Clin. exp. Immunol. (1973) **15**, 507–516.

ANTIGEN DIFFERENCES, DETECTED BY INDIRECT IMMUNOFLUORESCENCE, BETWEEN HUMAN PERIPHERAL AND THORACIC DUCT LYMPHOCYTES, AND HUMAN CULTURED LYMPHOBLASTS

D. THOMAS AND D. C. EDWARDS

Department of Biological Chemistry, Wellcome Research Laboratories, Beckenham, Kent

(*Received* 13 *June* 1973)

SUMMARY

Rabbits were immunized with peripheral cells, thoracic duct cells and various lymphoblast cell lines.

Bleedings were taken approximately 4 weeks after each injection corresponding to the primary and secondary immune response. The immunofluorescence (IMF) titre of each serum was estimated using the specific antigen cell and the cells used to raise the other sera. In general the titres were higher when the specific antigen cells were used. In absorption experiments it was found that anti-peripheral cell sera and anti-thoracic duct cell sera were readily absorbed by the specific or non-specific cells, but the anti-lymphoblast sera, although readily absorbed by the specific or other lymphoblast cells, could not be completely absorbed with peripheral and thoracic duct cells.

The IMF test was, by minor modifications, adapted to allow preliminary studies to be made of the desorption of anti-human lymphocyte globulin from the lymphocytes and it is concluded from these investigations that, while peripheral cells, thoracic duct cells and cultured human lymphoblasts may contain similar and/or identical antigens, they also express similar but not identical antigens, while cultured lymphoblasts in addition possess antigens absent from peripheral and thoracic duct lymphocytes.

INTRODUCTION

A wide variety of lymphoid cells (Carraz *et al.*, 1967; Najarian *et al.*, 1969; Seiler *et al.*, 1970; Dormont *et al.*, 1970; Woiwod *et al.*, 1970; Barnes *et al.*, 1972a), particulate fractions of lymphoid cells (Lance *et al.*, 1968; Zola *et al.*, 1971) and soluble fractions of such cells (Zola *et al.*, 1970) have been used in the large scale production of anti-human lymphocyte serum (AHLS) and anti-human lymphocyte globulin (AHLG).

One of the major factors limiting the use of AHLG in clinical work has been the difficulty

Correspondence: Mr D. Thomas, Department of Biological Chemistry, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS.

in obtaining sufficient antigen at a convenient time and in adequate quantity and purity for the immunization of large groups of animals. The human cultured lymphoblast has much to offer in these respects and has been used in several studies (Najarian *et al.*, 1969; Barnes *et al.*, 1972b; Seiler *et al.*, 1972; Guthöhrlein *et al.*, 1972; Groth *et al.*, 1972). In our own work the ready availability of such cells has proved very advantageous in that a more standardized antigen is available to immunize groups of animals, thus leading to a more uniform end product.

It is well known that malignant transformation of the mammalian cell is accompanied by a number of modifications of the cell surface; usually these modifications lead to the appearance of new antigens (Baldwin, 1970; Prehn, 1965, 1967). Lymphoblast cell lines, obtained from continuous prolonged culture of normal lymphoid cells, although remaining diploid are none the less pleomorphic and caution in the use of these materials has been advised (Perper *et al.*, 1970). Many of these cell lines contain viruses and in addition antigenic modification may occur on prolonged culture, certain antigenic specificities being lost while others may be acquired (Dumonde, 1966). By preparing antiserum to peripheral blood lymphocytes, thoracic duct cells and cultured human lymphoblasts and investigating their cross-reactivity and affinity for each other, it was felt that data relevant to the question of the antigenic differences between lymphoblasts and lymphocytes from various sources could be obtained, as well as information on the relative usefulness of cultured cells in the large scale preparation of AHLS and AHLG for clinical use.

