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HETEROLOGOUS SPECIFIC ANTISERUM FOR IDENTIFICATION OF HUMAN T LYMPHOCYTES*

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SUMMARY

Peripheral blood lymphocytes (PBL) from two patients with Bruton-type agammaglobulinaemia were used for the preparation of heterologous anti-human T cell-sera which were absorbed with cultured B lymphoblast cells, peripheral blood lymphocytes from a patient with chronic lymphatic leukaemia and 'adherent' cells. Using multiple criteria, one antiserum (ATCS) was shown to be specific for T lymphocytes. This antiserum asserts the existence of human-specific T-lymphocyte antigen(s) (HTLA) and provides another method for identifying human T cells. In the presence of rabbit complement, ATCS was cytotoxic for 65·5% (range 49–78) of normal PBL and 97% of thymocytes (the latter cells having also a higher surface density of HTLA than PBL). The study of PBL from a variety of patients showed that the percentage of ATCS-sensitive cells was high in Bruton-type agammaglobulinaemia, variable from patient to patient and from time to time in common variable hypogammaglobulinaemia and generally low in active lepromatous leprosy, in patients under antilymphocyte globulin therapy and in chronic lymphatic leukaemia. Cultured lymphoblasts from various B cell lines or from a Burkitt lymphoma cell line were resistant to ATCS.

INTRODUCTION

From numerous studies, focused on the thymus and the bursa of Fabricius, the developmental and functional heterogeneity of the lymphocyte population has been recognized and is now universally accepted (Stutman & Good, 1972). Lymphocytes differentiating under the influence of the thymus become 'T cells' and are mostly involved in processes of cell-mediated immunity, while those from the bone marrow developing under the influence of the bursa of Fabricius in birds—or possibly of a bursal equivalent in mammals—become 'B cells' and will synthesize and secrete antibodies. Besides these two major cell populations ('two-component concept' (Good, 1972)) there exists a population of lymphocyte-like, marrow-derived cells which can transform into monocytes (Elves, Gough & Israels, 1966).

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We call these cells 'M cells' (Stutman & Good, 1972). Also, among the lymphoid cells of the bone marrow and, to a lesser degree, of the peripheral blood, there are stem cells (Bryant & Cole, 1967; Bennett & Cudkowicz, 1967; McCredie, Hersh & Freireich, 1971) which we will call 'S cells'.

Improved knowledge of B and T lymphocytes, and especially of their functions, has derived from the discovery of surface markers in several animal species, particularly in the mouse. Contrary to T cells, B lymphocytes have, for instance, a high density of surface immunoglobulin (Ig) (Pernis, Forni & Amante, 1970; Unanue *et al.*, 1971; Wilson & Nossal, 1971) and a receptor for the third component of complement (Dukor, Bianco & Nussenzweig, 1971; Pincus, Bianco & Nussenzweig, 1972). Moreover, in mice, B cells possess the heteroantigen(s) MBLA (mouse-specific B lymphocyte antigen (Raff, Nase & Mitchison, 1971)) while T cells have other antigenic markers, including the θ alloantigen (Thy-1) (Reif & Allen, 1964; Aoki *et al.*, 1969; Raff, 1971), the Ly alloantigens (Itakura *et al.*, 1972) and the heteroantigen(s) MSLA [mouse-specific lymphocyte antigen(s) (Shigeno *et al.*, 1968)]. In humans, the existence of antigenic markers specific for T lymphocytes has not yet been established, although it seems quite likely. Preparations of anti-human T-cell sera are still infrequent and the study of these antisera is only beginning. Such antisera would undoubtedly provide significant information about human T lymphocytes and their functions in normal as well as in pathological states. We describe herein our efforts to prepare a heterologous anti-human T cell serum, to evaluate its specificity and to use it as a reagent for studying immunodeficiency diseases and malignant haemopathies.

MATERIALS AND METHODS

Preparation of anti-human T-cell serum

Lymphocytes for animal immunization. Two adolescents with Bruton-type agammaglobulinaemia were the source of peripheral blood lymphocytes (PBL) for immunization of thirteen rabbits and one goat. In both cases, the diagnosis of X-linked agammaglobulinaemia (X-LA) was established in early childhood on the basis of very characteristic manifestations: early onset of infections with encapsulated bacterial pathogens; serum immunoglobulins almost absent; agammaglobulinaemia in their brothers; absence of plasma cells and germinal centres in lymphoid tissues; inability to produce demonstrable antibody responses to several antigenic stimulations. Studies with fluorescein-labelled anti-human Ig serum revealed a virtually absolute lack of blood lymphocytes with surface Ig.

Blood was drawn by venepuncture. Lymphocytes were separated on a Ficoll–Hypaque gradient (Thorsby & Bratlie, 1970), and resuspended in RPMI 1640 tissue culture medium. A portion of the cells was injected immediately, the other part being frozen in DMSO–glucose–MEM (Wood *et al.*, 1972) and stored for booster injections.

Immunization schedules. Four protocols of immunization with low doses of lymphocytes were tried.

Each of six rabbits was given one intravenous injection, three times, every other week, of $8{\cdot}5 \times 10^6$ lymphocytes from the first donor, without adjuvant. The animals were bled 1 week after the last injection.

Three more rabbits were given one subcutaneous injection three times, every other week, of $1{\cdot}4 \times 10^7$ lymphocytes from the same donor, emulsified with Freund's complete adjuvant (FCA), and were bled 1 week after the last injection.

