the contact with the cells and not be caused by specific immune reactions.

According to this scheme the cytotoxic effect of immune lymphoid cells would depend on the presence of specific receptors facilitating attachment to the target cell and the death of these cells be complement independent, in contrast to antibody-induced cytotoxicity.

The present finding that pretreatment of the target cells with humoral antibodies inhibited the cytotoxic effect of immune lymphoid cells directed against the same isoantigens as the antibodies, is in agreement with the hypothesis above: the antibodies react with the antigenic determinants of the target cells, thereby blocking the site of attachment of the lymphoid cells. As a result, no close contact can be established between two incompatible cells and cytotoxicity cannot occur. Thus, it seems likely that cell-bound and

humoral antibodies have the same immunological specificity in this system, in contrast to other immunological systems (25), where cell-bound antibodies have been shown to be directed against a larger part of the antigen molecule compared to serum antibodies. If this were the case in the present system, inhibition by humoral antibodies would probably not occur, unless factors not related to immunological specificity such as steric hindrance, were responsible. It is not possible as yet to exclude steric factors in the present test system, however.

Humoral antibody-induced inhibition of cell-bound as well as humoral immunity has been demonstrated in a variety of immunological systems (for reference see 26). The antibody effect has been demonstrated to occur at different levels and to have different effects, but is in all cases dependent upon the reaction with the antigen. Humoral antibodies may interact with the antigen at an "afferent" level, thereby inhibiting the initiation of the immune response, or at an "efferent" level as in the present case leading to protection of the target cells from destruction by immunologically competent cells. Both mechanisms are responsible for the phenomenon of immunological enhancement. In addition, however, 7S antibodies are capable of inhibiting cellular proliferation of 19S antibody-producing cells after antibody synthesis has been initiated (23). These manifold effects of humoral antibodies all work in the same direction and tend to inhibit new antibody production. As suggested elsewhere (23), humoral antibodies may constitute a feed-back mechanism regulating the extent of antibody synthesis and preventing the risks of hyperimmunization against an individual antigen.

SUMMARY

The ability of specifically immunized lymphoid cells to kill H-2 incompatible target tumor cells in tissue culture was shown to depend on the source of the lymphoid tissue (spleen *versus* lymph nodes). Marked cytotoxic effects were obtained with regional lymph node cells 7 to 10 days after primary immunization, whereas spleen cells from the same animals had little or no effect. Hyperimmunization did not decrease the cytotoxic efficiency of lymph node cells. Experiments were performed to test the possibility that the weak effect of spleen cells is a result of humoral antibody production, antagonizing the cell-bound immunity.

Humoral antibodies were cytotoxic *in vitro* in the presence of complement only. Their effect was manifested after 2 hours, whereas immune lymph node cells did not require complement and cytotoxicity was not expressed until 24 to 48 hours' incubation. Tumor cell cultures treated with specific humoral antibodies in the absence of complement became resistant to the cytotoxic effect of subsequently added immune lymph node cells, while no such protection was seen when normal serum was added. Thus, humoral antibodies led to

an "efferent" inhibition of cell-bound immunity *in vitro*, in analogy with previous results *in vivo.*