The immunfluorescence test (IMF) (Thomas *et al.*, 1971; 1972) shown to be highly predictive of immunosuppressive potency of anti-mouse lymphocyte serum (AMLS) and anti-mouse lymphocyte globulins (AMLG) (Edwards *et al.*, 1972) and to be as good as any other *in vitro* test for AHLS and AHLG, was the method of choice for the investigatory study of cross-reactivity and antigenic differences. Further, by minor modification, it could be adapted to allow a preliminary study to be made of the desorption of AHLG from the lymphocytes and thus the 'affinity' of antiserum for antigen.

MATERIALS AND METHODS

Isolation, preparation and storage of lymphocytes

Human peripheral cells. Small lymphocytes were obtained from normal healthy donors by the following procedure. 250 ml of blood were collected from the donor into acid/citrate/dextrose (ACD) and the buffy coat obtained by centrifugation. The buffy coat was defibrinated manually as described by Wilson & Grimes (1968) and the erythrocytes sedimented by treating the resultant cell suspension with Dextran/EDTA/heparin mixture for 1 hr. Full details of these techniques are given elsewhere (Woiwod *et al.*, 1970).

The lymphocytes were isolated from the leucocyte-rich supernatant by the method of Thomson *et al.* (1966), which involved the removal of polymorphonuclear leucocytes by filtration through a column of polystyrene beads. The cell suspensions consisted of 98% small lymphocytes, of which 95% were viable (Trypan Blue dye exclusion). No platelets were visible in any of the preparations. The yield of lymphocytes ranged between 2×10^7 and 4×10^7 cells from the 250 ml of blood processed.

Cultured human lymphoblasts. Five human cultured lymphoblast lines were used in this study—three originating from Biomedic Associates, U.S.A. (1788, 4098 and 7249) and two from the Wellcome Research Laboratories (7 and 50). The cells were established and main-

tained in tissue culture by Dr A. Whitaker, of these laboratories, and supplied to us in quantity as required.

Human thoracic duct cells. Thoracic duct cells were obtained from patients awaiting renal transplantation. The cells were harvested by centrifugation and washed exhaustively in medium 199.

Storage of cells. All peripheral lymphocytes, cultured lymphoblasts and thoracic duct cells not required immediately were stored at −196°C in liquid nitrogen after equilibration with dimethyl sulphoxide and cooling at a controlled rate (Symes *et al.*, 1966).

Rabbit immunization. In all the immunization schedules described here, the freshly prepared cells or those recovered from storage in liquid nitrogen, were washed exhaustively in medium 199. All subcutaneous (s.c.) and intramuscular (i.m.) injections were administered in a volume not exceeding 1·0 ml.

A total of seventeen Californian rabbits, weighing approximately 2·5 kg, were injected with the lymphoid cells according to the schedules set out in Table 1. The rabbits immunized with human peripheral cells were injected on day 0 and day 98 with between 5 and $7{\cdot}5 \times 10^6$ cells, whilst those receiving cultured cells (1788, 4098 and 7249) were injected on day 0 and 56 with 1×10^6 cells. The rabbits immunized with cultured cells (7 and 50) and thoracic duct cells, were injected on day 0 and 63 with 1×10^6 cells. The cell dose for each rabbit was made up in 0·5 ml of medium 199, an equal volume of Freund's complete adjuvant (FCA) added and the mixture emulsified.

Blood was removed from the marginal ear vein of each rabbit 4 weeks after each injection, corresponding to the primary and secondary response. The blood was allowed to clot at room temperature, the clear serum obtained by centrifugation and inactivated at 56°C for 1 hr. The antisera thus obtained were stored at −30°C until required.

Immunofluorescence test

Immunofluorescence titres (IMF) of all antisera were determined against lymphoid cells by the method described by Thomas *et al.* (1971, 1972). The IMF activity of the primary and secondary response of each serum was measured by employing the specific cell used as antigen as well as the other cell types used to produce the other antisera (Tables 2 and 3).