One goat was injected intravenously with $4{\cdot}5 \times 10^7$ lymphocytes from the same donor, without adjuvant, boosted six times, every other week, with the same number of cells and bled 1 week after the last injection.

Four rabbits were injected intravenously with 9×10^6 lymphocytes from the second donor, without adjuvant, boosted five times by intravenous injections of the same number of cells, every other week, and bled 1 week after the last injection.

Absorption of antisera. After heat inactivation (56°C for 30 min) antisera were absorbed with human erythrocytes and, more importantly, with human B cells. Two sources of B cells were tried: a patient with chronic lymphatic leukaemia (CLL) and a lymphoblast cell line, from a normal donor, which has been in established culture for 2 years. More than 85% of the cells from cultures of this line were shown to possess a high density of surface Ig. Incubation with a variety of concentrations of cells was carried out. The anti-human T cell serum (ATCS) used for all further studies resulted from absorption of one batch of antiserum (a pool of serum from two of the six rabbits immunized according to the first protocol described) with 10^8 cells/ml, at 37°C, for 1 hr, followed by centrifugation to remove the cells. The absorption was repeated twice.

Two-stage microlymphocytotoxicity test

The antisera and especially ATCS were assayed for cytotoxicity with lymphocytes from various sources and donors, using a technique carried out in two stages as described by Amos *et al.* (1969).

Peripheral blood lymphocytes from patients or normal individuals were always separated on Ficoll–Hypaque gradients and resuspended in barbital buffer (Consolidate Laboratories Incorporated, Chicago Heights, Illinois).

Thymocytes were obtained from a piece of thymus kindly provided by Drs W. Lindsay and J. Foker, and removed from a 24-year-old patient undergoing cardiac surgery. The cells were separated by gentle disruption in Potter homogenizer followed by filtration through a steel mesh screen. To subject the thymocytes to the same preparation procedure as PBL, the cells were centrifuged on a Ficoll–Hypaque gradient before two incubations in Tris-buffered NH_4Cl (37°C for 10 min) with three washes in barbital buffer.

A suspension of 2×10^6 blood lymphocytes or thymocytes per ml was incubated with antiserum in the well of microtest tissue culture plate (Falcon Plastics 3034, Los Angeles California), then washed, and rabbit complement (C) (Grand Island Biological Company, Grand Island, New York) was added at the dilution of 1:2. All cell samples were studied with several dilutions of ATCS; every test was done in duplicate. The Trypan Blue exclusion method was used to evaluate the percentage of live and dead cells. Three hundred cells were counted in each well. The cytotoxic index was calculated as follows:

$$\text{cytotoxic index} = \frac{\text{percentage kill with ATCS and C} - \text{percentage kill with normal rabbit serum and C}}{100 - \text{percentage kill with normal rabbit serum and C}}$$

In some experiments a goat anti-human Ig serum was used and the cytotoxicity assay was performed with this antiserum in the same way as with ATCS. HL-A antigens on lymphocyte surface were determined with a similar two-stage microlymphocytotoxicity method, in Dr E. J. Yunis' laboratory, using forty antisera to identify twenty-one specificities.

Other techniques

Thymus-dependent rosette formation. Human rosette-forming cells (RFC) were quantified with a technique previously described (Kiszkiss, Choi & Good, 1974a), using untreated sheep erythrocytes, in the absence of serum. Much evidence has linked this 'non-immune' rosette formation to the T-cell population (Kiszkiss *et al.*, 1974b; Jondal, Holm & Wigzell, 1972; Wybran, Carr & Fudenberg, 1972; Kiszkiss *et al.*, 1973; Bach, 1973).

Anti-immunoglobulin-coated column. Separation of lymphocyte populations according to the density of surface Ig was achieved by filtration of cells through an anti-Ig coated column following the method of Wigzell, Sundqvist & Yoshida (1972): Degalan beads (Degalan V26, Degussa Wolfgang Ag., Hanau am Main, Germany) coated with pooled human γ-globulins plus excess goat anti-human Ig serum.

Immunofluorescent assays. Cell staining was performed using a double immunofluorescence technique according to a modification of methods applied by others (Pernis *et al.*, 1970; Lamelin *et al.*, 1972). 10 μl of fluorescein-labelled goat anti-human Ig serum (Meloy Laboratory Incorporated, Springfield, Virginia) absorbed with normal rabbit serum were added to a suspension of 1·8 to 2×10^6 cells in 0·1 ml Hanks' balanced salt solution (BSS) containing 5% bovine serum albumin (BSA) (Sigma Chemical Company, St. Louis, Missouri). After 30 min incubation at 0°C followed by three washings in Hanks' BSS with 5% BSA, the cells were resuspended in 0·1 ml of the same medium. The cell suspension was then incubated for 30 min at 0°C with 5 μl of ATCS diluted 1:3, washed 3 times and resuspended in 0·1 ml of medium. The third incubation (30 min at 0°C) of the cells was performed with 10 μl of rhodamine-conjugated goat anti-rabbit IgG serum (Cappel Laboratories Incorporated, Downingtown, Philadelphia).

