Clin. exp. Immunol. (1981) **43**, 311–318.

Analysis of the peanut agglutinin-binding site as a differentiation marker of normal and malignant human lymphoid cells

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(Accepted for publication 12 September 1980)

SUMMARY

The lectin peanut agglutinin (PNA), which interacts specifically with D-galactosyl residues, was studied for its binding to human normal and malignant lymphoid cells at various stages of differentiation. As previously reported, PNA binds to thymocytes; however, it does not interact with the prothymocytes which precede the cortical thymocyte differentiation stage. No mature peripheral cells in any of the lymphoid organs bind PNA. In contrast to the normal T differentiation pathway, the expression of the PNA-binding site does not seem to coincide with that of T cell characteristics in the various malignant lymphoid cells studied. We therefore conclude that more information is needed about the nature of the PNA-binding site before it can be used as a differentiation marker in malignant lymphoid cells.

INTRODUCTION

The lectin peanut agglutinin (PNA) binds to D-galactosyl residues and to sequences of D-galactose and *N*-acetyl galactosamine (Novogrodsky *et al.*, 1975; Pereira *et al.*, 1976). Agglutination and fluorescence-staining studies have demonstrated specific PNA binding to the immunoincompetent cortical thymocytes of mouse and man (Reisner, Linker-Israeli & Sharon, 1976; London, Berrih & Bach, 1978; Reisner *et al.*, 1979). Further studies indicated that the immunocompetent thymic-derived (T) cells do not bind PNA since the surface galactosyl residues are covered with sialic acid groups (Novogrodsky *et al.*, 1975; Reisner *et al.*, 1979) which appear to be mounted on the glycoproteins during the course of cortical thymocyte differentiation (Despont, Abel & Grey, 1975; Haessli, Bron & Pink, 1980). In view of the above findings, PNA binding is now regarded as a characteristic of immature T cells, and may serve as a marker for identifying malignant lymphoid cells of T origin (Reisner *et al.*, 1979; Levin *et al.*, 1980).

The purpose of the present study was to investigate the PNA-binding capacity to normal human lymphocytes obtained from various lymphoid organs, including prothymocytes which precede the stage of cortical thymocyte differentiation. In parallel, the PNA-binding capacity of a variety of leukaemic and cultured human lymphoblastoid cells was studied in order to evaluate whether this membrane characteristic may serve as a marker for distinct subsets of leukaemic cells.

MATERIALS AND METHODS

Normal human lymphoid cells. Spleen tissues and lymph nodes were obtained from donor cadavers for kidney transplants and thymic tissues from individuals undergoing open-heart sur-

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0099-9104/81/0200-0311$02.00

gery. Fetal thymic tissues obtained from aborted fetuses (17–23 weeks of gestation) in the course of routine pathological examination were also used. Initially the tissues were cut with scissors, passed through a stainless-steel mesh and resuspended in RPMI 1640 medium. Dead cells and erythrocytes were removed by buoyant density centrifugation using a 9% Ficoll–Hypaque suspension. Peripheral blood lymphocytes (PBL) and bone marrow mononuclear cells were also isolated by Ficoll–Hypaque gradient centrifugation. Granulocytes were obtained from the sedimenting fraction of the blood buffy coat centrifuged on Ficoll–Hypaque, and monocytes were separated from the PBL suspension by adherence to glass coverslips.

Isolation of prothymocytes from fetal thymic cell suspensions. The human prothymocytes were functionally defined as thymic lymphocytes which lacked the capacity to form E rosettes. These cells comprise 20–50% of the fetal thymic cells, but only a minor proportion (< 0·5%) of the postnatal thymocytes. Prothymocytes are larger than the more mature cortical and medullary thymocytes, and long-term cultures of these cells result in the expression of the cortical thymocyte characteristics, including the capacity for E rosetting (Gatien, Schneeberger & Merler, 1975; Galili *et al.*, 1980). Fetal thymic lymphocytes were allowed to form E rosettes, and thereafter the non-rosetting cells were isolated by centrifugation on a Ficoll–Hypaque solution, while the rosetting cells sedimented through the gradient. The sheep erythrocytes within the rosetting fraction were lysed by 0·85% NH_4Cl solution. Both prothymocytes and E rosetting thymocytes were washed twice and resuspended in RPMI 1640 + 10% fetal calf serum.