In absorption experiments (Table 4), three samples were selected, corresponding to the secondary immune response of each of the antigens used. Three absorptions were carried out on each antiserum using the specific cell type and the other cell types used in this study. The IMF titres were measured, before and after each absorption, by employing the specific cell, used as antigen, as well as the other cell types used to produce the other antisera.

Absorption technique

The cells used for the absorption were recovered from storage in liquid nitrogen and washed exhaustively in saline (0·85% w/v). The cells were packed by centrifugation (1000 *g* for 15 min), the supernatant removed by decantation, and 50 μl (equivalent to $2{\cdot}5 \times 10^7$ cells) was added to 50 μl of each of the antisera under test. The cells and antisera were allowed to react for 1 hr at room temperature (RT), after which the cells were removed by centrifugation (1000 *g* for 15 min). Two additional absorptions were carried out; one at +4°C for 18 hr and the second at RT using similar quantities of cells and antisera as described.

The antisera were tested for IMF activity, against the specific and other cells used to raise the antisera before and after the absorptions.

Desorption studies

The IMF test, used in the assessment of AHLS activity, is based upon the fact that the antibody reacts with, and binds to, the antigen. It seemed possible, therefore, that by using this basic technique in a modified form it might be possible to study the rate of desorption of antibody from the specific as well as the other antigens used in this study. Four sets of slides were prepared from each of the cell types, as described by Thomas *et al.* (1971, 1972).

Three individual dilutions of the peripheral cell AHLS were prepared such that the IMF activity, when tested against the various cell types, gave a titre in all cases of 1/16. Serial dilutions were then prepared in phosphate-buffered saline (PBS) of these dilutions and each set of slides was treated for 15 min with the appropriate set of AHLS dilutions. After this, the AHLS was removed and the washing of the sets of slides commenced in PBS. At intervals over a period of 120 min, a set of slides was removed and the bound antibody for each dilution was determined by treating the antigen–antibody with fluorescein isothiocyanate antiglobulin (FITC) (Wellcome Reagents Ltd., Beckenham, Kent, U.K.). The conjugate was allowed to react for 15 min, followed by exhaustive washing in PBS. The slides were mounted in non-fluorescent oil and viewed for the uptake of fluorescence (Wild M-20 microscope, Wild-Heerbrug, Switzerland). The end point was taken as the highest dilution which gave fluorescent staining (Thomas *et al.*, 1972). Thus, the desorption of the peripheral cell AHLS antibody from the various antigens was determined.

Cultured human lymphoblast AHLS and thoracic duct cell AHLS were studied in exactly the same manner using the same cell types, and similarly the desorption of antibody from the various antigens was determined.

RESULTS

The results presented in Tables 2 and 3, in general, show that when the specific cell type was used as the target cell in the IMF test, the titres were higher than when the IMF titres were determined with the other cell types. Antisera to peripheral cells and thoracic duct cells

TABLE 1. Rabbit anti-human lymphocyte serum (immunization schedules)

Rabbit number	Day	Dose administered* number of cells	Source of antigen
1 and 2	0 and 98	$6{\cdot}0 \times 10^6$	Human peripheral cells, donor A
3 and 4	0 and 98	$7{\cdot}5 \times 10^6$	Human peripheral cells, donor B
5 and 6	0 and 98	$5{\cdot}0 \times 10^6$	Human peripheral cells, donor C
7 and 8	0 and 56	$1{\cdot}0 \times 10^6$	Cultured cells 1788
9 and 10	0 and 56	$1{\cdot}0 \times 10^6$	Cultured cells 4098
11 and 12	0 and 56	$1{\cdot}0 \times 10^6$	Cultured cells 7249
13	0 and 63	$1{\cdot}0 \times 10^6$	Cultured cells 7
14 and 15	0 and 63	$1{\cdot}0 \times 10^6$	Cultured cells 50
16 and 17	0 and 63	$1{\cdot}0 \times 10^6$	Human thoracic duct cells

* Cells given subcutaneously (s.c.) on day 0+FCA and intramuscularly (i.m.) on days 98, 56 or 63+FCA.