After four washings in Hanks' BSS + 5% BSA, one drop of cell suspension was laid on a slide and a cover slip was sealed to the carrier slide with nail polish. The preparations were kept at 0°C until examined using a mercury lamp Zeiss photo microscope (Carl Zeiss Incorporated, New York). Each field was studied by phase contrast and by u.v. light, varying filters to obtain a selective visualization of either fluorescein or rhodamine.

Monocyte separation. For the separation of monocytes either of two techniques was used: (a) to remove monocytes, the cell suspension was filtered through a cotton column, which retains monocytes (Rocklin, Meyers & David, 1970); (b) to study monocytes, their glass adherence property was exploited. Cells were suspended in Eagle's minimum essential medium supplemented with L-glutamine (2 mM/ml), heat-inactivated AB serum (10%), penicillin G (50 μg/ml) and streptomycin (50 μg/ml). The cell suspension was incubated overnight in Petri dishes at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Then Petri dishes were repeatedly washed to remove the majority of lymphocytes.

RESULTS

Evaluation of the antilymphocyte sera before and after absorption

Among our antisera, the one prepared in rabbits with lymphocytes of the first donor, in absence of adjuvant and absorbed twice with cultured lymphoblasts (10^8 cells/ml), was the most extensively studied. This absorption was indeed sufficient to remove all the cytotoxic activity of this batch of antiserum for cultured lymphoblasts (Fig. 1). The serum thus prepared (ATCS) was still cytotoxic for a certain percentage of normal peripheral blood lymphocytes and this cytotoxic activity was not decreased by further absorption (Fig. 1).

The cytotoxic titres of the initial antiserum and of ATCS were determined with other sources of normal blood lymphocytes (Fig. 2). The cytotoxic activity of both antisera was relatively low, which is not surprising in view of the very low number of cells injected and of the rather short course of immunization. Yet a plateau of cytotoxic activity is outlined for dilutions of ATCS between 1:4 and 1:1.

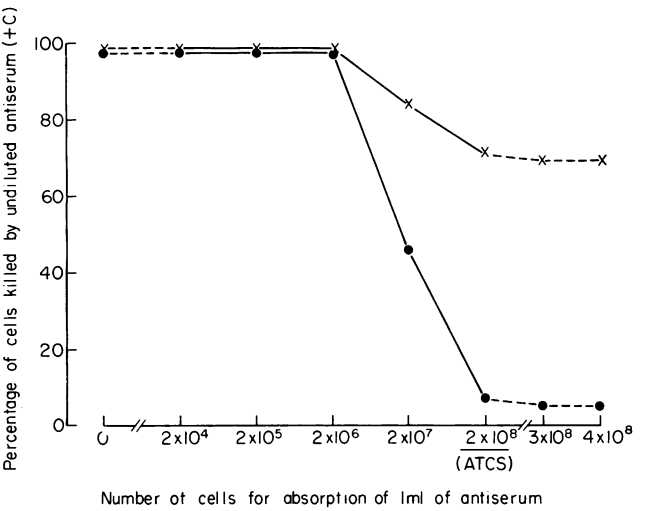

FIG. 1. Quantitative absorption of antiserum with cultured B lymphoblasts. Each absorption was performed at 37°C, for 1 hr. After the absorptions, undiluted antisera were assayed in cytotoxicity on (×) normal peripheral blood lymphocytes (PBL) and (●) cultured lymphoblasts, in the presence of rabbit complement (C). ATCS = anti-T cell serum.

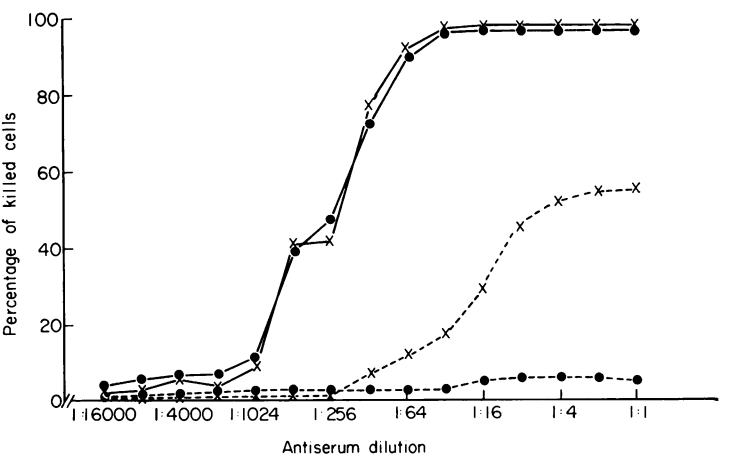

FIG. 2. Cytotoxic activity of antiserum before and after two absorptions with 10^8 lymphoblasts/ml. Activity of unabsorbed antiserum for (×—×) normal PBL and (●—●) lymphoblasts. Activity of ATCS for (× – – ×) normal PBL and (● – – ●) lymphoblasts.

When blood lymphoblasts from one patient with CLL were substituted for cultured lymphoblasts in an attempt at absorption, the initial antiserum kept its cytotoxic activity for virtually all normal blood lymphocytes, even after three sequential incubations with 10^8 cells/ml.

The other antilymphocyte sera produced were not so suitable for our studies: the antiserum raised with lymphocytes emulsified with FCA was surprisingly so weak that it could not kill more than 30% of normal blood lymphocytes in presence of complement, even when the serum was undiluted and unabsorbed (Fig. 3). To the contrary, the last two antisera outlined in the methods (one prepared in the goat, with lymphocytes of the same patient, the other in rabbit with lymphocytes from the second donor) were stronger and remained active on all normal blood lymphocytes after two absorptions with 10^8 cultured lymphoblasts/ml (Fig. 3). However, after four absorptions, the activity of the goat antiserum seemed to be comparable to that of ATCS.

