Clin. exp. Immunol. (1974) **18,** 491–498.

SHARED ANTIGENIC DETERMINANTS BETWEEN HUMAN BRAIN AND HUMAN T-CELL LINE

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(*Received* 15 *February* 1974)

SUMMARY

Rabbits were immunized with MOLT cells (T cells) and RPMI number 1788 (B cells) to get respective antisera. These sera were absorbed with human liver, human brain, or various B cells (RPMI number 1788, SOMMER-B cell, B-35M cells and B-411 cells). The results indicate that the cytotoxicity of rabbit anti-MOLT serum against MOLT cells could be absorbed to some extent with human liver and various B cells but not completely. Human brain could completely absorb anti-MOLT activity from rabbit anti-MOLT serum. Human brain could not remove anti-B-cell activity from rabbit anti-RPMI number 1788 (B cells). Rat brain did not remove anti-MOLT activity from anti-MOLT serum.

These data suggest that human brain has antigenic determinants identical or very similar to those found on MOLT cells and thus possibly human thymocytes.

INTRODUCTION

Murine thymus cells possess unique differentiation markers called θ, which have allowed their identification (Raff, 1970; Reif & Allen, 1964). Besides θ, several markers such as TL, Ly, H-2 exist on thymus cells. θ antigen is known to be present on the brain, skin, and peripheral T cells (Boyse, Old & Scheid, 1971). Heteroantiserum against mouse brain has been shown to be T cell-specific (Golub, 1971). Clagett *et al.* (1973) and Peter *et al.* (1973) demonstrated that rabbit anti-mouse brain cross-reacted with thymus cells of mouse and rat. So far, it is not known whether human T cell-specific antigen can produce antibody which cross-reacts with human brain. Thymus-specific antigens on human T cells were reported by Ablin & Morris (1972, 1973). Recently, Minowada, Ohnuma & Moore (1972) and Minowada & Moore (1973) have succeeded in establishing permanent cell lines (MOLT) from the peripheral blood of a patient with acute lymphoblastic leukaemia. These cells did not produce immunoglobulins, and did not have receptors for immunoglobulins or the Fc portion of immunoglobulins, which are considered to be characteristics of cells of the B line

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(Bianco, Patrick & Nussenzweig, 1970; Fröland, Natvig & Berdal, 1971; Rabellino *et al.*, 1971; Raff, 1970; Shevach *et al.*, 1972). On the other hand, MOLT cells bound sheep red blood cells (SRBC) upon exposure (Minowada *et al.*, 1972; Minowada & Moore, 1973; Moore & Minowada, 1973). Wilson & Nossal (1971) have indicated that acute lymphoblastic leukaemia (ALL) is a leukaemia of T cells and chronic lymphoblastic leukaemia (CLL) is that of B cells. Minowada & Moore (1973) have shown the selective cytolysis of cells of ALL (but not of CLL) by rabbit anti-MOLT serum.

We immunized rabbits with MOLT cells and cells of B-cell line and report that human brain shares antigenic determinants with MOLT cells and thus possibly human T cells.

MATERIALS AND METHODS

Cell lines

MOLT cells, T line, were obtained as reported previously (Minowada *et al.*, 1972; Minowada & Moore, 1973). Four B-cell lines were used for absorption and immunization: SOMMER B cells from ALL; RPMI number 1788; RPMI number 411–4 from healthy male adults; B35M from a Burkitt lymphoma patient. They were grown at 37°C in a suspension culture using the nutrient medium RPMI number 1640 supplemented with 10% (v/v) heat-inactivated foetal calf serum, penicillin (100 u/ml) and streptomycin (50 μg/ml).

Immunization

One millilitre of MOLT cells ($1{\cdot}5 \times 10^7$) or RPMI number 1788 ($1{\cdot}5 \times 10^7$) was mixed with 1 ml of Freund's complete adjuvant (FCA) and each 0·5 ml was injected into bilateral footpads, the rest (1 ml) being injected into a neck muscle. One week later 2×10^7 cells were injected intravenously, and another week later $1{\cdot}5 \times 10^7$ cells with FCA were injected into footpads and neck muscle. Blood was drawn 2 weeks after the last injection. After separation, serum was inactivated by heating at 56°C for 30 min.

