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Lymph Node Proliferation in Patients with Urological Tumours

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Summary. Reactivity of regional lymph node cells was determined in 87 patients with urological cancer by measuring the S-phases of the cell cycle using flow cytophotometry. Compared with a control group of 28 individuals with and without infectious disease regional to the extirpated lymph nodes, analysis of 250 lymph nodes in tumour patients exhibited either normal, reduced, or elevated S-phases. No relation could be established between the reduced or elevated pattern of reactivity of regional lymph node cells and any known tumour parameter, such as organ manifestation, histology, stage, and grade. Whether the determined S-phases correlate with the prognosis remains to be determined.

Key words: Regional lymph node reactivity, Cancer, Flow cytophotometry, S-phase.

Introduction

The reactivity of regional lymph node cells (RLNC) has been examined in a variety of cancer types in experimental animals [2, 4, 5, 9, 10, 15] as well as in humans [6–8, 12–14, 16, 17, 20]. In almost all of these studies RLNC activity was tested by immunological methods, e.g. mixed lymphocytes culture, mitogen stimulation, lymphocyte migration test, cytotoxicity etc. The results show variable patterns of RLNC activation. In most of these clinical studies in which the influence of tumour load was examined, RLNC reactivity was shown to be enhanced or depressed in patients with high or low tumour stages respectively [6, 12, 16, 17]. Also, in animal experiments this correlation has been confirmed [2, 9, 10, 15]. Table 1 summarises these data derived from the literature. Apart from immunological techniques, histological evaluation of lymph nodes in categories of normal, stimulated and depleted [12, 13] seems to be simple; however, errors due to subjective misinterpretation may be difficult to exclude. The common morphological parameter for this conventional histological evaluation is the proliferative activity of the nuclear DNA. Flow cytophotometry (FCM) on the other hand, seems to be a suitable method for objective measurement of RLNC proliferation by determining the S-phase of the cell cycle [21]. Using this method, tumour-induced proliferative RLNC changes can also be assessed [5].

Material and Methods

Regional lymph nodes were extirpated at the time of routine lymph node dissection for urological cancer. Lymph nodes which histologically proved to be free of metastases were used for FCM analysis. One half of a lymph node was processed in our laboratory and the rest of the material was sent for routine histological examination. In a total of 87 patients with cancer of the kidney (n = 24), urinary bladder (n = 11), prostate (n = 9), testicle (n = 40), penis (n = 2), and ureter (n = 1), 250 histologically ascertained tumour free lymph nodes from regional sites were evaluable. For controls, 28 lymph nodes from 28 patients with benign diseases operated on for various reasons were extirpated. In seven of these cases marked non-specific inflammation of neighbouring organs was present at the time of surgery.

After fixation of the lymph nodes in ethanol, the minced pieces were passed through a 75 μm nylon mesh. Further preparation and staining of the cells with Sulforhodamine SR101 and DAPI was performed as described by Goerttler and Stöhr [11]. The single cell suspension was measured in the impulse cytophotometer (ICP) 22 (Phywe Comp., Göttingen) which was connected to a multichannel analyser and IN96B minicomputer (German Intertechnique Comp., Mainz). The DNA histograms were fitted by Intertechnique computer program according to Stöhr et al. [19] and cell cycle analysis was performed [18].

For the purpose of this study S-phases of the RLNC's were determined and further analysed. Fig. 1 gives two examples for normal and pathologically elevated S-phases in tumour patients.

Results

In tumour-free controls with and without regional inflammatory disease, the mean value for the S-phase was $7.8 \mp 2\%$. Fig. 2 shows the distribution frequency of the determined S-phases in relation to the lymph nodes examined. It is of

Table 1. Regional lymph node cell reactivity (RLNCR) in human and experimental tumours with regard to methodology and tumour stage (References)