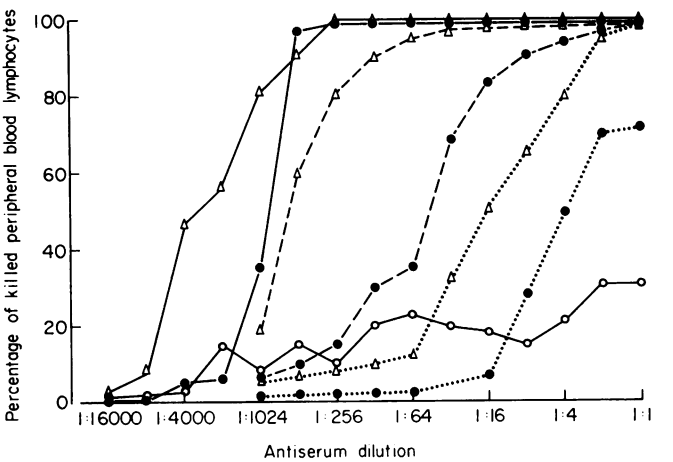

FIG. 3. Cytotoxic activity of other antilymphocyte antisera before and after absorption. ○—○ Rabbit antilymphocyte serum (FCA), unabsorbed. (●—●) Goat antilymphocyte serum unabsorbed, (● – – ●) absorbed twice with 10^8 lymphoblasts/ml and (● · · · · ●) absorbed four times with 10^8 lymphoblasts/ml. (△—△) Rabbit antilymphocyte (second donor) serum unabsorbed, (△ – – △) absorbed twice with 10^8 lymphoblasts/ml and (△ · · · · △) absorbed four times with 10^8 lymphoblasts/ml.

Hence, and to exclude any factor of variability due to the batches of antisera, all further studies with anti-T cell serum were performed using only the first batch described and all the results of cytotoxic assays presented below were obtained with this undiluted ATCS.

Specificity of ATCS

The specificity of ATCS for T vs B cells was attested by the following evidence. (a) After the absorption of antiserum with one B-cell line, virtually no cytotoxic activity remained against the lymphoblasts used for absorption or against six other cultured normal cell lines presumably of B type (see below). (b) This same exhaustive absorption with lymphoblasts has yet left a consistent cytotoxic activity for a certain percentage of normal PBL. At least a part of this activity was affecting T cells, as suggested by the strong inhibitory property of

ATCS for such T-cell functions as PHA and Con A stimulation or 'non-immune' rosette formation, with or without complement (Kiszkiss *et al.*, 1973a; Kiszkiss *et al.*, 1973b). (c) The filtration of PBL through an anti-Ig coated column, which retains specifically B lymphocytes with surface Ig (Wigzell *et al.*, 1972), increased very significantly the proportion of ATCS-sensitive cells (Table 1). (d) As mentioned below, the percentage of blood lymphocytes killed by ATCS in presence of complement was found to be high in patients with X-LA, who are known to lack B cells (Gajl-Peczalska *et al.*, 1973b). (e) The number of cells

TABLE 1. Enrichment in ATCS-sensitive cells and in RFC after passage through an anti-Ig-coated column

Cells	Experiment 1		Experiment 2	
	ATCS-sensitive cells	RFC	ATCS-sensitive cells	RFC
Peripheral blood lymphocytes	60%	43%	56%	41%
Peripheral blood lymphocytes passed through anti-Ig-coated column	90%	55%	97%	50%

TABLE 2. Additive cytotoxic effect of anti-T-cell serum and of an anti-human Ig (anti-γ+anti-κ+anti-λ) serum on normal peripheral blood lymphocytes

Cytotoxic assay with	Percentage of peripheral blood lymphocytes killed		
	Donor A	Donor B	Donor C
Anti-T-cell serum	63	59	56
Anti-Ig	15	18	20
Anti-Ig+anti-T-cell serum	80	78	65

TABLE 3. Immunofluorescent study of the specificity of anti-T-cell serum for non-Ig bearing lymphocytes

Cells	Peripheral blood lymphocytes
Rho positive* Fl negative cells	70·8%
Rho negative Fl positive cells†	19·1%
Rho positive Fl positive cells	1·7%
Rho negative Fl negative cells	8·4%

* Rho (rhodamine) positive cells are cells sensitive to anti-T cell serum.

† Fl (fluorescein) positive cells are cells bearing surface Ig.

Peripheral blood lymphocytes from one normal donor were stained with fluorescein-labelled anti-human Ig serum and rhodamine-labelled anti rabbit IgG serum (after incubation of cells with anti-T-cell serum).

sensitive to an anti-human Ig serum seemed to be grossly additive to the number of ATCS-sensitive cells (Table 2). (f) Using a double immunofluorescence technique with selective visualization of either fluorescein or rhodamine, it was shown that ATCS at a 1:3 dilution is labelling a different cell population than is an anti-Ig serum. The results of a typical experiment are summarized in Table 3. Less than 2% of normal PBL possessed both fluochromes and on each of these few double-stained cells, either fluorescein or rhodamine was highly predominent.

