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Peanut Lectin: A Useful Tool for Detecting Hodgkin Cells in Paraffin Sections

Peter Möller

Institut für Allgemeine Pathologie und Pathologische Anatomie
der Universität Heidelberg, Im Neuenheimer Feld 220, D-6900 Heidelberg,
Federal Republic of Germany

Summary. Using an extended peroxidase-antiperoxidase method receptors for peanut lectin (PNL) can be visualized in routinely fixed paraffin embedded tissue sections. PNL binding sites are numerous in human tissue. Each tissue, however, displays its specific binding spectrum and cellular binding pattern. 35 cases of Hodgkin's disease containing all histological subtypes were examined. A prominent, constant, and characteristic binding pattern in Hodgkin- and Reed-Sternberg-cells was found. PNL is proposed as an aid for detecting these cells in diagnostic histology. It might turn out to be a very useful reagent particularly in identifying the early lesion in Hodgkin's disease in which Hodgkin cells are small and scarce.

Key words: Peanut lectin – Peroxidase – antiperoxidase – Hodgkin cell

Peanut lectin (Arachis hypogaea agglutinin) reacts specifically with D-galactose beta-(1-3)-N-acetyl-D-galactosamine (Lotan et al. 1975) which is part of various glycolipids and glycoproteins. For that reason detectable amounts of PNL-receptor sites are wide-spread in biological structures (Reisner et al. 1977; Rose et al. 1981; Watanabe et al. 1981). Each tissue, however, has its characteristic binding spectrum. This report describes a focal aspect of PNL binding in the human lymphoreticular system that, apart from its scientific significance, might become a useful tool in detecting Hodgkin cells in the early stages of Hodgkin's disease or in equivocal lymph nodes.

Material and Methods

35 cases of Hodgkin's disease dating from 1980 and 1981 were obtained from the files of the Pathological Institute of Heidelberg University.

2 μ-thick sections were made from routinely processed and paraffin embedded tumour tissue using an microtome with disposable blades (Feather, Japan) to minimize cutting artifacts.

Offprint requests to: P. Möller at the above address

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Sections were streched and air dried at 37° C to avoid shrinking artifacts. The peroxidase-antiperoxidase (PaP-) technique (Graham and Karnovsky 1966; Sternberger et al. 1970; Taylor and Burns 1976) was applied with the following extension: after blocking the endogenous peroxidase first PBS-rinsing was followed by an incubation with commercially available PNL (E.Y. Lab. Inc., San Mateo CA 94401, USA) at a dilution of 1:60 (corresponding to 0.017 mg/ml lyophlized substance) in PBS pH 7.4 at room temperature in a moist chamber for 30 min. After a three step washing in PBS of 30 min duration rabbit- anti-PNL (E.Y. Lab. Inc., San Mateo, CA, USA) was used as primary antibody at a dilution of 1:120. A swine-anti-rabbit and a rabbit PaP-complex (both Dako, Denmark) were used as linking and labelling antibody, respectively. Peroxidase reaction was visualized with 3,3-diaminobenzidine (DAB) (Fluka, Switzerland) as chromogen. This procedure was followed by counterstaining with Mayer's hemalaun, dehydration and mounting with Eukitt. Instrinsic controls omitting the lectin, or one of the antibodies, or adding the specific sugar to the working solution of lectin incubation abolished the specific staining. A part of the sections were immunostained using a computerized incubation system (Hisiostainer, R. Jung GmbH, Nußloch, FRG).

Results

In all the cases examined and thus in each histological subtype of Hodgkin's disease the vast majority of Hodgkin cells and Reed-Sternberg cells have a peculiar dot-like paranuclear binding site for PNL (Figs. 1, 2) which is situated at the area of a nuclear inlet in mononuclear elements and surrounded by the nuclei in the centre of giant cells (Fig. 3a, b). This spot stains very brightly in cases of lymphocytic-predominance type and is small and clearly defined in lacunar cells of nodular sclerosis type (Fig. 4, arrows).