Lymphoblastoid cell lines (LCL). The human LCL listed in Table 4 were maintained in RPMI 1640 + 10% fetal calf serum (GIBCO) supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml). The LCL derived from normal B lymphocytes were obtained by infecting normal PBL with EB virus (EBV).

Peanut agglutinin-binding assay. Fluorescein-bound peanut agglutinin (FITC-PNA) (250 μg/ml) was used for studying the binding of the lectin to various cells, according to Reisner *et al.* (1979). A pellet of 1×10^6 cells was resuspended in 20 μl FITC-PNA solution and incubated for 20 min at room temperature. Thereafter the cells were washed twice and resuspended in 0·1 ml cold saline. The percentage of positive stained cells was assessed in a Zeiss u.v. microscope.

E rosette formation. Aliquots of 0·1 ml of lymphocyte suspension (2×10^6 cells/ml) were mixed with 0·1 ml of sheep erythrocyte suspension (1%) in RPMI 1640. The mixture was centrifuged at 200 *g* for 5 min and incubated for 1 hr at room temperature. Thereafter the pellet was gently resuspended and the proportion of cells forming E rosettes was scored in a haemocytometer.

RESULTS

PNA binding by cells from lymphoid organs. Human lymphoid cells obtained from bone marrow, thymus, spleen and lymph node tissues were studied for their capacity to bind FITC-PNA (Table 1). The only cells which bound the lectin were thymocytes. Thymus- or B-derived lymphocytes, populations isolated from the spleen, lymph nodes or bone marrow, did not stain with the

Table 1. PNA binding by normal lymphocytes

Cell type	Per cent E rosettes	Per cent PNA-stained cells
Thymocytes	98 ± 0·7*	96 ± 1·1*
PBL	68 ± 2·1	3 ± 0·6
Spleen lymphocytes	20 ± 1·3	2 ± 0·3
Lymph node lymphocytes	35 ± 3·6	< 2
Bone marrow mononuclear cells	12 ± 2·0	2 ± 0·4

* Mean ± s.e. of results obtained from five to eight subjects.

fluorescent lectin, and a variety of haematopoietic cells within the bone marrow also failed to interact with PNA.

Interaction of PNA with different leucocyte populations from the peripheral blood. The normal lymphocytes in the blood did not bind PNA (Table 2). The residual binding detected among the PBL could be attributed to the ability of monocytes to interact with the lectin, because the majority of glass-adherent monocytes, derived from PBL, were stained by FITC-PNA. Normal granulocytes and T lymphocytes which were activated *in vitro*, either by allogeneic stimulation in mixed lymphocyte culture or by the mitogen PHA, also lacked the capacity to bind PNA entirely.

PNA binding by fetal thymic cells. In order to determine whether cells of the prothymocyte subset, which precedes the differentiation stage of cortical thymocytes, also express the membrane site for PNA binding, their ability to interact with the lectin was studied. As seen from Table 3, the FITC-PNA-binding capacity of fetal thymic cells coincides with the expression of the E receptor. Thus E rosetting thymocytes bound the lectin, whereas prothymocytes, which failed to form E rosettes, did not bind PNA. Fetal splenic lymphocytes (Table 3), like their adult counterparts, also lack PNA-binding capacity completely.