TABLE 4. Effect on cytotoxic index of elimination of B cells and monocytes or of absorption of anti-T-cell sera with monocytes

Cells	Cytotoxic index with antisera		
	ATCS†	Sp‡	Sd§
PBL*	0·56	0·27	0·28
PBL passed through anti-Ig-coated column	0·97	0·47	0·45
PBL passed through anti-Ig coated column, then cotton column	0·97	0·42	0·40

* PBL = peripheral blood lymphocytes.
† ATCS = anti-T-cell serum.
‡ Sp = ATCS absorbed on cells retained in a short cotton column.
§ Sd = ATCS passed through a column without cells and thus subjected to the same dilution as Sp but not to absorption with monocytes (control antiserum for Sp).

The specificity of ATCS for T cells vs other, non-B, mononuclear cells (mostly monocytes) was more difficult to analyse. (a) The cytotoxic activity of ATCS for cells adhering to Petri dishes has been found high but, besides monocytes, some lymphocytes are often difficult to remove during the washing procedures. Even if B cells seem to be slightly more adherent than T cells (Shortman *et al.*, 1971; N. Hogg cited in Raff, 1971), this glass adherence property would in fact be shared by a sub-class of cells from each of the two main lymphocyte populations (Shortman *et al.*, 1972). A part, at least, of cells sensitive to ATCS in this experiment could thus be T lymphocytes not removed by four successive washings. (b) When PBL, depleted in B cells by filtration through an anti-Ig-coated column, were passed through a cotton column, the percentage of ATCS-sensitive cells was not modified (Table 4) but this percentage was already so high (97%) after the first filtration that it could hardly be increased. This could indicate either the presence of antibodies against M cells in ATCS or the low concentration of such cells in the suspension studied, especially after filtration through the anti-Ig column. (c) The absorption of ATCS with the cells retained in a short cotton column led to a serum (Sp) whose cytotoxic activity was not decreased as compared to the serum (Sd) passed through a sham column without adherent cells (Table 4). (d) Finally, the percentage of ATCS-sensitive cells was usually somewhat higher than that of RFC (some examples are reported in Table 1). This might be explained either by some degree

TABLE 5. Absence of significant amount of anti-HL-A antibodies in anti-T-cell serum (ATCS)

Donor of peripheral blood lymphocytes	First segregant series								Second segregant series													Percentage of ATCS-sensitive PBL
	HL-A-1	HL-A-2	HL-A-3	HL-A-9	HL-A-10	HL-A-11	W-19	W-28	HL-A-5	HL-A-7	HL-A-8	HL-A-12	HL-A-13	W-5	W-10	W-14	W-15	W-17	W-18	W-22	W-27	
Donor for rabbit immunization (→ATCS)			+				+		+										?			
1			+				+		+										?			62
2		+	+									+			+							50
3	+						+					+									+	52
4				+			+			+		+										60
5				+					+												+	53
6	+								+								+					58
7	+	+													+							57
8		+										+	+									61
9	+			+						+		+										72

of anti-monocyte activity of the antiserum or more likely by the fact that cells forming rosettes with this technique are representing only a sub-population of T cells (Kiszkiss *et al.*, 1973a; Bach, 1973; Touraine *et al.*, 1973a).

In order to know if there were any significant anti-HL-A antibodies in ATCS, the HL-A typing of the donor used for the immunization of the rabbit was performed and the anti-T-cell serum was assayed with lymphocytes from individuals having various leucocyte typings. The number of ATCS-sensitive cells did not appear to be dependent on the number of HL-A specificities—0 to 3 or 4—shared with the donor used for immunization (Table 5). Furthermore, we shall see that this very donor had one of the lowest cytotoxic indices with ATCS among the patients with X-LA tested.

On the whole, the ATCS described above did not contain a significant amount of anti-human specificity antibodies (except those of low affinity), anti-HL-A antibodies, anti-Ig and anti-B cell antibodies. It did possess an anti-T lymphocyte activity and possibly a small anti-monocyte activity. It thus seems to be reasonably specific for T cells and may be used as a reagent for the study of this cell population.

TABLE 6. Cytotoxic index of anti-T-cell serum with cells from various sources

Normal peripheral blood lymphocytes	0·655 ± 0·087*
Cultured cell lines	
from normal donors	0·02, 0·02, 0·04, 0·05, 0·06, 0·14, 0·16†
from Burkitt lymphoma	0·00
Thymocytes	0·97
Peripheral blood lymphocytes (from the same patient as thymocytes)	0·50
Bone marrow lymphocytes	0·04

* Mean ± SD (twenty-five normal subjects).
† Individual values.

ATCS-sensitivity of lymphocytes from various sources

Lymphoblast cells from eight different cultured lines (seven normal and one Burkitt lymphoma cell lines) were assayed with ATCS and found to be essentially resistant to this antiserum: cytotoxic index between 0·0 and 0·16 (Table 6). The normal cell lines were most probably of B type since the majority of cells had a high density of surface Ig.

Among PBL from twenty-five normal subjects, an average of 65·5% (range 49–78) was found to be sensitive to ATCS (Table 6). The number of individuals so far tested is too small to allow conclusions regarding the influence of age (Sabolović, Sabolović & Dumont, 1972) on this percentage of T cells.

Less than 5% of bone marrow cells were sensitive to ATCS (Table 6). The thymocytes from one young adult were also analysed: 97% of them were killed by ATCS in the presence of complement, contrasting with 50% of cells killed among PBL from the same subject (Table 6).