N.B. The bleedings studied for IMF activity were taken approximately 4 weeks after each injection, corresponding to the primary and secondary immune response.

TABLE 2. Rabbit anti-human lymphocyte serum. The results of *in vitro* IMF* activity of serum samples from primary and secondary immune response

Antigen	Rabbit number	Primary response				Secondary response			
		Target cell				Target cell			
		Peripheral cells	1788	4098	7249	Peripheral cells	1788	4098	7249
Peripheral cells									
Donor A	1	64	4	2	4	32	8	2	8
	2	32	4	2	4	16	8	2	2
Donor B	3	32	<2	2	4	64	4	2	4
	4	64	<2	2	4	64	4	2	2
Donor C	5	32	4	<2	<2	16	4	2	2
	6	64	4	<2	<2	32	4	<2	2
Human lymphoblasts									
1788	7	32	>512	8	8	128	<512	64	128
	8	16	256	16	32	64	128	64	128
4098	9	8	256	64	64	32	256	256	64
	10	32	256	64	64	16	128	128	64
7249	11	16	128	16	256	32	128	64	128
	12	8	128	32	128	16	64	64	>512

* Immunofluorescence titres expressed as reciprocals of dilutions tested against the various cell types (peripheral cells, lymphoblasts 1788, 4098 and 7249).

TABLE 3. Rabbit anti-human lymphocyte serum. The results of *in vitro* IMF* activity of serum samples from primary and secondary immune responses

Antigen	Rabbit number	Primary response				Secondary response			
		Target cell				Target cell			
		Peripheral cells	7	50	TD†	Peripheral cells	7	50	TD†
Human lymphoblasts									
7	13	8	32	32	4	32	64	48	4
50	14	12	128	128	4	32	128	128	16
	15	8	8	32	<2	64	96	128	16
Thoracic duct cells	16	16	2	2	32	64	8	8	64
	17	16	4	2	128	128	16	16	128

* Immunofluorescence titres expressed as reciprocals of dilutions tested against the various cell types (peripheral cells, lymphoblasts 7 and 50, thoracic duct cells).

† Thoracic duct cells.

TABLE 4. Rabbit anti-human lymphocyte serum. Results of *in vitro* IMF activity* of representative antisera absorbed† (×3) and tested against the various cell types

		Absorbed with PBL‡ IMF activity against							Absorbed with TDL§ IMF activity against							Absorbed with lymphoblasts IMF activity against						
Antigen	Rabbit number	PBL	1788	4098	7249	7	50	TDL	PBL	1788	4098	7249	7	50	TDL	PBL	1788	4098	7249	7	50	TDL
Peripheral cell AHLS	1 Secondary immune response	<2	<2	<2	<2	<2	NT	<2	<2	<2	<2	<2	<2	NT	<2	<2	<2	<2	<2	<2	NT	<2
Cultured lymphoblast AHLS	7 Secondary immune response	<2	12	6	6	4	NT	<2	<2	24	6	6	6	NT	<2	<2	<2	<2	<2	<2	NT	<2
Thoracic duct cell AHLS	16 Secondary immune response	<2	<2	<2	<2	<2	NT	<2	<2	<2	<2	<2	<2	NT	<2	<2	<2	<2	<2	<2	NT	<2

* Immunofluorescence titres expressed as reciprocals of dilutions when tested against the various cell types indicated.

† Absorptions carried out using equal volumes of the packed cells indicated and AHLS: (1) at RT for 1 hr followed by (2) 4°C for 18 hr and (3) 1 hr at RT.

‡ Peripheral blood lymphocytes.

§ Thoracic duct lymphocytes.

