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A Preliminary Note on the Composition of Lymphocytes in Human Peripheral Lymph

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Summary

Pilot experiments on the composition of lymphocytes in human peripheral lymph, mainly from healthy human volunteers, are presented. The experiments undertaken so far show a reduced proportion of B cells and an increased proportion of "Ia" positive lymphocytes in peripheral lymph as compared to peripheral blood. If confirmed, these data would suggest that lymphocyte circulation through extravascular subcutaneous tissues is a highly selective process.

Introduction

Studies on the composition of macromolecules on the cell surface of lymphoid cells over the last years have uncovered extensive heterogeniety in the T cell compartment (1) of the immune system. A similar level of heterogeniety of lymphocytes belonging to separate differentiation pathways may also be present in the B cell compartment (2). This heterogeniety is likely to be mirrored by a similar functional heterogeniety.

Our previous studies in patients with chronic lymphocytic leukemia (CLL) and other malignancies have shown that the B/T cell ratio in peripheral lymph is significantly lower than in central lymph and peripheral blood; peripheral lymph contains a high percentage of T cells, but almost no B cells (3–4).

We have addressed ourselves to the question of whether the T cell population in peripheral lymph has the same membrane characteristics as the T cell population in peripheral blood and if the very low B/T cell ratio in peripheral lymph in patients with malignancies can be demonstrated also in healthy volunteers. A preliminary account of this study is given. Our data suggest that the B/T cell ratio and T cell subpopulations in normal peripheral lymph are different from that in the blood, indicating that lymphocyte circulation through extravascular subcutaneous tissue is a highly selective process.

Materials and Methods

The investigation was carried out on 5 healthy men, age 24–29 years,and one male patient, age 53 years, with advanced bronchial carcinoma. All had a superficial lymph vessel cannulated on one or both legs as previously described (5). These vessels drain skin, subcutaneous tissue and the perimuscular facia of the leg. Lymph was collected in plastic syringes with 20 IU heparin, taped to the leg throughout a 7 days period. Sampling of lymph for the present study was performed on day 3 or 4 after the cannulation had been established and contained lymph from 8 pm to 9 am. In this period the mean flow was 0,39 ml/hr range 0,06 (no. 242, table 1) and 0,54 (no. 260 and 262, table 1).

Surface marker studies

The cells from peripheral lymph were spun down (200 x g x 15 min), washed twice in Eagles MEM with Hepes buffer, resuspended in an appropriate volume of Eagles MEM, and cell counts were made. Peripheral blood lymphocytes were prepared as described previously (6).

The methods for sheep red cell rosettes (E-rosettes) and detection of surface immunoglobulin have also been described elsewhere (6, 7). Human "Ia"-like determinants were detected by indirect fluorescence. As a first layer, rabbit anti human "Ia" antigen (ASO18) from Sera Labs, Sussex, England, was used 1:20. As a second layer pig anti-rabbit immunoglobulin labelled with Fluorescein isothiocyanate (FITC) (F2190) from Dakopatts, Copenhagen, was

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Tab. 1 Surface marker studies on lymphocytes in peripheral lymph as compared to peripheral blood

Subject no.		E-rosette+ cells (T-cells)		Ig+ cells (B-cells)		Ia+ cells	
		Blood	Lymph	Blood	Lymph	Blood	Lymph
232	(N)*	44%	50%	15.5%	1.5%	29.5%	66%
234	(N)	58%	66%	20.5%	2%	34%	48%
242	(N)	52%	8%	–	–	–	–
256	(P)**	–	35%	–	2%	–	76%
260	(N)	33%	<4%	17%	1%	31%	68%
262	(N)	50%	4%	23%	0.5%	32%	71%
Mean (range)		47(58–33)	27(66–0)	19(23–15.5)	1.4(2–0.5)	32(34–29.5)	66(76–48)

*n = healthy men **P = patient

used in a dilution 1:40. Normal rabbit serum (1:20) + F2190 (1:40) served as controls.

Results and Discussion

As shown in Table 1, the proportion of Ig+ cells, i.e. B cells, are reduced by at least 90% in peripheral lymph. The low number of cells obtained from lymph made it impossible to count more than up to 200 cells. Thus, the number of Ig+-positive cells is somewhat uncertain and could be even lower.