Interaction of LCL with PNA. A number of LCLs were examined for staining with FITC-PNA (Table 4). The only T-derived line which exhibited strong PNA binding was Be13, while HD-MAR cells which readily form E rosettes interact very weakly with the lectin. Other T lines such as MOLT-4 and PEER which regularly express the human T antigen on their surface, completely lacked the PNA-binding capacity. In contrast to these T cell lines, all the B-derived LCL, which originated from either African (Epstein–Barr nuclear antigen-positive) or American (EBNA-negative) type Burkitt's lymphoma, exhibited strong binding of FITC-PNA. This binding is specific to galactosyl residues since the PNA can be detached from the cell surface by incubation of the PNA-stained LCL with free 0·2 M galactose (not shown) (Flandrin & Brouet, 1974). Other B cell lines, originating from normal, EBV-converted peripheral blood B cells, also bound the lectin. Cells from the myeloerythroid cell line (K562) did not bind PNA while cells from the REH line, originating from ALL, showed only a partial capacity to interact with the lectin.

Table 2. PNA binding by various peripheral blood leucocytes

Cell type	Per cent PNA-stained cells
Lymphocytes	3±0·3*
Granulocytes	<2
Monocytes	82±3·7
MLC-transformed T lymphocytes	<2
PHA-transformed T lymphocytes	<2

* Mean ± s.e. of results obtained from five subjects.

Table 3. PNA binding by fetal thymic subsets

Cell type	Per cent E rosettes	Per cent PNA-stained cells
Whole thymic suspension	68±7·5*	78±8·3*
E rosetting cells (thymocytes)	95±0·8	97±1·1
Non-E rosetting cells (prothymocytes)	6±1·0	9±2·3
Spleen lymphocytes	1	<2

* Mean ± s.e. of results obtained from four subjects.

Table 4. PNA binding by various lymphoblastoid cell lines

Designation	Type	Reference	Origin	Per cent E rosettes	PNA % staining
MOLT-4	T	Minowada *et al.*, 1972	ALL	0	0
HD-MAR	T	Ben-Bassat *et al.*, 1980	Hodgkin's disease	80	20
PEER	T	Ravid *et al.*, 1980	ALL	0	0
Be13	T	Galili *et al.*, (in prep.)	ALL	40	100
Raji	B	Pulvertaft, 1965	Burkitt lymphoma	0	100
Daudi	B	Klein *et al.*, 1968	Burkitt lymphoma	0	100
Bjab	B	Klein *et al.*, 1975	American Burkitt lymphoma	0	100
Bjab-HR_1K	B	Klein *et al.*, 1975	EBV-infected Bjab	0	100
Ramos	B	Fresen & Zur Hausen, 1976	American Burkitt lymphoma	0	100
Ramos-HR_1K	B	Fresen & Zur Hausen, 1976	EBV-infected Ramos	0	100
K562	Myeloid (CML)	Lozzio & Lozzio, 1975	CML	0	2
Reh	Non-B, non-T ALL	Rosenfeld *et al.*, 1977	ALL	0	50
EBV-converted PBL	B		Normal blood	0	80

PNA binding by various leukaemic cells. Of the series of leukaemic patients studied, in only two cases of acute lymphoblastic leukaemia (ALL) did the leukaemic cells exhibit PNA binding (Table 5). In one of these patients (M.M.) the leukaemic cells were also able to form E rosettes, whereas in the other (U.R.) they lacked E rosetting capacity completely. In other ALL patients, the leukaemic cells which in some cases exhibited positive paranuclear acid phosphatase staining (regarded as a T cell marker), unlike normal circulating PBL, almost completely lacked the capacity to bind PNA. No chronic lymphocytic (CLL) or chronic myeloid leukaemia (CML) cells bound FITC-PNA, while in three AML patients the leukaemic cells showed partial PNA binding. Cells obtained from three non-Hodgkins' lymphoma patients and from one patient with hairy cell leukaemia also bound FITC-PNA.

DISCUSSION

The data obtained in this study confirm earlier findings (Reisner *et al.*, 1979; Levin *et al.*, 1980) showing that PNA binding is a characteristic of human thymocytes, while peripheral blood lymphocytes lack this capacity. In addition it is also evident that lymphocytes isolated from spleens, lymph nodes or bone marrows do not bind this lectin. In view of the above findings and the inability of fetal splenocytes to bind PNA, it can generally be assumed that immunocompetent human lymphocytes lack surface sites for PNA binding. This indicates that no free galactosyl residues are expressed on the surface of mature lymphoid cells.