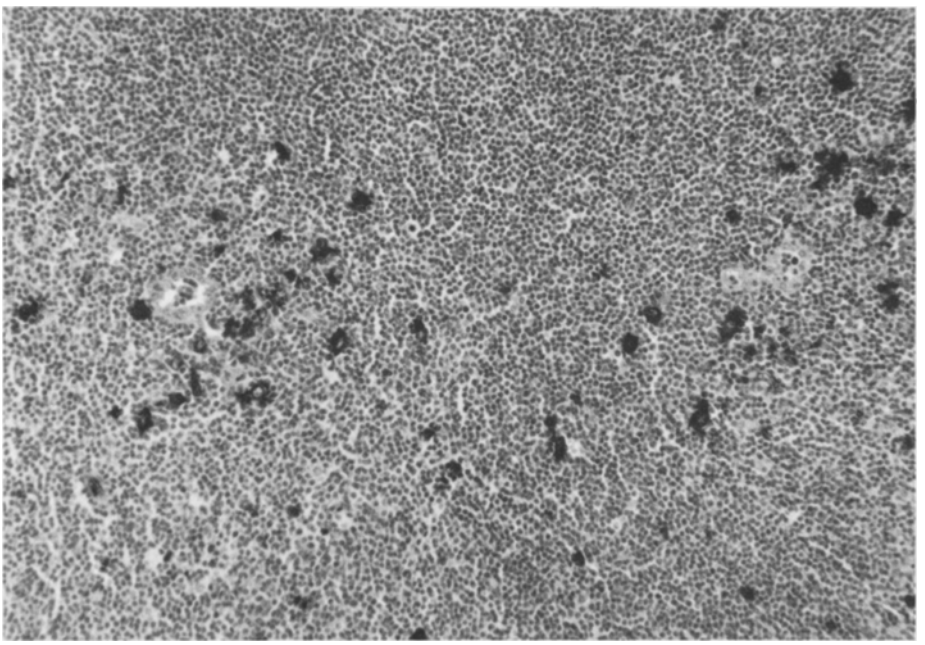
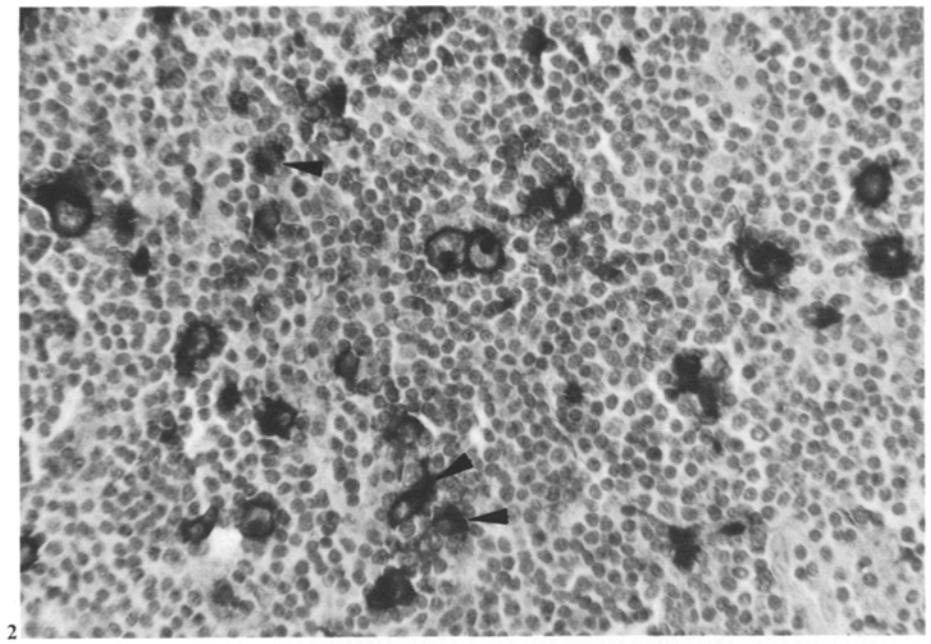