With regard to E-rosette positive cells, the peripheral lymph showed tremendous variation, but was within the range of peripheral blood in three out of the six cases tested. In the other three cases, the number of E-rosette forming cells was very low. The reason for these variations is not clear, but technical shortcomings should be considered.

The volunteers belonged to various experimental groups where cannulation was performed primarily for other reasons than cell studies. Therefore, there are some variations in the procedures which have to be considered. Thus, the patient (256, Table 1), had radiotherapy which might cause a shift in B/T cell ratio (8). No. 232, 234, and 242 had peroral Penicillin treatment which would prevent bacterial growth in lymph samples. These volunteers were also cannulated without using Patent Blue Violet (PVB) which might have caused a local inflammatory reaction on the dorsum of the foot in the others where this hyperosmolar dye was used. Obviously, more studies have to be done. Another possibility to be considered is whether or not T cells of peripheral lymph may represent a subpopulation with reduced E-rosette forming capacity.

In our view the most interesting finding in this study, so far, is the high proportion of "Ia" positive cells in the peripheral lymph. The "Ia" determinant does not seem to be absorbed passively from the lymph as peripheral blood lymphocytes incubated in autologous lymph did not aquire "Ia" positivity. The nature of these cells remains obscure. They have the morphology of small lymphocytes and therefore appear unlikely to be monocytes. Their numbers are also too high to be monocytes (see *Sokolowski* and *Jakobsen,* this volume). It seems more likely that they represent an Ia-positive non-B, non-T lymphocyte or an Ia-positive subpopulation of T cells.

The last possibility is attractive for several reasons. First, in the subjects with high numbers of E-rosette-positive cells in the peripheral lymph, a large proportion of E-rosette-positive cells also carry "Ia" determinants.

Secondly, *Cohen* and *Livnat* (9) have described a subpopulation of T cells in mouse, called Initiator-T-Lymphocytes (ITL), which apparently carry antigen to the draining lymph node and there recruite effector T cells. The ITL may also be Ia+ (1).

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Discussion

Bremer: We have also done some marker studies on peripheral lymph lymphocytes of normal and lymphoma patients. Using the E-rosette technique we found a percentage of 70–90% T-cells in the lymph of all patients studied in contrast to your relatively low percentages, which to my opinion can only be explained by some methodological differences. The application of Patent-Blue seems to be of no importance, because all our patients had Patent-Blue to visualize peripheral lymphatics; however, for the B- and T-cell determinations we did not use the first lymph samples, which were blue, but we took the latest ones, which have been sampled at the 5–7th day of peripheral lymph drainage. The use of the first lymph samples may alter your findings to some degree; nevertheless, we cannot confirm this low percentage of T-cells in the peripheral lymph. Concerning the B-cells we agree that no significant numbers of B-cells can be detected, they are less than 1% even looking for the presence of other B-cell receptors such as Fc and C3 receptors on the peripheral lymph lymphocytes.

Ford: Would you be provoked into speculating whether the subset recirculating through nonlymphoid tissue represent "early" or virgin T lymphocytes or effector T lymphocytes corresponding to the Ly-2, 3^+ subset in the mouse?

Godal: It is possible that we are dealing with subsets of T-cells, which may or may not have a common marker. This slide is taken from Snell in a recent issue of the Immunological Reviews (vol. 38, 1977), and they have speculated a lot on your question, and I think what they are really thinking is that T-cell in the afferent lymph is the so called initiator T-cell (ITL), which would then be a so called early T-cell which has both the LY 1, 3 and d 3 markers, Ia positive, and possibly Fc receptor. There is some indirect evidence so far that this cell is a very interesting cell in the sense that it may carry the antigen to the lymph node and might be the cells that recruit other cells to respond to the antigen and to develop them into various kinds of effector cells. The critical question here is what about Ia determinants on these other cells, and as you see it seems that the number of Ia positive T-cells in the mice is increasing for the moment, so it is hard to make distinction based on this marker only.

They are definitely Ig negative. But if you do not do your test appropriately, then you tend to get them also positive because they will take up the antibody through the Fc receptor. In addition to be negative for immunoglobulin and the E-rosette receptor, the monocyte is of course phagocytic. You have to examine whether they have the capacity to phagocytose particles. That is the only way, otherwise you cannot make a distinction between non-T, non-B lymphocytes and macrophages on the basis of surface markers. I am sorry that immunologist is not more helpful in the particular area for the moment. There are macrophage antisera around, but I do not think one should rely too much on their specificity for the moment.

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