The comparative absorption capacity of thymocytes and PBL from this individual was studied. As shown on Fig. 4, a higher concentration of peripheral lymphocytes than of

thymocytes was needed for the absorption of the anti-T-cell activity of ATCS. Although our results are not perfectly suitable for an accurate quantitative evaluation, it can be noted that a tenfold concentration separates the two otherwise similar curves. As ATCS-sensitive cells accounted for half the PBL from this subject and almost all the thymocytes, it might be concluded that the absorptive capacity of thymocytes was approximately five times greater than that of peripheral T lymphocytes.

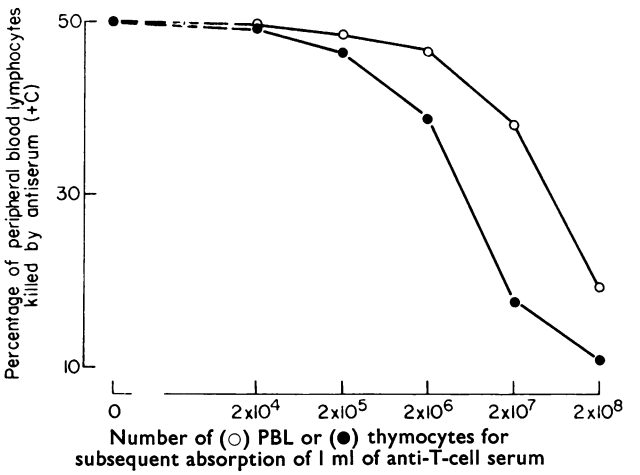

FIG. 4. Comparative capacity of peripheral blood lymphocytes and thymocytes (from the same donor) to absorb the cytotoxic activity of the anti-T cell serum (previously absorbed with lymphoblasts). (○) PBL = peripheral blood lymphocytes. (●) Thymocytes.

ATCS-sensitive PBL in immunodeficiencies and haemopathies

Fig. 5 shows the results obtained in some patients by the cytotoxic assay with ATCS.

PBL from four patients with X-LA were studied and, in each case, a high percentage of these cells was found to be sensitive to ATCS: 89%, 88%, 75%, and in the donor used for immunization, 77%.

In common variable hypogammaglobulinaemia (CVH) the proportion of T cells, as evaluated with ATCS, was very different from one patient to another (range 42–93%).

Two cases of ataxia–telangiectasia (A-T) were studied. In these patients, the deficiency of cell-mediated immunity seemed very mild and the number of T cells was not decreased. On the other hand, a somewhat low number of T cells was present in a case of benign thymoma with myasthenia gravis (thymectomy) and systemic lupus erythematosus as well as in a 9-year-old male patient whose clinical and laboratory syndrome, although difficult to classify, included a deficiency of cellular immunity and a low PHA response of lymphocytes *in vitro*.

The number of T cells was below the normal range in five out of thirty-six patients with leprosy. These decreased figures were observed among the twenty cases of active lepromatous leprosy. Moreover, the mean percentage of T cells in the whole group of patients with this form of the disease is significantly lower than the normal mean ($P<0{\cdot}01$), in contrast to the mean T cell number in inactive lepromatous leprosy, borderline or indeterminate