Fig. 1. Hodgkin's disease, lymphocyte predominance type. Scattered and clearly stained cells being exclusively Hodgkin cells and reticulum cells forming rosettes with small lymphocytes. (PNL, anti-PNL; PaP technique, diaminobenzidine (DAB)/hemalaun, × 148



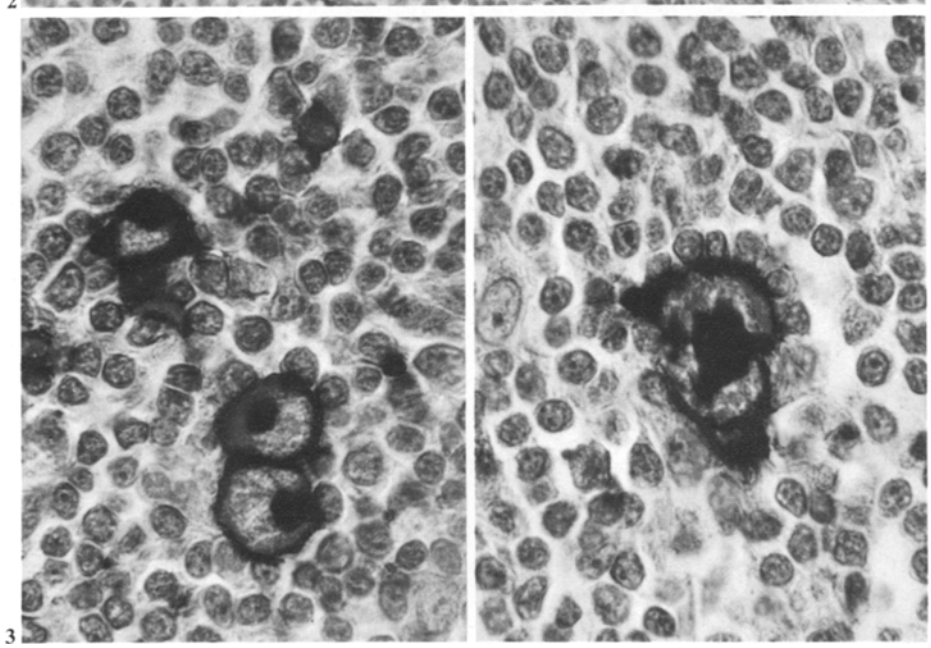


Fig. 2. Lymphocyte-predominance type of Hodgkin's disease. Hodgkin cells detectable by the dot-like paranuclear PNL-binding, in this case with a strong additional cytoplasmic staining. *Arrows* show some of the reticulum cells forming rosettes with small lymphocytes, clearly distinguishable from Hodgkin cells by cytomorphology. (PNL, anti-PNL; PaP technique, DAB/hemalaun, ×370

Fig. 3. a Three Hodgkin cells of the case depicted above; prominent spot in the paranuclear area at a nuclear inlet and a strong cytoplasmic staining. Note the attached lymphocytes on the cell surface. **b** Multinuclear Reed-Sternberg cell of mixed cellularity type; paranuclear dots form a confluent gross patch in the cell centre; brightly stained cytoplasm. Note the lymphocyte rosetting at the cell surface. (Both, (**a**) and (**b**) PNL, anti-PNL; Pap technique, DAB/hemalaun, ×932