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BIBLIOGRAPHY

1. Govaerts, A. J., Cellular antibodies in kidney homotransplantation, *J. Immunol.*, 1960, **85**, 516.
2. Rosenau, W., and Moon, H. D., Lysis of homologous cells by sensitized lymphocytes in tissue culture, *J. Nat. Cancer Inst.*, 1961, **27**, 471.
3. Koprowski, H., and Fernandes, M. V., Autosensitization reaction *in vitro*. Contactual agglutination of sensitized lymph node cells in brain tissue culture accompanied by destruction of glial elements, *J. Exp. Med.*, 1962, **116**, 467.
4. Perlmann, P., and Broberger, O., *In vitro* studies of ulcerative colitis. II. Cytotoxic action of white blood cells from patients on human fetal colon cells, *J. Exp. Med.*, 1963, **117**, 717.
5. Wilson, D. B., The reaction of immunologically activated lymphoid cells against homologous target tissue cells in vitro, *J. Cellular and Comp. Physiol.*, 1963, **62**, 273.
6. Vainio, T., Koskimies, O., Perlmann, P., Perlmann, H., and Klein, G., In vitro cytotoxic effect of lymphoid cells from mice immunized with allogeneic tissue, *Nature*, 1964, **204**, 453.
7. Brondz, B. D., Interaction of immune lymphocytes *in vitro* with normal and and neoplastic tissue cells, *Folia Biol. (Prague)*, 1964, **10**, 164.
8. Rosenau, W., Interaction of lymphoid cells with target cells in tissue culture, *in* "Cell-Bound" Antibodies, (B. Amos, and H. Koprowski, editors) Philadelphia Wistar Institute Press, 1963, 75–83.
9. Holm, G., Perlmann, P., and Werner, B., Phytohemagglutinin-induced cytotoxic action of normal lymphoid cells on cells in tissue culture, *Nature*, 1964, **203**, 841.
10. Möller, E., Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens, *Science*, 1965, **147**, 873.
11. Möller, G., Studies on the mechanism of immunological enhancement of tumor homografts. III. Interaction between humoral isoantibodies and immune lymphoid cells, *J. Nat. Cancer Inst.*, 1963, **30**, 1205.
12. Batchelor, J. R., and Silverman, M. S., Further studies on interaction of sessile and humoral antibodies in homograft reactions, *Ciba Found. Symp., Transplantation*, 1962, 216.
13. Möller, E., and Möller, G., Quantitative studies of the sensitivity of normal and neoplastic mouse cells to the cytotoxic action of isoantibodies, *J. Exp. Med.*, 1962, **115**, 527.
14. Möller, E., Isoantigenic properties of tumors transgressing histocompatibility barriers of the H-2 system, *J. Nat. Cancer Inst.*, 1964, **33**, 979.

15. Jerne, N., and Nordin, A. A., Plaque formation in agar by single antibody-producing cells, *Science*, 1963, **140**, 405.
16. Gorer, P. A., and O'Gorman, P., The cytotoxic activity of isoantibodies in mice, *Transplant. Bull.*, 1963, **3**, 142.
17. Amos, D. B., The use of simplified systems as an aid in the interpretation of mechanisms of graft rejection, *Progr. Allergy*, 1962, **6**, 468.
18. Hellström, K. E., and Möller, G., Immunological and immunogenetic aspects of tumor transplantation, *Progr. Allergy*, 1965, **9**, 158.
19. Snell, G. D., The immunology of tissue transplantation, *in* Conceptual Advances in Immunology and Oncology, M.D. Anderson Hospital Symposium Texas, 323, 1963.
20. Boyse, E. A., Old, L. J., and Stockert, E., Some further data on cytotoxic isoantibodies in the mouse, *Ann. New York Acad. Sc.*, 1962, **99**, 574.
21. Möller, E., Quantitative studies on the differentiation of isoantigens in newborn mice, *Transplantation*, 1963, **1**, 165.
22. Winn, H. J., The participation of complement in isoimmune reactions, *Ann. New York Acad. Sc.*, 1962, **101**, 23.
23. Möller, G., and Wigzell, G., Antibody synthesis at the cellular level. Antibody induced suppression of 19S and 7S antibody response, *J. Exp. Med.*, 1965, **121**, 969.
24. Möller, G., and Möller, E., data to be published.
25. Benacerraf, B., and Gell, P. G. H., Immunological specificity of delayed and immediate hypersensitivity reactions, *in* Mechanism of Cell and Tissue Damage Produced by Immune Reactions, (P. Grabar and P. Miescher, editors) Basel, Benno Schwabe and Co., 1961, 136–145.
26. Möller, G., Antibody induced depression of the immune response. A study of the mechanism in various immunological systems, *Transplantation*, 1964, **2**, 405.