NT, not tested.

cross-reacted only very slightly with lymphoblasts. On the other hand, thoracic duct cell antisera reacted well with human peripheral cells, while moderate reactions were found when antisera to lymphoblasts were cross-reacted with peripheral and thoracic duct cells.

In absorption experiments, however (see Table 4), it was found that the IMF activity of thoracic duct cell AHLS and peripheral cell AHLS could be completely absorbed with cultured lymphoblasts as well as with thoracic duct cells and peripheral cells. By contrast the IMF activity of cultured cell AHLS, which was readily absorbed with the specific cultured lymphoblast, was not fully absorbed with thoracic duct cells or peripheral cells, even though three absorption stages were always included.

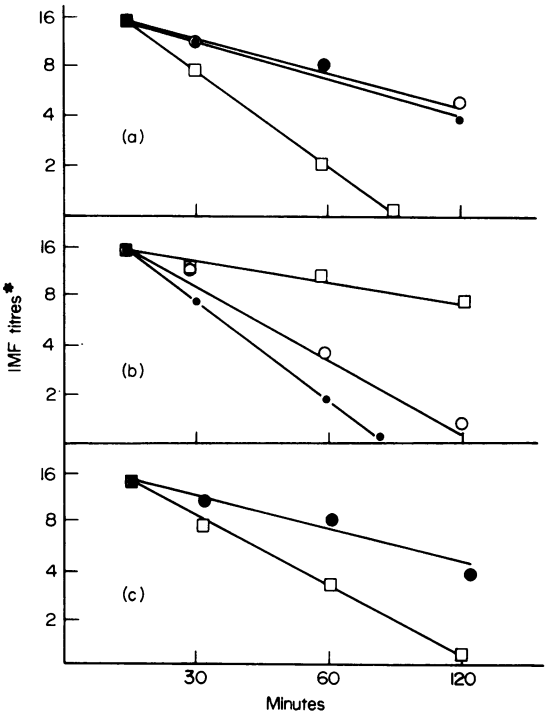

FIG. 1. Rates of desorption of *in vitro* IMF activity from the various cell types used as antigens. (a) Peripheral cell AHLS. (b) Cultured lymphoblast AHLS. (c) Thoracic duct cell AHLS. Each tested against peripheral cells (○), thoracic duct cells (●) and cultured lymphoblasts (□).

* IMF titres expressed as reciprocals of dilutions.

These results demonstrated the degree of cross-reactivity of rabbit antisera to peripheral cells, thoracic duct cells and human cultured lymphoblasts, and clearly showed that the cell types were similar in antigenic composition, that is to say all three expressed at least a number of similar or identical antigens on their surfaces. The results of the absorption experiments, in addition, suggested that cultured human lymphoblasts express on their surface an additional antigen or antigens not present on peripheral or thoracic duct cells.

It should be mentioned here that a very limited number of antisera prepared against cell lines 7 and 50 and thoracic duct cells were investigated for their cross-reactivity against the

other lymphoblast lines (1788, 4098 and 7249) and antisera to lymphoblast cell lines 1788, 4098 and 7249 were similarly investigated against lymphoblast cell lines 7 and 50, as well as thoracic duct cells. The results were in general agreement with those already described.

In order to investigate more fully the question of similarity and identity of the common antigen(s), the experiments shown in Fig. 1 were carried out. In these experiments the rate of desorption of bound antibody was measured in three representative antisera.

