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BINDING OF PEANUT LECTIN TO GERMINAL-CENTRE CELLS: A MARKER FOR B-CELL SUBSETS OF FOLLICULAR LYMPHOMA?

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Summary.—The binding of horseradish-peroxidase-labelled peanut lectin (HRP-PNL) to cryostat sections of tonsil, lymphoma lymph nodes, reactive lymph nodes and miscellaneous tumours demonstrated that PNL binds selectively to lymphocytes in germinal centres. Lymph nodes from 21 patients with non-Hodgkin's lymphomas were phenotyped as cell suspensions for PNL binding, and the following surface markers: E rosetting, C3d, SIg, OK markers of T-cell subsets, Ig heavy-chain and light-chain classes. There was a positive correlation between PNL binding and cells with SIg and C3d receptors. 4/5 cases of centroblastic/centrocytic follicular lymphoma had a PNL$^+$ SIg$^+$ C3d$^+$ phenotype. Both cases of centroblastic/centrocytic diffuse lymphoma were PNL$^-$. There was no correlation between PNL binding and heavy- or light-chain Ig class. PNL binding and presence of C3d receptors were not always positively correlated, indicating that follicular cells may be either PNL$^+$ SIg$^+$ C3d$^+$ or PNL$^+$ SIg$^+$ C3d$^-$. The binding pattern of PNL to 1 case of thymic hyperplasia and 2 cases of malignant lymphoma lymphoblastic T type suggested that some but not all cortical thymocytes bind PNL.

THE LECTIN derived from the seeds of the peanut plant *Arachis hypogaea* (peanut lectin, PNL) binds to single terminal galactose residues, but more avidly to the disaccharide D-galactose β' 1-3 D-N, acetylgalactosamine (Pereira *et al.*, 1976). This sugar is part of the oligo-saccharide array on cell surfaces, but it is commonly covered by sialic acid. Thus PNL will not bind to most lymphocytes unless they are pre-treated with neuraminidase. However, cortical thymocytes of mouse and man (Reisner *et al.*, 1976; 1979), small numbers of peripheral T cells in the mouse (London *et al.*, 1978; Roelants *et al.*, 1979) and germinal-centre lymphocytes of mouse (Rose *et al.*, 1980) bind PNL *without* neuraminidase treatment. Experiments from this laboratory have also revealed that horseradish peroxidase-labelled PNL (HRP-PNL) binds to germinal-centre lymphocytes in human tonsil (Rose & Malchiodi, 1981). The selective binding of PNL by human cortical thymocytes and follicular lymphocytes prompted a search for the PNL-binding characteristics of lymphoblastic lymphomas of cortical T-cell type, and of follicular lymphoma. In this paper we describe the binding of HRP-PNL to frozen sections of tonsil, lymphoma lymph nodes, reactive lymph nodes and some other non-lymphoid tumours, and the binding of fluorescein–isothiocyanate-labelled PNL (FITC-PNL) to cell suspensions from various tissues and non-Hodgkin's lymphomas. The results confirm the restricted binding of peanut lectin to subsets of B and T

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lymphocytes, and illustrate its potential use as a marker of follicular lymphoma, in section or in cell suspensions.

MATERIALS AND METHODS

Surgical biopsy specimens of the lymph nodes studied were obtained from patients admitted to the Royal Marsden Hospital, Fulham Road, London and Sutton, or the Medical Oncology Unit, St Bartholomew's Hospital, London. Conventional histological diagnoses on paraffin-embedded sections were performed by either Dr John Sloane (Sutton) or by Dr A. E. Stansfeld (Bart's Hospital).

Binding of HRP-PNL to cryostat sections.— Blocks from unfixed surgical biopsy materials were snap-frozen in liquid N_2 and stored in liquid N_2 before use. Embedded (Ames TC compound) frozen blocks were cryostat-sectioned at 4 μm, and air-dried. HRP-PNL (prepared as previously described by Rose *et al.*, 1980) was reacted with sections in a concentration of 20 μg/ml in phosphate-buffered saline (PBS, pH 7·2). The reaction was allowed to proceed for 30 min at 20°C, and was terminated by washing sections $\times 3$ in a large excess of PBS. Sites of fixation of HRP-PNL were identified by development with 3,4,3′,4′ tetra-aminobiphenyl hydrochloride (B.D.H.).