Tumour site	No. of cases	Method	RLNCR	Relation to tumour stage	Author
Human tumours:					
Breast	62	^{3}H-thymidine uptake	↑ ↓	Not examined	[8]
Osteogenic sarcoma	11	Cytotoxicity	↑	Not examined	[1]
Bladder	47	Histology	↑ ↓	Yes	[12]
Breast	30	Cytotoxicity	↑	No	[7]
Prostate	9	Histology, mitogen stimulation	↑ ↓	–	[13]
Bladder	7	Mixed lymphocyte culture	↓	–	[14]
Breast	69	Histology, lymphocyte migration test	↑ ↓	Yes	[16]
Breast	33	Mitogen stimulation	↑ ↓	Yes	[6]
Kidney, bladder, prostate	14	Mitogen stimulation	↓	–	[3]
Lung	100	Histology	↓	–	[20]
Stomach, colon	55	Mitogen stimulaton	↑ ↓	Yes	[17]
Experimental tumours:					
Breast (mouse)	–	Neutralisation test, cytotoxocity	↑ ↓	Yes	[9]
Different tumors (mouse)	–	Mitogen stimulation	↑ ↓	Yes	[10]
Hepatoma (rat)	–	Histology, cytotoxocoty	↑ ↓	Yes	[15]
Sarcoma (mouse)	–	Cytotoxocity	↑ ↓	Yes	[2]
Bladder (mouse)	–	Flow cytophotometry	↑	Not examined	[5]
Breast (rat)	–	Morphometry	–	Not examined	[4]

Table 2. Relevant data of patients with urological tumours and decreased or elevated S-phases with regard to tumour stage and grade

Tumour site (total No. of cases)	Stage (grade)	S-Phases	
		< 4%	> 12%
Kidney (n = 24)	$pT_3\ pN_0\ M_0\ (G_1)$	(n = 1)	
	$pT_{1-3}\ pN_{0-2}\ M_0\ (G_{1-2})$		(n = 6)
Bladder (n = 11)	$pT_3\ pN_0\ M_0\ (G_{2-3})$	(n = 2)	
	$pT_{2-3}\ pN_{0-3}\ M_0\ (G_{2-3})$		(n = 3)
Prostate (n = 9)	$pT_2\ pN_0\ M_0\ (G_3)$	(n = 2)	
	$pT_{1-3}\ pN_0\ M_0\ (G_{1-3})$		(n = 5)
Penis (n = 2)	$pT_2\ pN_0\ M_0\ (G_2)$		(n = 1)
Testicle (n = 40)	$pT_{1-4}\ pN_{0-4}\ M_0$	(n = 4)	
	$pT_{1-4}\ pN_{0-4}\ M_0$		(n = 19)

interest to note that S-phases in patients with significant infections do not differ from non-inflammatory controls. For further evaluation, the upper and lower limits of normal S-phases were defined at 12% and 4%, respectively.

In all urological tumour patients, S-phases from 250 regional lymph node sites revealed a mean value of 9.8 ± 5%, the difference to the control group being statistically significant by the F-test ($p < 0{,}001$). In comparison with the control group, S-phases of urological cancer patients showed a broader pattern of distribution (Fig. 3). Most of these values fell into the normal range between 4%–12%. A small portion of S-phases, however, showed values below 4% whereas a significant number exceeded the upper limit of 12%. A similar distribution of S-phases applied for each category of tumour, i.e. cancer of the kidney, bladder, prostate, and testicle. Those cancer patients who exhibited either elevated or decreased S-phases deserve special interest. Table 3 gives the relevant data of these patients with reference to tumour site, stage, and grade. The results of this compilation show a lack of correlation between the S-phases and any known tumour parameter. For examination of the influence of the primary tumour side on the retroperitoneal lymph nodes, we further analysed our patients with testicular cancer. The results in Table 3 show that no correlation exists between the extent of retroperitoneal tumour spread (N-stage) and the S-phases of tumour-free lymph node cells. In the same way, no effect of the primary tumour stage (T-stage) or the histological tumour type could be corroborated.