Reisner *et al.* (1976) have pointed out that the maturation of immunoincompetent cortical thymocytes into immunocompetent medullary cells involves the mounting of sialic acid groups on galactosyl residues, which abolish the capacity of immunocompetent T cells to bind PNA. Several independent studies support this view. These include the demonstration of increased electrophoretic mobility of lymphocytes due to increased negative surface charge (i.e. sialic acid) (Wiig, 1974; Häyry *et al.*, 1975), activity of sialyl transferase in thymocytes (Despont *et al.*, 1975), and demonstration of an increased content in sialic acid on cell surface (Wiig, 1974) and on the Thy-1 antigen (Haessli *et al.*, 1980) of the immunocompetent T cells when compared with thymocytes. It is of interest to note that despite the similarities between cortical thymocytes and immunoactivated T cells in respect of their ability to form stable E rosettes (Galili & Schlesinger, 1976) and to express the natural attachment phenomenon (Galili *et al.*, 1978; Galili, Häyry & Klein, 1979), activities which relate to the lack of surface-negative charge (Galili *et al.*, 1979), no PNA binding was detected among the MLC-activated and PHA-transformed human T cells. Similar findings were encountered with PHA-transformed murine T cells (Rose *et al.*, 1980). The ability of the immunoincompetent cortical thymocytes to bind PNA has led some investigators to the assumption that this capacity characterizes T cells at an early stage of differentiation (Reisner *et al.*, 1979; Levin *et al.*, 1980). The data obtained from this study of fetal thymic cells indicate that the PNA-binding capacity is confined to the cortical thymocyte stage, and coincides with the expression of the E receptor. On the other hand, prothymocytes lack both these characteristics. The fetal thymic cells which do not form E rosettes indeed precede the stage of the cortical thymocytes, as demonstrated by long-term *in vitro* cultures of the prothymocytes, which resulted in spontaneous expression of the cortical thymocyte characteristics within 3 days of incubation (Gatien *et al.*, 1975; Galili *et al.*, 1980). These findings are supported by results of earlier studies by London, Berrih & Papiernik (1979) on the PNA binding by murine thymocytes. They showed that the lymphoid cells obtained from murine embryonic thymuses did not display PNA-binding capacity until the 14th day of gestation. Thereafter this characteristic was gradually expressed on the maturing thymocytes. The lack of PNA binding by isolated human mononuclear bone marrow cells also supports the assumption that PNA binding is not generally a characteristic of other immature cells.

Unlike the well-defined data obtained with normal lymphocytes at various stages of maturation, PNA binding does not seem to characterize early T-type LCL exclusively. All the Burkitt's lymphoma-derived LCL exhibited strong PNA binding, which did not seem to depend on the presence of the EBV genomes. Accordingly, the EBNA-negative American Burkitt-derived LCL bound PNA just as well as the EBNA-positive Burkitt-derived LCL. Similarly, LCL derived from