The antisera selected were (1) a peripheral cell AHLS, (2) a cultured lymphoblast AHLS and (3) a thoracic duct cell AHLS, and the desorption of each AHLS was determined using the specific cell type and the other two cell types as described earlier (see Materials and Methods). Thoracic duct cell AHLS desorbed slowly from thoracic duct cells and from peripheral cells, whilst it desorbed rapidly from cultured lymphoblasts. Conversely, cultured lymphoblast AHLS desorbed slowly from cultured cells but rapidly from both thoracic duct cells and peripheral cells. Peripheral cell AHLS was similar to thoracic duct cell AHLS in that the antiserum had bound more firmly to thoracic duct cells and peripheral cells and poorly to cultured lymphoblasts. It follows, therefore, that the cultured lymphoblast AHLS had a low affinity or binding capacity for both the peripheral cell and thoracic duct cell. The peripheral cell AHLS and the thoracic duct cell AHLS, on the other hand, had a high affinity or binding capacity for both the peripheral and thoracic duct cell but a low affinity for the lymphoblast, and suggested to us that the cross-reacting antigen expressed by the lymphoblast was not identical with the antigen expressed by the other normal lymphoid cells.

DISCUSSION

Since AHLS and/or AHLG presumably act by binding to the lymphocyte (Lance, 1970) and perhaps more specifically to the long-lived thymus processed recirculating lymphocyte or T cell, it seemed reasonable to postulate that a measure of the binding of AHLS to peripheral cells would provide an idea of the *in vivo* efficacy of a given AHLS. The IMF test used throughout this study has been shown to correlate particularly well with immunosuppression in the mouse system (Thomas *et al.*, 1971, 1972; Edwards *et al.*, 1972) where the titres are determined against a specific cell type, namely the thymocyte.

As might be expected, the data provided by the present study indicate that each antiserum was found to exhibit its maximum binding as indicated by IMF activity when the IMF was determined using the specific cell type to which the antibody was raised. However, the degree of cross-reaction found suggested that peripheral lymphocytes, thoracic duct lymphocytes and cultured lymphoblasts express on their cell surface some similar or identical antigens. This was confirmed by the absorption studies in which the IMF activity of peripheral cell AHLS and thoracic duct AHLS was found to be completely absorbed by absorptions with either peripheral cells, thoracic duct cells or lymphoblasts. The IMF activity of cultured lymphoblast AHLS, although markedly reduced by such absorptions with peripheral cells and thoracic duct cells, could not be removed completely with subsequent absorptions. This activity, however, could be removed completely when absorbed with either the specific lymphoblast used in raising the antiserum or by other lymphoblast cell lines, suggesting that the lymphoblast, in addition to expressing similar and/or identical antigen(s), also expresses antigens not found on either peripheral or thoracic duct lymphocytes.

In addition, it was thought that cultured lymphoblasts, as well as expressing specific antigens, might well also express shared antigens that were not completely identical to those

expressed by the peripheral and thoracic duct lymphocyte. If the assumption was correct, this would give rise to variations in the affinity or binding capacity of the antisera to the various cell types, and such differences were shown in the desorption experiments. These results clearly demonstrated that the cross-reacting antigens expressed by the lymphoblast were not identical with the antigens expressed by the other normal lymphoid cells.

Analysis of the histocompatibility antigens (Dr J. A. Sachs, personal communication) of one of the lymphoblast lines (cell line 7) used in this study, revealed that the line itself not only expressed all the antigens present on the donor cell but also expressed an additional specificity not detected on the parent cell.

It is, of course, possible that foreign macromolecules and haptenic groups are absorbed from the culture media onto the surface of the lymphoblast cell lines, giving rise to these observed antigenic differences. It is known, for example, that cultured HeLa cells absorb such macromolecules onto their surface which in turn contribute to their antigenic make up (Hamburger, Pious & Mills, 1963). Foetal calf serum antigens have also been shown to be present on cell lines (Eng and Landon, 1971). More recently, however, Steel *et al.* (1973) suggested that antigenic disparity between fresh lymphocytes and cell lines derived from these lymphocytes, arose through modification of histocompatibility determinants and were not an artefact of the cell culture technique.

The implications of this study, if the binding of antibody to lymphocytes is a factor determining the efficacy of AHLS in producing immunosuppression, are that peripheral or thoracic duct lymphocytes are the cells of choice in the production of AHLS.