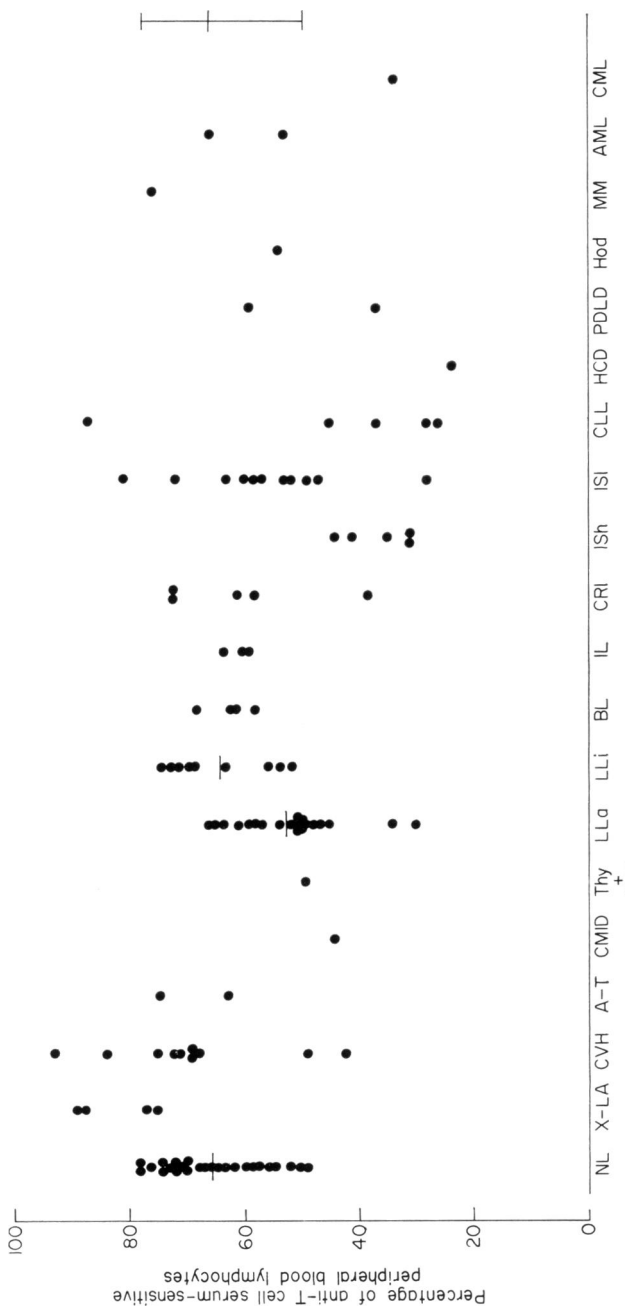

FIG. 5. Percentages of peripheral blood T-cells (anti-T-cell serum-sensitive lymphocytes) in immunodeficiencies and haemopathies. NL = normal subjects. X-LA = X-linked agammaglobulinaemia. CVH = common variable hypogammaglobulinaemia. A-T = ataxia-telangiectasia. CMID = cell-mediated immunodeficiency of undefined origin. Thy + SLE = thymoma, thymectomy and systemic lupus erythematosus. LLa = active lepromatous leprosy. LLi = inactive lepromatous leprosy. BL = borderline leprosy. IL = indeterminate leprosy. CRI = chronic renal insufficiency. ISh = high doses of immunosuppressive agents. ISl = low doses of immunosuppressive drugs. CLL = chronic lymphatic leukaemia. HCD = heavy chain disease. PDLP = poorly differentiated lymphocytic diffuse lymphoma. Hod = Hodgkin's disease. MM = multiple myeloma. AML = acute myelogenous leukaemia. CML = chronic myelogenous leukaemia.

leprosy (Lim *et al.*, 1973). In one patient with an initial cytotoxic index of 0·50, a slight increase was observed after leucocyte infusion therapy.

In patients undergoing renal transplantation, the following results were obtained. (a) Nearly normal figures for ATCS-positive lymphocytes before treatment, in spite of chronic renal insufficiency. (b) Consistently low percentage of T cells and even lower absolute number (because of lymphopenia) at the time of heavy immunosuppressive therapy including high doses of intravenous antilymphocyte globulin (ALG). (c) Almost normal cytotoxic index a few weeks after the level of immunosuppression was reduced and ALG stopped.

Among the patients with malignant haematological disorders that we studied, those with a CLL gave us the most interesting results (Fig. 5). The five patients were seen before treatment and at a time when lymphocyte proliferation was moderate ($<100{,}000$ white cells/mm^3). In four of them, the percentage of ATCS-sensitive cells among PBL was significantly decreased, but the absolute number was in the normal range or slightly increased in all four cases. In the fifth patient, a consistently high percentage of ATCS-sensitive cells was observed and this unusual result will be discussed below.

DISCUSSION

The present studies show the feasibility of preparing heterologous antiserum to human T lymphocytes by proper immunization and absorption. As suggested by Gozzo, Wood & Monaco (1971) immunization of rabbits with low doses of lymphocytes leads to antilymphocyte antisera with moderately high cytotoxic titres. In our restricted experience, cultured B lymphoblasts were better than CLL cells for absorption of species-specific antibodies. Both sources of B cells could be criticized on the ground that the absorbing cells may lack several antigenic determinants other than those characteristic of the T-cell specificity and that are present on the immunizing cells. HL-A antigens and a variety of other surface antigens might be lost in cultured cells as well as in leukaemic cells.

The more extensively studied antiserum (ATCS) was convincingly shown to be specific for T lymphocytes and its very existence testifies to the presence of antigenic markers on the surface of human T cells. This is reinforced by a few recent reports of other heterologous anti-human T-cell sera prepared by different protocols (Aiuti & Wigzell, 1973a; Williams *et al.*, 1973; Smith *et al.*, 1973; Aisenberg *et al.*, 1973; Ablin & Morris, 1973; Wortis, Cooper & Brown, 1973). It has also been suggested that, in some infectious mononucleosis sera, there are antibodies reacting with a subpopulation of T cells (Thomas, 1972).

Because of similarity with the antigens described in the mouse (Raff *et al.*, 1971; Reif & Allen, 1964; Aoki *et al.*, 1969; Raff, 1971; Itakura *et al.*, 1972; Shigeno *et al.*, 1968) we proposed to name this anti-T-cell specificity human-specific T lymphocyte antigen(s) (HTLA). As suggested by the comparative absorption studies of ATCS with PBL and thymocytes, the density of HTLA would be higher on the surface of thymocytes than on peripheral T lymphocytes. Thus, as far as the membrane constitution is concerned, the post-thymic T cells seem to have undergone significant alterations with, for instance, an increased density of histocompatibility antigens (HL-A) contrasting with a decreased representation of differentiation antigens (HTLA).

The identification of peripheral T lymphocytes with ATCS in normal subjects and in a variety of conditions was significantly extended since our preliminary report (Touraine *et al.*,

1973b). The percentage of normal PBL sensitive to ATCS (49–78%) is an agreement with the results of other methods for evaluation of T cell number. For instance the percentage of RFC in normal human peripheral blood has been found to be as high as 52–81% by some authors (Jondal *et al.*, 1972). Also cell electrophoretic mobility of PBL from young adults indicated 65% of presumed T cells (Sabolović *et al.*, 1972). In the CBA strain of mice, 60–85% of PBL have been shown to be sensitive to anti-*0* serum (with a possible overestimation as lymphocytes were separated on glass–wool column which could eliminate a few B cells in addition to the monocytes) (Raff, 1971).