Table 5. PNA binding by various leukaemic and lymphoma cells

Disease type	Patient	Count	Paranuclear acid phosph.	Blasts (%)	E rosettes (%)	PNA binding (% stained cells)
ALL	U.M.	18,000	n.d.	>85	2	<2
	R.Z.	20,000	+	>85	6	<2
	S.F.	18,000	−	70	23	<2
	B.I.	70,000	+	>85	2	<2
	I.B.	20,000	−	>85	2	<2
	U.R.	40,000	n.d.	>85	<1	95
	A.H.	80,000	n.d.	>85	<1	<2
	M.B.	250,000	n.d.	>85	25	<2
	N.B.	Bone marrow	n.d.	>85	<1	<2
	E.F.	Bone marrow	+	>85	<1	<2
	J.S.	Cell line	+	100	0	30
	S.A.	Cell line	+	100	0	<2
	J.K.	50,000	+	>85	<1	<2
	A.H.	Bone marrow	n.d.	>85	<1	<2
	M.M.	50,000	−	>85	67	100
CLL	S.K.	300,000			<1	<2
	H.M.	40,000			3	<2
	H.P.	300,000			<1	<2
	J.S.	60,000			2	<2
	M.L.	20,000			25	<2
	B.Z.	200,000			<1	<2
	S.R.	80,000			<1	<2
	B.P.	16,000			20	<2
	M.K.	17,000			<1	<2
Non-Hodgkin's lymphoma	B.A.	Pleural effusion	−	80	2	95
	G.M.	Cell line	−	100	0	98
	L.I.*	20,000	−	>85	2	40
AML	E.P.	40,000		>85	8	5
	S.T.	80,000		>85	2	<2
	A.Z.	15,000		>85	2	<2
	A.K.	20,000		>85	3	<2
	D.G.	70,000		>85	2	<2
	E.C.	20,000		>85	5	35
	A.N.	40,000		>85	<1	38
	N.K.	35,000		>85	1	<2
	P.S.	30,000		80	5	80
	A.A.	65,000		>85	2	<2
	S.M.	50,000		>85	2	<2
CML	S.M.	35,000		>85	5	<2
	R.V.	35,000		>85	<1	<2
	M.K.	70,000		>85	2	<2
	Z.K.	80,000		>85	2	10
Hairy cell leukaemia	S.S.	Cell line		100	0	98

* Lymphosarcoma.

normal B cells immortalized by infection with EBV had PNA-binding capacity. Unlike the B-derived LCL, not all the T-derived LCL interacted with the lectin. The cells from the T cell lines MOLT-4 and PEER did not bind PNA, whereas the cells of the HD-MAR LCL line which readily form E rosettes exhibited a low PNA-binding capacity. In contrast to the normal T differentiation pathway the appearance of the PNA-binding site in ALL cells did not seem to coincide with the expression of the E receptor, and ALL cells which did not form E rosettes were able to bind the lectin. Similar non-rosetting, PNA-binding patterns in non-E rosetting ALL cells have also been reported in other studies (Reisner *et al.*, 1979; Levin *et al.*, 1980).

At present the nature of the PNA-binding site is unclear. Its expression on human and murine thymocytes in parallel stages of maturation suggests that it may be of importance in the differentiation of normal T lymphocytes. It is possible that this molecule serves as a base for the mounting of sialic acid during further maturation, thereby conferring to the T lymphocyte its negative surface charge. This negative surface charge seems to be essential for normal T lymphocyte circulation in the body (Woodruff & Gesner, 1969).

The isolation and characterization of the PNA-binding molecule from the thymocyte membrane, and generation of specific antibodies against it, are required in order to determine whether this binding site appears *de novo* during the prothymocytes' differentiation into cortical thymocytes. Furthermore such a study may indicate the degree of similarity between the PNA-binding molecules on thymocytes and those found on the surface of related non-T-derived malignant lymphoid cells, such as the Burkitt lymphoma-derived LCL. It is possible in the latter lymphoma cells that some of the normal surface glycoproteins are synthesized without the terminal group of the sialic acid, thus exposing galactosyl residues which are normally attached to it (Novogrodsky *et al.*, 1975). Yet another explanation for the PNA binding by the non-T LCL may be related to the synthesis of new types of surface glycoproteins which bear the galactosyl residues as their end-groups. PNA-binding capacity has also been encountered on both normal and malignant non-lymphoid cells. Epithelial cells from lactating mammary glands and well-differentiated tumour cells from the same origin bind PNA, whereas less differentiated tumour cells of the same origin do not (Newman, Klein & Rudland, 1979). In another study, PNA was found to interact with undifferentiated murine embryonal cells, but not with differentiated derivatives (Reisner *et al.*, 1977). Thus it seems that a variety of surface glycoprotein molecules with free galactosyl residues may interact with the PNA in different cells.

In view of the above data and the lack of correlation between PNA-binding capacity and the expression of T cell differentiation characteristics encountered in this study and by others (Reisner *et al.*, 1979; Levin *et al.*, 1980), it is suggested that more information concerning the nature of the lectin-binding site is required before it can be used as a differentiation marker in well-defined malignant states.

