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Lymphocyte Subpopulations in Human Lymph Nodes: A Normal Range

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Summary

Detailed surface marker studies were performed on 21 nodes from control subjects, 12 normal and 9 showing reactive hyperplasia. Reactive nodes showed a significantly decreased proportion of T lymphocytes (median 44%) and increased proportion of B lymphocytes (median 32%) when compared with normal nodes (median 68% and 18%, respectively). The increase in B lymphocytes was seen in all immunoglobulin classes studied. The values for normal nodes were comparable with three previous studies of nodes confirmed to be normal by histology. On the other hand, the results from reactive nodes were similar to the majoritiy of previously published "control" nodes, many of which were histologically abnormal. The importance of ensuring that control nodes are histologically normal is emphasized by this study.

Introduction

Surface marker studies have recently been carried out in lymphoid tissues of patients with lymphoma and leukaemia. It has been established that the majoritiy of cases of adult non Hodgkin's lymphoma (1, 2, 3) and chronic lymphocytic leukemia (4, 5) are neoplasms of B lymphocytes. Malignant T lymphocytes are found in Sezary's syndrome, mycosis fungoides, and a proportion of cases of childhood acute lymphoblastic leukaemia (3).

Surprisingly, the normal range of the lymphocyte subpopulations in lymphoid tissues has received only superficial attention. Furthermore, much of the published data on control nodes has been obtained from tissues which were not demonstrated to be normal by histology. Because of the paucity of similar studies we report our normal range, and a range from nodes showing reactive hyperplasia.

Materials and Methods

The preparation of lymphocyte suspensions from nodes, and the surface marker studies were performed using established methods, reported elsewhere (6, 7). 'T' lymphocytes were assessed by rosette formation with sheep erythrocytes, and 'B' lymphocytes by immunofluoroscence with polyvalent immunoglobulin (1g) antiserum and mono-specific antisera for 1gG, 1gM, 1gA, 1gK and 1gλ. In addition the 'B' lymphocytes forming rosettes with mouse erythrocytes ('B1' lymphocytes, 7) and the non-specific Fc receptors for 1gG were estimated. Results were determined for each receptor as a percentage of total lymphocytes, after ensuring cell viability.

Lymph nodes were taken directly from the operating theatre for preparation. The following patients were studied: 12 subjects undergoing surgery for conditions unrelated to lymph node disease had nodes removed from regions well clear of any obvious abnormality. Nodes were macroscopically and histologically normal. They were from the mesenteric or gastric groups in 6 patients undergoing laparotomy for diagnosis (3 patients) or for treatment of duodenal ulcer (3 patients); from the external iliac or inguinal region in 4 patients undergoing prostatectomy, hernia repair, saphenous vein ligation or excision of a naevus; and from the axilla in 2 patients having a biopsy elsewhere (breast, prostate). Nine other patients had nodes removed for diagnostic purposes from the axilla (5 patients), groin (2 patients), or neck (2 patients). These nodes were enlarged, and palpable on clinical examination. Although there was some variation in

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the histology, all showed reactive follicular hyperplasia; one showed prominent sinus histiocytosis. The clinical conditions were variable, but included rheumatoid arthritis, polymyositis, toxoplasmosis and mammary dysplasia. The surface marker studies were performed without knowledge of node histology. However, clinical information was available, and only in the diagnostic group was there any suspicion of lymphoma.

Results

The median, and range, of the lymphocyte populations from normal and reactive nodes are shown in Table 1. Reactive nodes showed significantly fewer 'T' lymphocytes and more 'B' lymphocytes. The 'B' cell increase was apparent in all 1g subclasses, although this failed to reach significance with 1gA. In Table 2 the percentage of total T and B lymphocytes is compared with the corresponding values from three previous studies with normal nodes (1, 9, 11). A similar comparison is made in Table 3 between the results from our reactive nodes and eight previous studies in which control nodes were stated to be (or were by inference) abnormal (1, 10–16). Except where stated, the methods used were similar to our own. We have excluded from the tables published figures which are not strictly comparable. For example there were three papers in which 'B' lymphocytes were measured by a non-specific complement receptor (17–19), one in which

Table 1 Percentage of lymphocytes in each class from normal and reactive lymph nodes. Median and range shown. *Wilcoxon rank sum test

		Lymphocyte Population								
Node histology	No	T SRBC	B PV 1g	B 1gG	B 1gA	B 1gM	B 1gK	B 1gλ	B MRBC	Fc
Normal	12	68 (61–77)	18 (12–28)	4 (2–15)	5 (0–9)	8 (3–16)	13 (7–21)	6 (3–11)	2 (0–10)	18 (2–35)
Reactive	9	44 (18–59)	32 (18–47)	14 (4–20)	7 (3–31)	20 (2–33)	18 (13–54)	11 (4–23)	5 (1–6)	14 (0–24)
Difference p*	–	< 0.01	< 0.01	< 0.05	NS	< 0.01	< 0.01	< 0.05	< 0.05	NS