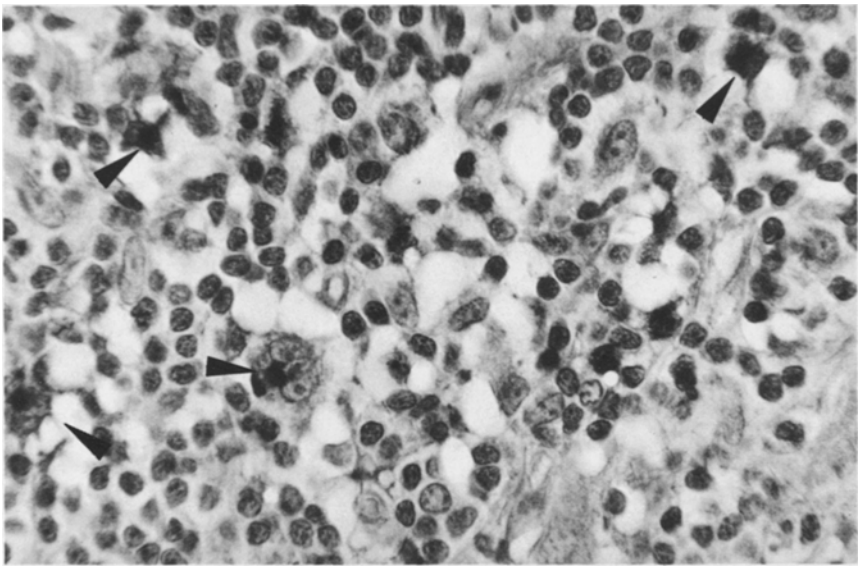


Fig. 4. Four lacunar cells of nodular sclerosis type. Note the retracted pale and non-stained cytoplasm; PNL-binding being restricted on the typical paranuclear dot-like area (*arrows!*). (PNL, anti-PNL; PaP technique, DAB/hemalaun. ×624

In the mixed and in the lymphocytic depletion type both, thick and minute paranuclear spots are visible.

As an inconstant feature many Hodgkin and Reed-Sternberg cells show a strong and granular cytoplasmic and others a surface-associated staining pattern (Figs. 2, 3b).

The surrounding lymphocytes, monocytes, eosinophils and fibroblastic reticulum cells, are always negative, however (Fig. 2). In cases of epitheloid cell reaction, these morphologically well-distinguishable cells exibit a faint and sometimes vague cytoplasmic PNL-binding. Apart from a perisinosoidal subgroup of secreting plasma cells there seems to be only one other cell within the T-region which constantly displays a very dense cytoplasmic PNL-binding. This medium-sized cell has a pale nucleus, an inconspicious nucleolus and characteristically forms rosettes with small lymphocytes (Fig. 2, arrows). Although extremely difficult to find in light microscopy this cell is a regular constituent of the normal lymph node (Möller, unpublished data).

To sum up, together with cytomorphology eluciated by the hematoxilin counterstain, PNL helps to detect the Hodgkin cells and their early form.

Discussion

In lymph node histopathology reactive changes, angioimmunoblastic lymphadenopathy, Lennert's lymphoma and (T-)CLL are occasionally difficult to differentiate from one of the various manifestations of Hodgkin's disease.

The method described above helps to detect the diagnostic Hodgkin cells by their peculiar PNL binding pattern. Examinating conventionally processed and paraffin-embedded tumour tissue, the patterns described were a regular result in the vast majority of Hodgkin and Reed-Sternberg cells whenever the method was followed exactly. False negative results can be due to inappropriate staining and have to be ruled out by looking for the intrinsic check points for positivity, such as the strongly staining and rosette forming cell of the T-region which is probably corresponds to the HLA-DR antigen positive interdigitating reticulum cell (Müller-Hermelink and Schwarting 1981; Janossy et al. 1981). In cases where there are preserved lymph follicles the centre macrophages have a granular cytoplasmic PNL binding site whereas follicular centre cells both, centrocytes and centroblasts show a faint surface binding (Rose et al. 1981; Möller, unpublished data). Diffuse, homogenous cellular or nuclear PNL-binding has to be regarded as an artifact and can be due to bad fixation or necrobiotic changes, apart from poor technique.

PNL has a substrate specificity for a disaccharide and is not a specific cell marker. The binding *pattern* however, used in addition to cytomorphology, might become a reliable tool for detecting cells such as the diagnostic Hodgkin cell at early stages of the disease or in equivocal cases.

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