Binding of FITC-PNL to cell suspensions.— FITC-PNL was prepared as previously described (Rose *et al.*, 1980). Cell suspensions from fresh surgical biopsy samples of lymph nodes were prepared in RPMI 1640, washed $\times 2$, and cell concentration adjusted to 10^7/ml. 100 μl of cell suspension was mixed with 100 μl of FITC-PNL in PBS at a concentration of 20 μg/ml. The resulting mixture was incubated at 4°C for 30 min and washed $\times 3$ in a large excess of PBS.

Phenotypic analysis of cell suspensions.— Cell suspensions were analysed for content of T and B cells by rosetting, and immunofluorescence techniques using standardized particle preparations and antisera as follows: T cells were quantitated using E rosettes (4°C), anti-HTLA (anti-human lymphocyte antigen serum raised against monkey thymus in rabbits, kindly provided by Drs M. Greaves, M. Roberts and G. Janossy) and in some instances monoclonal mouse antisera against human T-cell subsets (OKT series antisera—Ortho diagnostics). B cells were quantitated using surface immunoglobulin (SIg) expression, with heteroantisera specific for total immunoglobulin (Ig) (μ, γ, α, δ heavy chains), and class-specific anti-Ig sera for μ, γ, α, δ heavy chains and κ and λ light chains (Habeshaw *et al.*, 1979). The expression of C3d receptors by lymphocytes was assessed concurrently, as previously described (Habeshaw *et al.*, 1979). HLA and Ia antigen expression was monitored with the monoclonal antisera W6/32 (anti-HLA-ABC) and Da_2 (anti-HLA-D, Ia-like antigen).

The phenotyping results, histological diagnosis, and HRP-PNL binding on frozen sections were all assessed independently, and combined after completion of the series.

RESULTS

PNL binding to tonsil and reactive lymphoid tissue

HRP-PNL was found to bind to the membrane of lymphocytes present in germinal centres, but not to the coronal small lymphocytes of all the 10 tonsils examined (Fig. 1). Whole-cell suspensions from tonsil indicated 10–20% of FITC-PNL-binding cells, whereas 25–40% of the same cell population was SIg^+ complement-receptor-positive. The PNL-binding cell populations found in reactive lymph nodes are shown in Table I. Where follicular hyperplasia was present, SIg^+ $C3d^+$ and PNL-binding cells appeared together, suggesting that follicular cells are SIg^+ $C3d^+$ PNL^+. The SIg positivity indicates that the cells concerned are B lymphocytes.

PNL binding to lymphoma lymph-node cells, reactive lymph nodes and miscellaneous tumours

Preliminary experiments examined the binding of HRP-PNL to frozen sections of a variety of malignant and non-malignant tissue (Table II). PNL was found to bind largely to lymphocytes of follicular lymphoma and reactive lymph nodes, and rarely to lymphocytes of other lymphomas. The binding of HRP-PNL to oat-cell sarcoma and rhabdomyosar-