Cytotoxicity

0·1 ml of cell suspension containing 2×10^6 viable cells per millilitre of 1X Eagle solution was mixed with 0·1 ml of either antiserum or normal rabbit serum (NRS) and the mixture was kept at 20°C for 30 min. Then 0·1 ml of guinea-pig complement was added to each tube and incubated at 37°C for 30 min. After incubation, the cell suspension was centrifuged for 15 min at 800 *g*, and the supernatants were removed. 0·3 ml of 1X Eagle solution and 0·1 ml of 0·2% Trypan Blue solution were added to each tube and mixed vigorously. The numbers of viable cells and dead cells were counted under a microscope. Viability (percentage of live cells) was calculated by dividing the percentage of viable cells in a tube with antiserum by the percentage of viable cells in a tube with normal rabbit serum, and multiplying by 100. The percentage of dead cells was obtained by subtracting the percentage of live cells from 100. The presence of cytotoxic substances in the serum unrelated to antibodies was tested by measuring cytotoxicity to cells without the addition of complement. These substances turned out to be negligible.

Absorption

One gram of human brain (cortex, mainly grey matter) or human liver, provided by Dr

U. Kim of our Institute, was homogenized in 3 ml of saline in a Potter homogenizer. The homogenate was centrifuged for 20 min at 2000 *g*. The supernatant was drawn and 3 ml of saline was added before the mixture was centrifuged again. After the second centrifugation, the pellet was mixed with 3 ml of either anti-MOLT or anti-RPMI number 1788 serum and kept for 30 min at 20°C. When antiserum was absorbed with B cells, 3 ml of antiserum was mixed with a 1-ml volume of the mixture of B-cell lines (RPMI number 1788, B35M, and number 411, and SOMMER-B cells). After incubation for 30 min the mixture was centrifuged for 20 min at 2000 *g* and the supernatant was used for cytotoxicity tests or further absorption. Rat brain (1 g) was used as a control and treated the same way. Human liver and brain from two different autopsies were used to show that the absorption was not specific to individuals but to the organ.

RESULTS

Cytotoxicity against MOLT cells of rabbit anti-MOLT sera absorbed with various tissues of human origin

Anti-MOLT sera obtained by injection of MOLT cells three times into rabbits were used: (····) without absorption; (o—o) after absorption once with human liver and twice with the mixture of four kinds of established cells of B line; (×– –×) after absorption once with

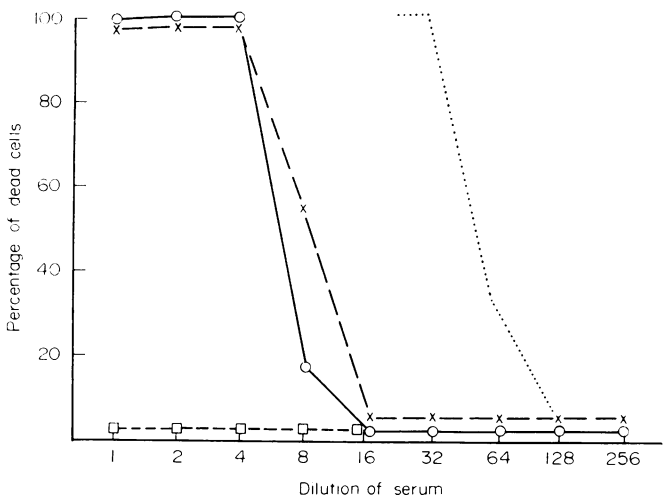

FIG. 1. Cytotoxic effects to MOLT cells of rabbit antisera against MOLT cells. (····) Antiserum was used without absorption. (o—o) Three millilitres of antiserum was absorbed with 1 g of human liver, and twice with the mixture of B cells (1 ml in the packed volume for each absorption). (×– –×) Three millilitres of antiserum was absorbed with 1 g of human liver, and three times with the mixture of B cells. (□– –□) Three millilitres of antiserum was absorbed with 1 g of human liver, and twice with the mixture of B cells, and once with 1 g of human brain.

0·1 ml of serially diluted antisera was mixed with 0·1 ml of MOLT cell suspension (2×10^6/ml), kept for 30 min at room temperature, then 0·1 ml of guinea-pig complement was added. After the mixture was incubated for 30 min at 37°C, it was centrifuged at 800 *g* for 10 min. The supernatant was removed and 0·3 ml of 1X Eagle solution was added to each and mixed vigorously. Finally 0·1 ml of 0·2% Trypan Blue was added to test the viability of the cells. The percentage of dead cells was expressed as described in Materials and Methods section.

human liver and three times with B cells; or (□– –□) after absorption once with human liver, twice with human B cells and once with human brain.