Discussion

Cell cycle analysis by flow cytophotometry is a relatively easy and reliable method to determine the S-phase, which in turn accurately reflects the proliferative activity of the cell population measured [21]. To our knowledge, this is the first report using this method to determine the lymph node reactivity in cancer patients. All types of urological cancer principally show decreased, normal, or elevated S-phases. In contrast, S-phases of tumour-free patients are within the normal range regardless of non-specific infections in the vincinity of the lymph nodes examined. Therefore, the decreased or elevated values in tumour patients must be considered as a specific effect of malignant disease. Thus

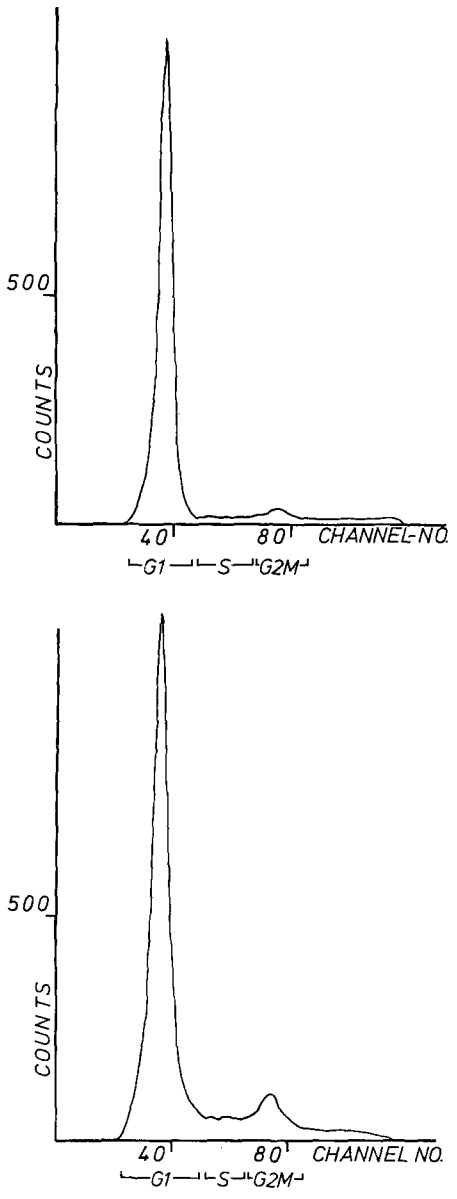

Fig. 1. a) DNA histogram of a left iliac lymph node from a patient with bladder cancer with normal S-phase (G_1 = 89, 7%; S = 6.7%; G_2M = 3,7%, CV = 3,7%). b) DNA histogram of a left obturator lymph node from a patient with bladder cancer (G_1 = 73,5%; S = 18,1%; G_2M = 8,4%; CV = 4,9%)

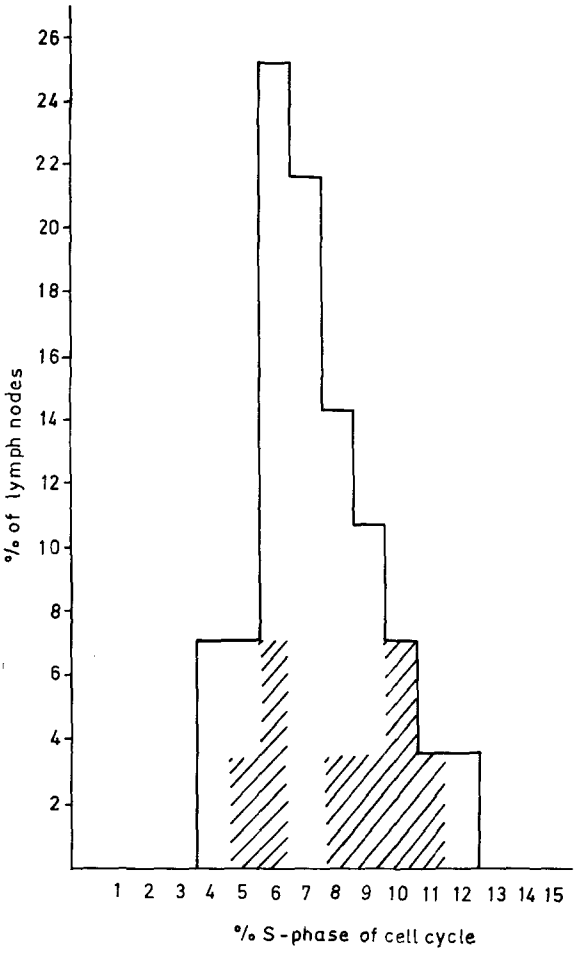

Fig. 2. Distribution frequencies of the determined S-phases in a control group with (▨) and without inflammatory disease (□) regional to the lymph nodes