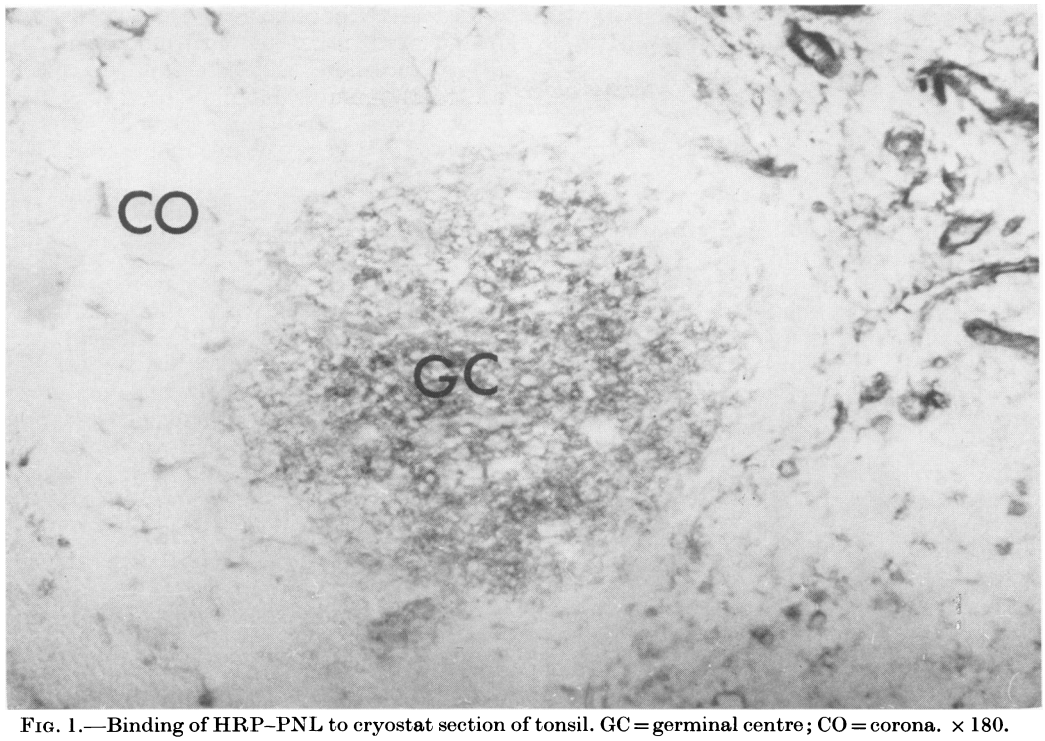

FIG. 1.—Binding of HRP–PNL to cryostat section of tonsil. GC = germinal centre; CO = corona. × 180.

TABLE I.—*PNL$^+$ cells in reactive (non-neoplastic) lymph nodes*

Patient	Age	Histology	% of total viable cells E	C3d	SIg$^+$	PNL	
JW	27	Follicular hyperplasia	30	37	45	20	FITC–PNL
CY	25	Follicular hyperplasia	18	40	40	17	FITC–PNL
BK	2½	Follicular reaction	33	24	42	+	HRP–PNL*
DHD	58	Sinus histiocytes	58	11	30	±	HRP–PNL*
EJ	47	Reaction to mammary dysplasia	51	14	40	±	HRP–PNL*
CP	16	Non-specific reactive follicular hyperplasia	ND	ND	ND	+	HRP–PNL*
GA	65	Follicular reaction	57	21	30	+	HRP–PNL*

* HRP–PNL on cryostat sections. + is PNL$^+$ lymphocytes; ± is lymphocytes PNL$^-$ but connective tissue PNL$^+$.

coma was seen as strong cytoplasmic staining, and distinct from the membrane staining of PNL to lymphocytes. Cryostat sections from PNL$^+$ follicular lymphoma show large areas of PNL-binding lymphocytes (Fig. 2). PNL was also found to bind to follicular dendritic cells, connective tissue and macrophages to varying degrees in different lymphomas, but it is not possible to say whether it was membrane or cytoplasmic binding. Electron-microscope studies have revealed HRP-PNL binding to the membrane of lymphoblasts from human tonsil (Lydyard & Birbeck, personal communication) and the fact that large numbers of FITC-PNL-binding cells can be recovered in cell suspensions from lymph nodes from patients with follicular lymphoma demonstrates that the binding of PNL to follicular lymphocytes is membrane binding. Where possible, retrospective surface marking was established for

TABLE II.—*Binding of HRP-PNL to frozen sections of lymphoma lymphnode cells, reactive lymph nodes and miscellaneous tumours*

Tumour	No. examined	No. containing PNL+ lympho-cytes
Hodgkin's lymphoma	8	1*
T-cell lymphoma	3	0
Reactive hyperplasia	7	5
Nodular follicular lymphoma	6	5
Diffuse lymphoma	1	0
Other lymphomas and tumours‡	14	2†
Wilms' tumour	1	CT only +

* Small discrete germinal centre at edge of node.
† Oat-cell carcinoma and rhabdomyosarcoma.
‡ Carcinoma of the larynx, leg sarcoma, angioblastoma, breast carcinoma, "secondary" carcinoma, non-endemic Burkitt's lymphoma, immunoblastic sarcoma, undifferentiated sarcoma, metastatic carcinoma, high-grade malignant lymphoma, malignant lymphoma lymphocytic, reactive sinus histiocytosis, oat-cell carcinoma, rhabdomyosarcoma.
CT = connective tissue.

the lymphoma patients of this series (Table III).