Fig. 1 shows that 50% cytolysis by the unabsorbed serum occurred at serum dilutions between 1:32 and 1:64. On the other hand, absorption with human liver and B cells (2X) resulted in an eight-fold decrease in cytotoxicity, indicating that antibodies against human-specific antigens on MOLT cells were absorbed with liver and B cells. Further absorption with B cells (three times) did not result in lower cytotoxicity, while further absorption with human brain completely eliminated the cytotoxicity of the sera against MOLT cells.

Cytotoxicity of rabbit anti-MOLT sera against B-cell lines

Fig. 2 shows that unabsorbed anti-MOLT serum was cytotoxic to SOMMER-B cells obtained from a patient of acute lymphoblastic leukaemia, but that the cytotoxicity was about eight-fold less than that against MOLT cells as compared with 50% cytolysis. Although anti-MOLT sera were cytotoxic to RPMI number 1788 cells obtained from a normal person, the cytotoxicity was very little.

Cytotoxicity of rabbit anti-MOLT sera absorbed with various tissues

Fig. 3 shows the results of experiments in which anti-MOLT sera were absorbed with human liver and rat brain, or human brain. (○—○) Indicates the results of cytotoxicity tests of unabsorbed anti-MOLT sera against MOLT cells. (△– –△) Indicates the results of cytotoxicity test of anti-MOLT sera absorbed with human liver (1 g) and rat brain (1 g). It is shown that human liver and rat brain did not absorb cytotoxic antibody in anti-MOLT sera against MOLT cells. (× · · · ×) and (●–·–●) Show the results of experiments in which anti-MOLT serum was absorbed once and twice with human brain, respectively. One absorption with human brain resulted in removal of anti-MOLT antibody to some extent, by absorbing twice, the anti-MOLT antibodies were completely removed.

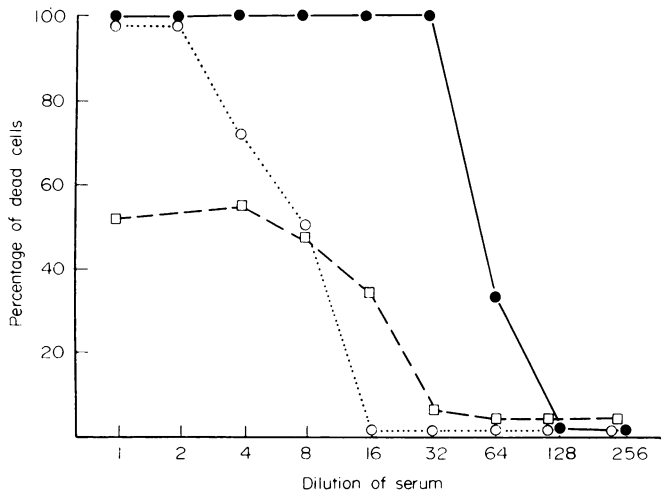

FIG. 2. Cytotoxic effects of rabbit antiserum against MOLT cells to establish human cell lines. Target cells are: MOLT cells (●—●); SOMMER-B cells (○· · ·○); RPMI number 1788 cells (□– –□).

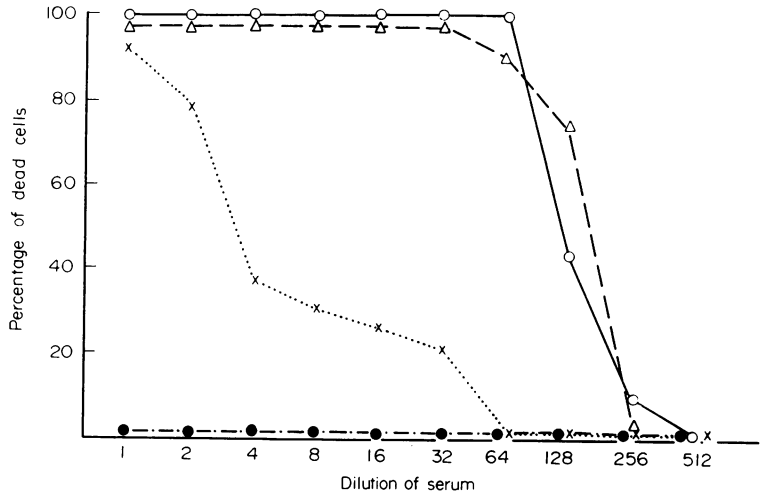

FIG. 3. Cytotoxic effect of rabbit antisera against MOLT cells after absorption with various tissues. (○—○) The serum was not absorbed. (△– –△) The serum (3 ml) was absorbed with human liver (1 g) and rat brain (1 g). (×· · ·×) The serum (3 ml) was absorbed with human brain (1 g). (●– · –●) The serum (3 ml) was absorbed twice with human brain (1 g).