our results confirm the findings obtained in experimental [2, 4, 5, 9, 10, 15] and human tumours [1, 3, 6–8, 12–14, 16, 17, 20] using conventional histological [12, 13, 15, 16, 20] or immunological [1, 3, 6, 7, 9, 10, 13–17] methods. Collste et al. [5] successfully employed FCM in determining the lymph node reactivity in a murine tumour system. In the literature, the pattern of reactivity was often related to the tumour stage with activated RLNC's in early disease and less reactive ones in advanced tumours [2, 6, 9, 10, 12, 15–17]. Analysing our patient data, however, no correlation could be established with any known tumour parameter such as organ manifestation, histological tumour type, stage, or grade. Whether RLNC reactivity may reflect the prognosis in cancer patients is difficult to assess since the follow-up, in most cases, is too short for drawing any conclusions.

In patients with primary tumours on the right or the left side one could expect a lateralised effect on the regional lymph nodes. To investigate this possible relationship, our large group of patients with testicular cancer, in whom lymph node metastastatic spread strictly follows homolateral pathways, was considered most suitable. Our results clearly show that RLNC reactivity in testicular cancer does not show any distinct pattern. Both normal and elevated S-phases on each side neither correlate with the extent of lymph node metastases nor with the stage or histology of the primary tumour. Analysing our extended material from lymph nodes in 87 patients there is no indication of a different side-dependent lymph node reaction, e.g. a weak stimulation of lymph nodes on the right side and a strong one on the left side.

From our material we conclude that the RLNC reactivity significantly varies in response to urological tumours.

Table 3. S-phases in testicular cancer patients in relation to the right of left side of the primary tumour with regard to the pN-stage

Side of testicular cancer	S-phases			
	Right side		Left side	
	Normal	Elevated	Normal	Elevated
Right (n = 23)	pN_0 (n = 8) pN_{1-3} (n = 4)	pN_0 (n = 6) pN_{1-4} (n = 5)		
Left (n = 17)			pN_0 (n = 3) pN_{1-4} (n = 8)	pN_0 (n = 3) pN_{1-2} (n = 3)

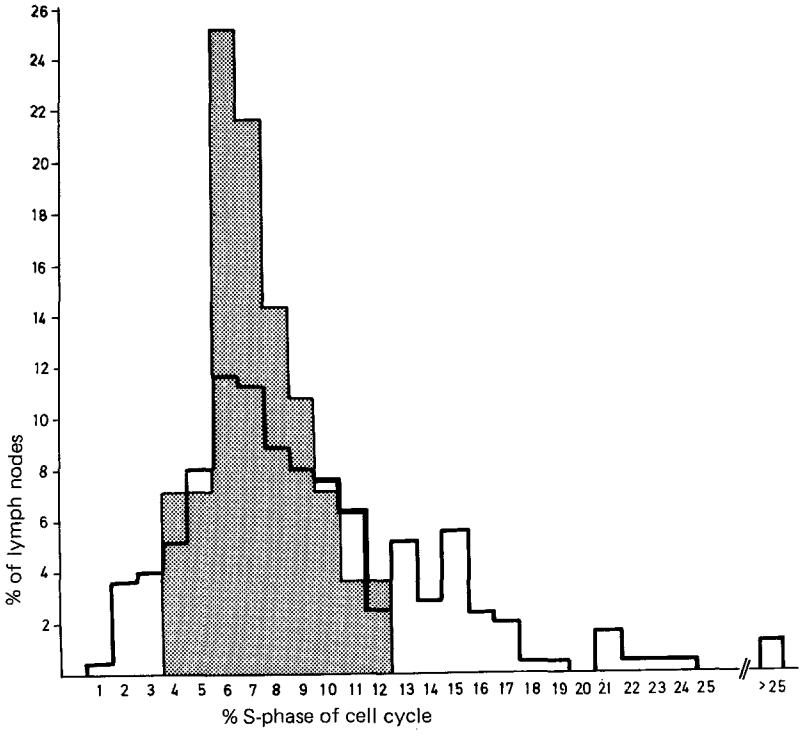

Fig. 3. Cumulative distribution frequencies of the determined S-phases in patients with urological cancer (□) in comparison with the control group (▨)

The biological implication of elevated or reduced S-phases, however, cannot be determined yet.

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