SRBC – Sheep Red Blood Cell Rosette
PV – Polyvalent
1g – Immunoglobulin
NS – Not Significant

Table 2 The percentage of T and B lymphocytes from normal nodes – 12 subjects in present series compared with 3 previous publications. Median and ranges shown

Reference	No. Nodes	Source of Nodes	Major Class "T"	Major Class "B"
Verma (9)	6	Mesenteric (5) and tracheobronchial – unrelated surgery	–	22 (18–31)
Aisenberg (1)	5	'Histologically normal'	22 (17–76)	12 (7–18)
Eremin (11)	23	Abdominal, Inguinal and other nodes – unrelated surgery, histologically normal	70 (58–80)	27+ (18–37)
Present series	12	Unrelated surgery, histologically normal	68 (61–77)	18 (12–28)

+ – mixed antiglobulin rosette method

Table 3 The percentage of T and B lymphocytes from nodes showing reactive changes – 9 subjects in present series compared with 8 previous publications. Median and/or ranges shown, except*

Reference	No. Nodes	Control Group	Major Class 'T'	'B'
Brouet (12)	?	"subacute lymphadenitis"	40–70	20–40
	?	benign hyperplasia	80–95	–
Cooper (10)	8	reactive hyperplasia	43 (26–64)	–
Aisenberg (1)	6	hyperplasia	45 (29–52)	17 (0–35)
Eremin (11)	5	inflammatory – planned diagnostic procedure	49 (26–65)	45+ (25–64)
Gajl-Peczalaska (13)	3	uninvolved nodes – Hodgkin's disease	55–57	37 (24–49)
Payne (14)	22	reactive hypoplasia or patients with cancer	*53 ± 12	or *47 ± 11+ 25 ± 13
Stuart (15)	13	10 "without signs of lymphoreticular disease"; 3 with "lymphadenopathy"	41 (18–59)	32 (12–50)
Desplaces (16)	5	from thyroidectomy or hysterectomy	*48 ± 4	*24 ± 1
Present series	9	reactive hyperplasia	44 (18–59)	32 (18–47)

+ – mixed antiglobulin rosette method; *mean ± standard deviation

the results were not expressed as a percentage of lymphocytes (20), and two where monospecific surface Ig only was assessed (21, 22).

Discussion

The results of studying patients who did not present with a palpable lymph node has enabled us to define a lymphocyte population range in histologically normal nodes which is clearly distinct from nodes showing reactive change. The very careful studies of *Eremin* and co-workers (11) yielded similar results and the 11 clearly normal nodes from 2 other studies (Table 2) have conformed to a similar pattern. An exception is the low T lymphocytes count from *Aisenberg* and *Long* (1) who however found a more comparable T cell count (64%) using anti-thymocyte globulin. It seems quite clear that the 'T' lymphocytes predominate in normal nodes. Thus in the only 2 series of any size (6, 11), the percentage of 'T' lymphocytes in normal nodes has been 58–80% compared with 18–65% in inflammatory or reactive nodes. We have identified only 12–28% 'B' cells (median 18%) in normal nodes, a lower figure than *Eremin* and his colleagues (11). Although our immunofluorescent method might produce a lower figure than a direct antiglobulin rosetting reaction (23), as shown in the results from *Payne* et al. (14) in Table 3, we feel that the 'B' cell population is unlikely often to exceed 30% in normal nodes.

On the other hand, there is an increase in the B lymphocyte population in nodes showing reactive hyperplasia. No one class of surface Ig appears to be involved, although such a specific reaction might well be lost in our results which were from a somewhat heterogenous collection of abnormal nodes.

That 'B' lymphocytes appear in greater numbers in nodes showing reactive follicular hyperplasia is not surprising, as 'B' lymphocytes are known to predominate in lymphoid follicles (24). What is more surprising is that recent work in mice suggests that 'B' lymphocytes accumulate in nodes in response to

stimuli (oxazolone or bacterial adjuvants) traditionally thought of as activating a thymus dependent or cell mediated reaction (25).

Table 3 shows the percentages of lymphocyte subpopulations in reactive nodes. Some of these values have in fact, been accepted as falling within the normal range. However, when compared with the results obtained from histologically confirmed normal lymph nodes (Table 2) a difference is apparent, and in our studies this difference is statistically significant (Table 1). The necessity for careful attention to the absolute normality of nodes cannot be overemphasised, and to our knowledge only two other papers have fulfilled these criteria (1, 11).

In the study of malignant lymphoproliferative disorders monoclonal populations of lymphocytes are identified and comparison with a normal range is not essential. However, current interest in the responses of lymph nodes draining local malignancies accentuates the need for a truly normal reference sample. Already it has been suggested that some of the early and subtle changes in such nodes may include an increased proportion of B cells (11), particularly of cells with surface IgM (27). Such reactions in human nodes are worthy of careful study, but they will not be interpretable unless a range of normality can be clearly defined.

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