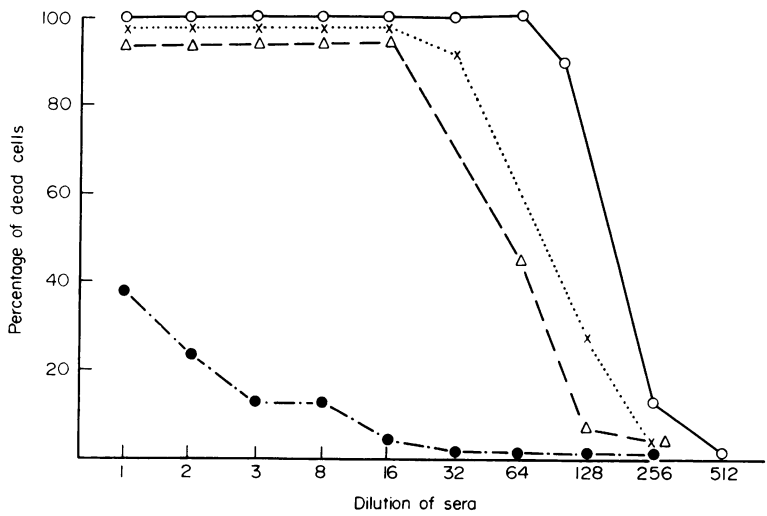

FIG. 4. Cytotoxic effects against B-35M cells of rabbit antisera against RPMI number 1788 cells and MOLT cells. Anti-MOLT serum was used without absorption (●— · —●). Antiserum against RPMI number 1788 cells was not absorbed (○—○) or 3 ml of the serum was absorbed with 1 g of human brain once (×· · ·×) or twice (△– –△).

Cytotoxicity of rabbit antisera against RPMI number 1788 *and MOLT cells to B-35M cells*

So far we have shown that injections of MOLT cells into rabbits resulted in the formation of antibody which could not be absorbed with human liver, human B cells, or rat brain, but could be absorbed with human brain. These results may indicate that antibody was formed mainly against human T cell-specific antigens and that antibodies against human-

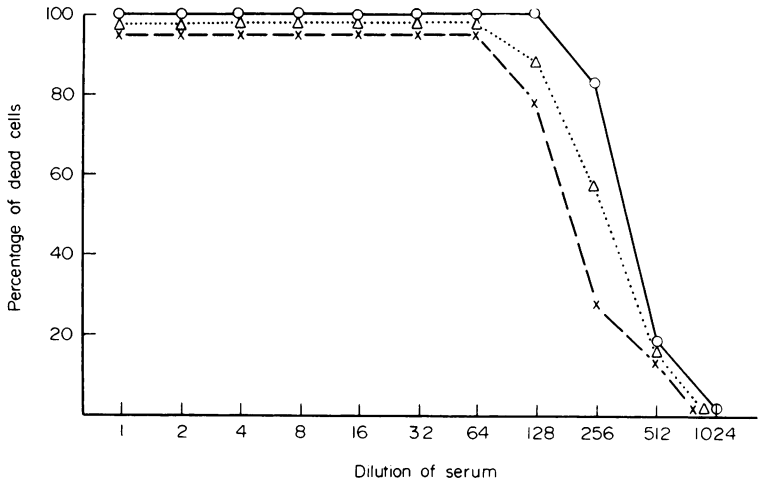

FIG. 5. Cytotoxic effects against SOMMER-B cells of rabbit antisera against RPMI number 1788 cells. The antiserum was not absorbed (○—○) or 3 ml of the antiserum was absorbed with 1 g of human brain once (△ · · · △) or twice (× – – ×).

specific antigens (such as HL-A) were negligible, since human liver could not absorb anti-MOLT antibodies. Fig. 4 shows the results of experiments in which cytotoxicity was tested against B-35M cells and one B-cell line with antisera against MOLT and RPMI number 1788 cells. As shown here, anti-MOLT serum hardly killed B-35M cells, whereas antiserum against RPMI number 1788 did. Antiserum against RPMI number 1788 was absorbed with human brain. Human brain only slightly absorbed antibodies against B-35M cells in anti-RPMI number 1788 serum.

Cytotoxicity of rabbit anti-RPMI number 1788 serum against SOMMER-B cells

Fig. 5 shows the results of experiments in which SOMMER-B cells were incubated with rabbit sera against RPMI number 1788 cells in the presence of complement. Rabbit antiserum was either untreated or absorbed with human brain (once or twice). Human brain did not remove cytotoxicity against SOMMER-B cells from the serum.