Several attempts have been made in the past to produce AHLS using peripheral cells but toxicity due to the development of anti-platelet antibody has prevented much progress (Woiwod *et al.*, 1970). Thoracic duct cells are not likely to be available for AHLS production and the present situation is that lymphoblasts, despite the low binding affinity and the antigenic differences, both histocompatibility antigens and AHLS/AHLG receptor sites, shown in the present work, are likely to continue to be used, until such time that past difficulties encountered with peripheral lymphocytes are overcome.

ACKNOWLEDGMENTS

The authors acknowledge the invaluable assistance of Mr J. Boak in donating certain materials essential for this study. We also wish to thank Mrs A. L. Bruton and Miss A. Kane for excellent technical assistance.

REFERENCES

BALDWIN, R.W. (1970) Tumour specific antigens associated with chemically induced tumours. *Europ. J. clin. biol. Res.* **15**, 593.

BARNES, A.D., HAWKER, R.J., COWDALL, E.I., DUKES, H., FESFAR, J. & GRIFFIN, J. (1972a) The production of antihuman thymocyte antiserum in various species. *ALG Therapy and Standardisation Workshop*, No. 51, p. 63. Published Behringwerke AG, Germany.

BARNES, A.D., DICKERSON, C. & HAWKER, R.J. (1972b) The production of antilymphoblast globulin for clinical use. *ALG Therapy and Standardisation Workshop*. No. 51, p. 66. Published Behringwerke AG, Germany.

CARRAZ, M., TRAEGER, J., FRIES, D., PERRIN, J., SAUBIER, E., BROCKIER, J., VEYSSEYNE, C., PREVOT, J., BRYON, P., JOUVENCEUX, A., ARCHIMBAUD, J.P., BONNET, P., MANUAL, Y., BERNHARDT, J.P. and TRAEGER-FOUILLET, Y. (1967) Preparation properties et activité d'immunoglobulines de cheval anti-lymphocytes humains. *Rev. Inst. Pasteur Lyon*, **1**, 17.

DORMONT, J., HORS, J., LAMY, R., EYQUEM, A., LAVERGNE, M., WALLON, C. & REYNAUD, M. (1970) Preparation of horse antilymphocyte serum using blood lymphocytes, thymocytes and cells from chronic lymphatic leukaemia as antigens. *Int. Symp. on ALS, Versailles*, **16,** p. 43. Karger, Basel.

DUMONDE, D.C. (1966) Tissue specific antigens. *Advanc. Immunol.* **5,** 245.

EDWARDS, D.C., THOMAS, D., MOSEDALE, B., WOODROOFFE, J.G. & ZOLA, H. (1972) The immunosuppressive potency of antilymphocyte sera and globulins predicted by the use of the immunofluorescence test. *ALG Therapy and Standardisation Workshop*, No. 51, p. 28. Published Behringwerke AG, Germany.

ENG, C.P. & LANDON, J.C. (1971) Antigenic studies on primate haematopoietic tissue culture cells. *In Vitro*, **6,** 387.

GUTHÖHRLEIN, G., SEILER, F.R., HEIDE, K., REBER, G. & SCHWICK, H.G. (1972) Large scale production of horse antihuman lymphocyte globulin (AHLG) for clinical use. *ALG Therapy and Standardisation Workshop*. No. 51, p. 81. Published Behringwerke AG, Germany.

GROTH, C.G., KASHIWAGI, N., MOORE, G.E., HUSBERG, B., BJORKEN, C., PUTNAM, C.W. & STARZL, T.E. (1972) Production of a standardised antilymphocyte globulin. *ALG Therapy and Standardisation Workshop*. No. 51, p. 86. Published Behringwerke AG, Germany.

HAMBURGER, R.N., PIOUS, D.A. & MILLS, S.E. (1963) Antigenic specificities acquired from the growth medium by cells in culture. *Immunology*, **6,** 439.