In X-LA the high percentage of T cells is in good correlation with the virtually total absence of B cells. It is not clear whether the 10–20% cells unsensitive to ATCS and not bearing Ig are B cells without surface immunoglobulin, T cells with a low concentration of HTLA or cells belonging neither to the B nor to the T lineage. Contrary to other authors (Aiuti & Wigzell, 1973b), we have found that the incidence of T cells in CVH is very different from one patient to another. The number of peripheral lymphocytes bearing surface Ig was very low in some of our patients with CVH, and normal—even occasionally high—in others (results not shown). The complementary variation of B and T cells was not only seen from one individual to another but also in the same patient at various times. The finding in some patients with CVH of a virtual absence of B cells and an increased number of T cells, as in X-LA, suggests that the pathogenesis of CVH is not, in every case, an inadequate differentiation of B cells into plasma cells, as proposed when an almost normal number of surface Ig-bearing lymphocytes has been found in a few cases studied (Cooper, Lawton & Bockman, 1971; Choi, Biggar & Good, 1972).

In patients with active lepromatous leprosy, the deficiency of cell-mediated immunity is well established (Bullock, 1968; Turk & Waters, 1969; Han, Weiser & Kau, 1971) and could be due to a reduction of the T-cell population (Turk & Waters, 1968), to an inadequate functional capacity of T cells (Talwar *et al.*, 1972) and/or to humoral inhibitory factors (Bullock & Fasal, 1971). Our studies establish that the percentage and the absolute number of peripheral T cells are significantly lower in this form of the disease than in other forms or in normal controls (Lim *et al.*, 1973). This is consistent with the increased number of surface Ig-bearing lymphocytes in these patients (Gajl-Peczalska *et al.*, 1973a). However, although statistically significant, the decrease in T cell number is not a major one and it may be only one of several factors responsible for the deficiency of cell-mediated immunity in active lepromatous leprosy. Furthermore, it is still debatable whether this deficit in T lymphocytes is primary or secondary in nature.

In renal insufficiency, the deficiency of cellular immunity has been shown to depend slightly on lymphopenia and mostly on humoral factors (Touraine *et al.*, 1970). This is in good correlation with the present finding of an almost normal proportion of T cells among PBL from uraemic patients. When these patients were receiving high doses of immunosuppressive agents, including ALG, for a renal transplant, a lymphopenia was noted, at least at the beginning, and the percentage of T cells was decreased. These results could indeed be expected since ALG is known to deplete mainly the circulating T-cell population (Martin & Miller, 1968). Furthermore, it has been shown that most blood lymphocytes from patients treated with horse ALG are neither agglutinable by an anti-horse globulin serum nor susceptible to binding of diluted antilymphocyte antibodies (Antoine *et al.*, 1971), which supports the hypothesis of the elimination from the blood of ALG-bound and ALG-sensitive cells (Touraine & Touraine, 1973). However, the proportion of ATCS-sensitive

cells returned to a normal level shortly after the immunosuppression was reduced and ALG stopped, suggesting that the production of significant numbers of cells belonging to the T lineage could be more rapid than is usually expected. This finding suggests that there is a subpopulation of T cells with a short life span and a rapid turnover.

In four out of five patients with chronic lymphatic leukaemia a relatively low percentage of PBL was sensitive to ATCS. This finding is in agreement with the observations suggesting that CLL cells are—most often or always—of a B cell type since they usually bear surface Ig and/or complement receptor sites (Wilson & Nossal, 1971; Pernis *et al.*, 1971; Preud'homme & Seligmann, 1972; Ross *et al.*, 1973). However, this proliferation of CLL B cells produces a dilution of T cells but not a reduction of the population of ATCS-sensitive cells. Our finding in this regard confirms the observation by Wybran *et al.* (1973) of a normally responsive T-cell population in such patients. In one other patient, a consistently high percentage of ATCS-sensitive cells was observed, contrasting with a low number of rosette-forming cells. The evaluation of surface Ig-bearing cells was difficult because of an especially weak reaction with fluorescein-labelled anti-Ig serum, whence variation in results on repeated testings. Rather than postulate that this case is a T cell chronic leukaemia (Piessens *et al.*, 1973; Dickler *et al.*, 1973), it could be understood as a CLL with B cells especially susceptible to antibodies which might contaminate our ATCS at such small concentrations that they are undetectable in cytotoxicity tests against normal lymphocytes. Such false reactions with leukaemic cells have already been observed when using anti-HL-A antisera which were thought to be monospecific (Ohayon *et al.*, 1972). Further studies of our case could show by absorption procedures that the leukaemic cells of this patient did not contain all surface antigens of either normal B cells or T cells.

In conclusion, the study of heterologous antisera to T lymphocytes establishes the existence of antigenic markers specific to the T-cell population in human as in other species. Such an anti-human T-cell serum is a useful tool for studying blood lymphocytes from patients with immunodeficiency states or haematological disorders and enables analyses of which of the cell-mediated immunodeficiencies are dependent on a reduction in T cell numbers. This antiserum may also prove to be useful in therapy (for instance, when trying to prevent or overcome GVHR in bone marrow allografting) with a similar but more specific mode of action than has 'conventional' ALG.

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