DISCUSSION

Since Iwakata & Grace (1964) succeeded in the permanent culture of a haematopoietic cell line from the peripheral blood of a patient with acute myelogenous leukaemia, many cell lines were successfully established. Lymphoid cell lines established from normal persons (Moore, Gerner & Franklin, 1967) appeared quite similar to each other. These cell lines produced immunoglobulins (Tanigaki *et al.*, 1966) and had receptors for complement and antigen–antibody complexes. The presence of surface-bound immunoglobulins (Jondal, Holm & Wigzell, 1972; Rabellino *et al.*, 1971; Raff, 1970) and of receptors for complement (Jondal *et al.*, 1972; Shevach *et al.*, 1972) and antigen–antibody complex on the membranes (Shevach *et al.*, 1972) were considered to be markers for B lymphocytes.

Minowada *et al.* (1972, 1973) succeeded in the establishment of lymphoid cell lines (called

MOLT cells) from the peripheral blood of a patient with acute lymphoblastic leukaemia. MOLT cells did not have immunoglobulins on the surface, nor receptors for antigen–antibody complex, but bound sheep red blood cells (SRBC) to form rosettes. Any of the B-cell lines so far established did not bind SRBC. The formation of spontaneous rosettes with SRBC in man was considered to be a marker for T cells (Jondal *et al.*, 1972; Lay *et al.*, 1971). Thus MOLT cells had characteristics of human T cells.

Wilson & Nossal (1971) suggested that acute lymphoblastic leukaemia might be the leukaemia of T-cell lineage. As Minowada *et al.* (1972) have shown, rabbit anti-MOLT sera specifically killed cells from most of the patients with acute lymphoblastic leukaemia, but did not kill cells from most of the patients with chronic lymphoid leukaemia. Furthermore, cytotoxicity of rabbit anti-MOLT serum was absorbed with fresh human thymocytes, but not with human B-cells or liver (unpublished observations).

By using MOLT cells, we wanted to know whether there are antigenic determinants shared by MOLT cells and human brain, but not shared by any of the B cells nor by human liver. Since MOLT cells had antigenic determinants shared by human thymocytes, the results may determine whether human brain shares antigenic determinants with human thymus. Shared antigenic determinant between thymus and brain has been shown in mice and rats (Clagett *et al.*, 1973; Golub, 1971; Peter *et al.*, 1973).

Fig. 2 shows that anti-MOLT serum was more cytotoxic to MOLT cells than to cells of two B-cell lines. Fig. 1 shows that cytotoxicity in anti-MOLT serum was absorbed by human liver and B cells to some extent but not completely. On the other hand, human brain absorbed cytotoxicity completely. These data indicate that human brain removed antibodies that could not be removed by B cells. As shown in Fig. 3, rat brain did not absorb cytotoxicity at all whereas absorbing anti-MOLT serum twice with human brain resulted in complete removal of cytotoxicity to MOLT cells. Finally, we have shown that human brain hardly removes cytotoxicity to B cells from anti-B-cell serum. As shown in Fig. 4, anti-MOLT serum hardly killed B35M cells. This means that B-35M cells do not share many antigenic determinants with MOLT cells. Thus, if human brain specifically removes anti-human T cell-specific antigens, anti-B-cell serum should not reduce its cytotoxicity to B cells upon absorption with human brain. Figs 4 and 5 show that cytotoxicity of rabbit anti-RPMI number 1788 serum against B-35M cells and SOMMER-B cells was hardly absorbed with human brain.

These data clearly indicate that there may be shared antigenic determinants between human brain and MOLT cells and thus between human brain and human thymocytes. It should therefore be possible to get antibody against human T cell-specific antigens by injecting human brain into animals. We obtained such sera and used MOLT cells and cells of B lineage to test cytotoxicity and many tissues to see if they would absorb these antibodies. The results further suggested that antigenic determinants were shared between human brain and human thymocytes and will be reported elsewhere.

ACKNOWLEDGMENTS

This work was supported in part by PHS grant CA-14413 from the National Cancer Institute, and AI-08899 from the National Institute of Allergy and Infectious Disease and General Research Support grant number RR-05648-08.

We are grateful to Dr Pressman of our Institute, and Dr Stefan Thierfelder, Head of the

Immunology Division, Institut für Hematologie, München, West Germany, for valuable discussions.

Excellent technical assistance by Harry Babbitt is greatly appreciated.

Sommer B cells were given to us by Dr G. E. Moore, Denver, Colorado, U.S.A.

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