LANCE, E.M. (1970) The selective action of antilymphocyte serum on recirculating lymphocytes. *Clin. exp. Immunol.* **6,** 789.

LANCE, E.M., FORD, P.J. & RUSZKIEWICZ, M. (1968) The use of subcellular fractions to raise antilymphocytic serum. *Immunology*, **15,** 571.

NAJARIAN, J.S., SIMMONS, R.L., GEWURZ, H., MOBERG, A.W., MERKEL, F. & MOORE, G.E. (1969) Antiserum to cultured human lymphoblast: preparation, purifications and immunosuppressive properties in man. *Ann. Surg.* **170,** 617.

PERPER, R.J., YU, T.Z. & KOOISTRA, J.B. (1970) The *in vitro* specificity of antilymphocyte sera produced by either cultured or non cultured lymphocytes. *Int. Arch. Allergy*, **37,** 418.

PREHN, R.T. (1965) Cancer antigens in tumours induced by chemicals. *Fed. Proc.* **24,** 1018.

PREHN, R.T. (1967) *Cross Reacting Antigens and Neoantigens.* (Ed. by J. J. Trentin), p. 105. Williams and Wilkins, Baltimore.

SEILER, F.R., HEIDE, K. & SCHWICK, H.G. (1970) Comparison of several ALG preparations; human lymphocytes of different origin studied in varied immunisation schedules. *Int. Symp. on ALS, Versailles*, Vol. 16, p. 37. Karger, Basel.

SEILER, F.R. (1972) Choice of antigen for raising AHLG. *ALG Therapy and Standardisation Workshop*. No. 51, p. 71. Published Behringwerke AG, Germany.

STEEL, C.M., HARDY, D.A., LING, N.R., DICK, H.M., MACKINTOSH, P. & CRICHTON, W.B. (1973) The interaction of normal lymphocytes and cells from lymphoid cell lines. III. Studies on activation in an autochthonous system. *Immunology*, **24,** 177.

SYMES, M.O. & RIDDELL, A.G. (1966) The viability of human spleen cells after cooling *in vitro*. *Brit. J. Surg.* **53,** 794.

THOMAS, D., MOSEDALE, B. & ZOLA, H. (1971) The use of the indirect fluorescent antibody technique in assessing the activity of antilymphocytic sera and antilymphocytic globulins. *Clin. exp. Immunol.* **8,** 987.

THOMAS, D., WOODROOFFE, J.G., MOSEDALE, B., WARD, P.D., ZOLA, H., EDWARDS, D.C. & BALNER, H. (1972) Antibody titres of antilymphocytic sera and globulins determined by immunofluorescence and their correlation with immunosuppressive activity. *Clin. exp. Immunol.* **11,** 569.

THOMSON, A.E.R., BULL, J.M. & ROBINSON, M.A. (1966) A procedure for separating viable lymphocytes from human blood and some studies on their susceptibility to hypotonic shock. *Brit. J. Haemat.* **12,** 433.

WILSON, J.D. & GRIMES, A.J. (1968) Defibrination of normal human blood for *in vitro* cell studies. *Nature (Lond.)*, **218,** 178.

WOIWOD, A.J., COURTENAY, J.S., EDWARDS, D.C., EPPS, H.B.G., KNIGHT, R.R., MOSEDALE, B., PHILLIPS, A.W., RAHR, L., THOMAS, D., WOODROOFFE, J.G. & ZOLA, H. (1970) The preparation and properties of horse antihuman lymphocyte serum and globulin. *Transplantation*, **10,** 173.

ZOLA, H., MOSEDALE, B. & THOMAS, D. (1970) The preparation and properties of antisera to subcellular fractions from lymphocytes. *Transplantation*, **9,** 259.

ZOLA, H., THOMAS, D., MOSEDALE, B. & COURTENAY, J.S. (1971) Immunosuppressive antisera to soluble extracts from mouse and human lymphocytes. *Transplantation*, **12,** 49.