These results clearly justified a more detailed study of the surface phenotype associated with large numbers of PNL+ lymphocytes. FITC-PNL binding was therefore undertaken on cell suspensions from lymphoid organs of a number of other patients in conjunction with description by other markers (Table IV). The results establish some interesting correlates of PNL binding with histology and with phenotype.

PNL− lymphomas

None of the cells from the nodes or blood of *lymphocytic lymphoma* patients bound PNL. The single case of T CLL (Patient EB) showed slight (8%) PNL binding of lymph-node cells. Patient AB, with MLL, who showed a phenotypic profile of SIgA-positive B cells expressing C3d receptors, did not bind PNL, evidence

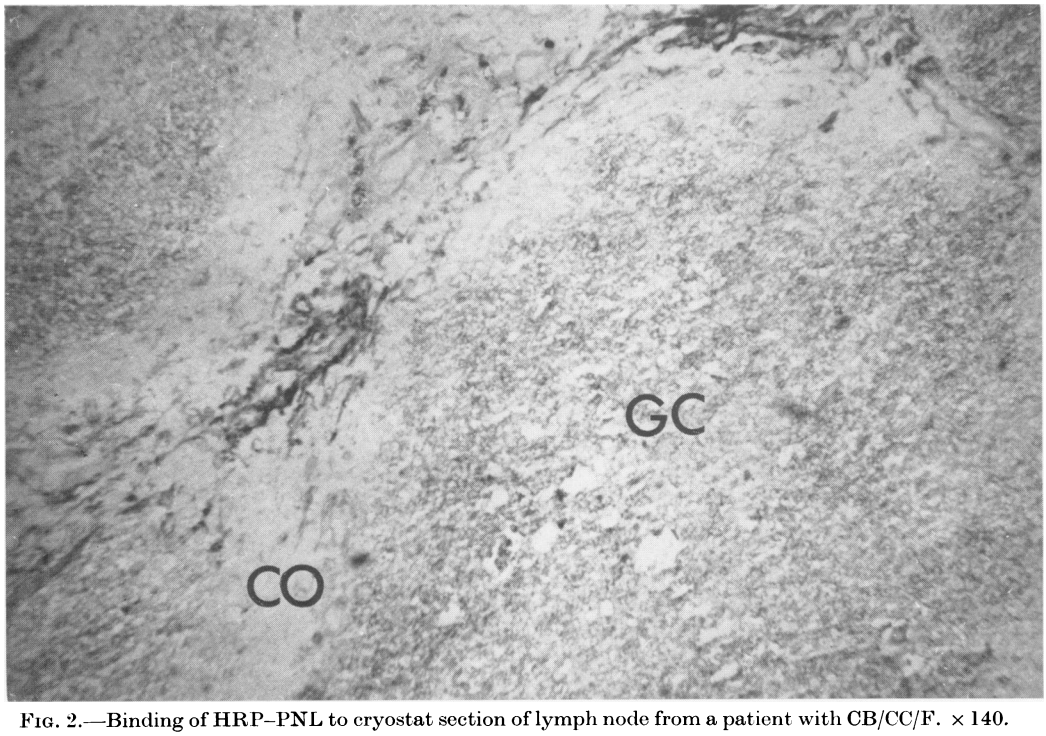

FIG. 2.—Binding of HRP–PNL to cryostat section of lymph node from a patient with CB/CC/F. × 140.