We wish to thank Professor N. Sharon for the Weizmann Institute, Rehovot, for his generous gift of FITC-PNA.

REFERENCES

BEN-BASSAT, H., MITRANI-ROSENBAUM, S., GAMLIEL, H., NAPARSTEK, E., LEIZEROWITZ, R., KORBESH, A., SAGI, M., VOSS, R., KOHN, G. & POLLIACK, A. (1980) Establishment in continuous culture of a T-lymphoid cell line (HD-MAR) from a patient with Hodgkin's lymphoma. *Int. J. Cancer*, **25**, 583.

DESPONT, J.P., ABEL, C.A. & GREY, M.M. (1975) Sialic acid and sialyl transferase in murine lymphoid cells: indication of T cell maturation. *Cell. Immunol.* **17**, 487.

FLANDRIN, G. & BROUET, J.C. (1974) The Sézary cell: cytologic cytochemical and immunologic studies. *Mayo Clin. Proc.* **49**, 575.

FRESEN, K.O. & ZUR HAUSEN, H. (1976) Establishment of EBNA expressing cell lines by infection of Epstein–Barr virus (EBV) genome-negative human lymphoma cells with different EBV strains. *Int. J. Cancer*, **17**, 161.

GALILI, U., GALILI, N., VANKY, F. & KLEIN, E. (1978) Natural species restricted attachment of human and murine T lymphocytes to various cells. *Proc. Natl. Acad. Sci. USA*, **75**, 2396.

GALILI, U., HÄYRY, P. & KLEIN, E. (1979) Loss of net negative surface charge during MLC stimulation of human T lymphocytes. Correlation to stable E formation and natural attachment to normal and malignant cells. *Cell. Immunol.* **48**, 91.

GALILI, U., KLEIN, E. & SCHLESINGER, M. (1977)

Human T lymphocytes receptors for sheep red blood cells and specific antigens: are they identical sites on the cell membrane? *J. Immunol.* **119,** 104.

GALILI, U., POLLIACK, A., OKON, E., LEIZEROWITZ, R., GAMLIEL, H., KORBESH, A., SCHENKAR, J.C. & IZAK, G. (1980) Human prothymocytes: membrane properties, differentiation patterns, glucocorticoid sensitivity and ultrastructural features. *J. exp. Med.* **152.** (In press.)

GALILI, U. & SCHLESINGER, M. (1976) The formation of stable E rosettes by human T lymphocytes activated in mixed lymphocyte reaction. *J. Immunol.* **117,** 730.

GATIEN, J.G., SCHNEEBERGER, E.E. & MERLER, E. (1975) Analysis of human thymocyte subpopulations using discontinuous gradient of albumin: precursor lymphocytes in human thymus. *Eur. J. Immunol.* **5,** 312.

HAESSLI, D., BRON, C. & PINK, R.L. (1980) T-lymphocyte differentiation is accompanied by increase in sialic acid content of Thy-1 antigen. *Nature*, **283,** 576.

HÄYRY, P., ANDERSSON, L.C., GAHMBERG, C., ROBERTS, P., RANKI, A. & NORDLING, S. (1975) Fractionation of immunocompetent cells by free flow cell electrophoresis. *Isr. J. Med. Sci.* **11,** 1299.

KLEIN, G., GIOVANELLA, B., WESTMAN, A., STEHLIN, J.S. & MURFORD, D. (1975) An EBV-genome-negative cell line established from an American Burkitt lymphoma: receptor characteristics, EBV infectability and permanent conversion into EBV-positive sublines by *in vitro* infection. *Intervirology*, **5,** 319.

KLEIN, E., KLEIN, G., NADKARNI, J.S., NADKARNI, J.J., WIGZELL, H. & CLIFFORD, P. (1968) Surface IgM-kappa specificity on a Burkitt lymphoma cell *in vivo* and in derived culture lines. *Cancer Res.* **28,** 1300.