TABLE III.—*HRP-PNL positivity in lymphoma: retrospective correlation with surface marking*

Patient	Age	Histology	E	C3d	SIg	SIg class	HRP-PNL*
CC	19	CB/CC/F	34	36	58	γ μ κ	+
SD	47	CB/CC/D	12	26	71	δ κ	−
JB	25	MLL	2	2	90	μ κ	−
HB	62	MLL	28	0	70	μ κ	−
LOD	59	MLL	5	3	88	δ μ κ	−
PMG	48	Plasmacytoma	4	15	22	α κ	+†
MC	23	MLHgU	64	12	20	pc	−

* Binding to lymphocytes unless otherwise stated.
† Connective tissue was especially positive.
MLIB = malignant lymphoma immunoblastic; MLHgU = malignant lymphoma high-grade unclassified; MLCC = malignant lymphoma centrocytic; MLL = malignant lymphoma lymphocytic; ML/LB:B = malignant lymphoma lymphoblastic B type; ML/LB:T = malignant lymphoma lymphoblastic T type; CB/CC/F = centroblastic and centrocytic follicular; CB/CC/D = centroblastic and centrocytic diffuse; HD = Hodgkin's disease; LP = lymphocyte predominant; ML/CC/SC = malignant lymphoma centrocytic small-cell; TdT = terminal deoxynucleotidyl transferase; pc = polyclonal.

TABLE IV.—*Percentage of FITC-PNL lymphocytes in malignant lymphoma–correlation with phenotype*

Patient	Age	Histology	E	C3d	SIg	SIg class	% FITC–PNL	Biopsy
JY	37	CB/CC/F*	24	—	76	γ only	3	Node
JN	66	CB/CC/F + HD	30	35	37	pc	36	Node
LP	34	CB/CC/F	1	41	70	α λ	66	Node
GB	53	CB/CC/F	20	39	30	α δ λ	20	Node
HS	69	CB/CC/F	26	12	66	γ μ λ	70	Node
SD	47	CB/CC/D‡	1	7	98	γ μ δ κ	4	Blood
SD	47	CB/CC/D	12	26	71	δ κ	0	Node
CB	9	ML/LB:B	2	50	80	μ κ	7	Node
LF	2½	ML/LB:B	2	3	97	μ δ κ	97	Node
GC	47	MLIB	63	16	32	μ γ α δ κ	8	Node
JB	64	MLIB	38	9	55	γ δ κ	0	Node
LC	78	MLCC	1	34	88	γ δ γ	0	Node
HB	62	MLL	28	0	70	μ κ	0	Node
JM	44	MLL	14	2	80	δ γ α κ	1	Blood
AB	72	MLL	4	44	94	α κ	1	Node
EB	64	"T" CLL	55	2	3	pc	8	Node
KF	5	ML/LB:T	80	4	4	—	1	Pleural effusate‡
LT	7	ML/LB:T	70†	1	1	—	0	Node‡
PMG	48	Plasmacytoma	4	15	22	δ κ Cy α κ	45	Stomach
FS	51	Lennert's lymphoma	80	7	6	pc	0	Node
FR	61	ML/CC/SC	12	13	84		0	Node

* Recurrent. † OKT6+, TdT+. ‡ Leukaemic. Cy = Cytoplasmic.
For key to abbreviations, see Table III.

that the PNL-binding site cannot be the same as the SIgA or C3d binding sites.

The 2 cases of *T-cell lymphoblastic lymphoma* (KF, LT) (Table IV) did not show PNL binding, though 80% of their lymphocytes expressed thymic cortical-lymphocyte antigen (OKT6+) and were TdT+. This suggests that not all cortical thymocytes bind PNL, a possibility which is supported by the profile of a thymus gland (Table V) where there is a discrepancy between the number of cortical thymocytes (84%) and the number of cells binding PNL (46%).

One case of *immunoblastic lymphoma* (GC, Table IV) showed only small numbers (8%) of PNL-binding cells, though some of the B cells in this patient expressed C3d receptors. Patient MC (Table III) with a similar profile to GC, also failed to show PNL positivity of lymphocytes in lymph-node sections stained with HRP-

TABLE V.—*PNL positivity in thymus*

1. Cell suspension

Markers	% Binding
FITC-PNL	46
E rosettes	96 (HTLA 90)
OKT6	84
HLA (W6/32)	43
Ia (Da_2)	12
TdT	+
SIg	1

2. Cryostat section
HRP-PNL—definite "follicular" staining in cortex with groups of PNL^+ cells.

PNL. One *lymphoblastic lymphoma* of B-cell type (CB, Table IV) had only 7% lymphocytes binding FITC-PNL, despite the presence of complement receptors on most B lymphocytes. In Patient JY (Table IV) with *follicular lymphoma*, PNL-binding cells were not detected. This biopsy sample was from a patient treated for recurrent disease over 2 years. Her phenotype was atypical in 2 previous biopsy samples, the tumour cells lacked C3d receptors and did not express light-chain Ig.