LEVIN, S., RUSSELL, E.C., BLANCHARD, D., MCWILLIAMS, N.B., MAURER, H.M. & MOHANABUMAR, T. (1980) Receptors for peanut agglutinin (*Arachus hypogea*) in childhood acute lymphoblastic leukemia: possible clinical significance. *Blood*, **55,** 37.

LONDON, J., BERRIH, S. & BACH, J.F. (1978) Peanut agglutinin. I. A new tool for studying T lymphocyte subpopulations. *J. Immunol.* **121,** 438.

LONDON, J., BERRIH, S. & PAPIERNIK, M. (1979) Peanut agglutinin. III. Study of T lymphocyte differentiation during murine ontogeny. *Dev. Comp. Immunol.* **3,** 343.

LOZZIO, C.B. & LOZZIO, B.B. (1975) Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood*, **45,** 321.

MINOWADA, J., OHNUMA, T. & MOORE, G.E. (1972) Rosette forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus derived lymphocytes. *J. Natl. Cancer Inst.* **49,** 891.

NEWMAN, R.A., KLEIN, P.J. & RUDLAND, P.S. (1979) Binding of peanut lectin to breast epithelium, human carcinomas, and a cultured mammary stem cell: use of the lectin as a marker of mammary differentiation. *J. Natl. Cancer Inst.* **63,** 1339.

NOVOGRODSKY, A., LOTAN, R., RAVID, A. & SHARON, N. (1975) Peanut agglutinin, a new mitogen that binds to galactosyl sites exposed after neuraminidase treatment. *J. Immunol.* **115,** 1243.

PEREIRA, M.E., KABAT, F.A., LOTAN, P. & SHARON, N. (1976) Immunological studies on the specificity of peanut (*Arachis hypogea*) agglutinin. *Carbohydr. Res.* **51,** 107.

PULVERTAFT, R.J.V. (1965) A study of malignant tumors in Nigeria by short-term tissue culture. *J. clin. Pathol.* **18,** 261.

RAVID, Z., GOLDBLUM, N., ZAEZOV, R., SCHLESINGER, M., KERTES, I., MINOWADA, J., VERBI, W. & GREAVES, M. (1980) Establishment and characterization of a new leukemic T-cell line (Peer) with an unusual phenotype. *Int. J. Cancer*, **25,** 705.

REISNER, Y., BINIAMINOV, M., ROSENTHAL, E., SHARON, N. & RAMOT, B. (1979) Interaction of peanut agglutinin with normal human lymphocytes and with leukemic cells. *Proc. Natl. Acad. Sci. USA*, **76,** 447.

REISNER, Y., GACHELIN, G., DUBOIS, P., NICOLAS, J.F., SHARON, N. & JACOB, F. (1977) Interaction of peanut agglutinin, a lectin specific for non-reducing terminal D-galactosyl residues with embryonal carcinoma cells. *Dev. Biol.* **61,** 20.

REISNER, Y., LINKER-ISRAELI, M. & SHARON, N. (1976) Separation of mouse thymocytes into two subpopulations by the use of peanut agglutinin. *Proc. Natl. Acad. Sci. USA*, **76,** 447.

ROSE, M.L., BIRBECK, M.S.C., WALLIS, F.J., FORRESTER, J.A. & DAVIS, A.J.S. (1980) Peanut lectin binding properties of germinal centres of mouse lymphoid tissue. *Nature*, **284,** 364.

ROSENFELD, C., GOUTNER, A., CHOQUET, C., VENUAT, A.M., KAYIBANDA, G. & PICO, J.L. (1977) Phenotypic characterisations of a unique non-T, non-B acute lymphoblastic leukaemia cell line. *Nature*, **267,** 841.

WIIG, J.N. (1974) Effect of neuraminidase on lymphoid cells. Differences in structure of B and T cells and thymocytes of mouse shown by cell electrophoresis and sialic acid determination. *Scand. J. Immunol.* **9,** 357.

WOODRUFF, J.J. & GESNER, B.M. (1969) The effect of neuraminidase on the fate of transfused lymphocytes. *J. exp. Med.* **129,** 551.