In the single case of CB/CC/diffuse lymphoma (SD, Tables III & IV) little or no binding of PNL could be shown, either by HRP-PNL in section, or by FITC-PNL in suspension. This patient was leukaemic in addition to showing nodal involvement, and blood lymphocytes on 9 repeat tests did not bind PNL. One centrocytic lymphoma (LC, Table IV) did not show FITC-PNL positivity, though the SIg^+ $C3d^+$ phenotype characteristic of these lesions (Habeshaw *et al.*, 1979) was clearly expressed.

PNL^+ lymphomas

Clear positive binding of PNL was found in 4/5 cases of follicular lymphoma (CB/CC/F), in plasmacytoma, and in lymphoblastic lymphoma of B-cell type. In each positive case studied in suspension, the positivity ranged from 20% to 97% of the lymph-node cells. In tissue sections, positivity in follicular lymphoma (CB/CC/F) was restricted to the nodules, and was not expressed on lymphocytes in surrounding internodular tissue. All PNL^+ follicular lymphomas showed a B-cell profile (SIg^+ $C3d^+$) characteristic of this lesion (Habeshaw *et al.*, 1979). PNL positivity was also present in *B lymphoblastic lymphoma* (*LF*, *Table IV*) in which complement receptors were not expressed. No obvious correlation between SIg isotype and PNL binding could be established from this series.

DISCUSSION

Discussion of these results centres around the PNL reactivity of follicular B-cell populations, and the usefulness of FITC-PNL or HRP-PNL as markers in malignant lymphoma.

It is clear from previous studies of mouse and man that PNL binding is expressed by cells of both T and B lineage. In T cells, PNL binding is restricted to cortical lymphocytes in mouse and man, and to small numbers of peripheral T cells in the mouse (London *et al.*, 1978). However, whereas PNL binds to 80% of murine thymocytes, and histologically this appears to correlate with all cortical thymocytes (Rose & Malchiodi, 1981), the results presented here (Table V) and other reports (Reisner *et al.*, 1979) suggest that PNL does not bind to all cortical thymocytes in man.

Lymphocytes (presumptive B cells) in germinal centres but not in the corona bind PNL. This has been demonstrated in mouse (Rose *et al.*, 1980) and here for human tonsil and other lymph nodes. Moreover, in the present series substantial numbers of PNL^+ cells were found concurrently with similar numbers of Ig^+ cells (Table IV). It is known that the peripheral lymphoid tissue of unstimulated mice contains $< 5\%$ PNL^+ cells, and that Ig^+ cells are PNL^- (Newman & Boss, 1980; Rose & Malchiodi, 1981). However, in the mitotically active Peyer's patches, which contain germinal centres and 30% PNL^+ cells, double-labelling studies have revealed that 80% of the PNL^+ cells bear surface Ig (Rose &

Malchiodi, 1981). It may be assumed that in the present series of non-T-cell lymphomas the majority of PNL^+ follicular cells are also Ig^+, and the question arises whether such cells share the other follicular phenotype of C3d positivity. It has been shown in this paper that C3d positivity does not always correlate with PNL positivity. It may be that PNL positivity identifies only one of the follicular B-cell subsets. This subset can present as a SIg^+ $C3d^-$ cell (as in Patient LF, Table IV) or as a cell expressing C3d receptors (as in Patients LP and GB, Table IV).

One unexpected feature was the unequivocal positivity of a case of plasmacytoma, in which the plasma-cell component of the lesion was PNL^+. Plasma cells, like cortical thymocytes and germinal-centre lymphocytes, are not actively recirculating lymphocytes. They may be only temporarily sessile while in a certain state of differentiation. We would like to suggest that PNL may be a marker of *sessile* lymphoid populations—of T or B class. This may have important implications in the prognosis of lymphoma, as the tendency of PNL^+ tumours to become leukaemic should be less marked than for PNL^- tumours of equivalent phenotype.

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