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ALTERED SURFACE TOPOLOGY AND MEMBRANE FUNCTIONS OF RAT THYMOCYTES ELUTED FROM NYLON WOOL COLUMNS

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SUMMARY

(1) Following incubation of thymocytes with nylon wool at 37 °C, the eluted cells showed an increase in the number of microvilli per cell and a concominant elongation of the microvilli (0.22 μm versus 1.15 μm).

(2) Cyclic adenosine monophosphate (cylic AMP) levels were lowered by 30–50 % in nylon wool-treated thymocytes.

(3) Nylon wool-treated cells showed an impaired Na^+-dependent amino acid transport system (2-aminoisobutyrate) whereas the Na^+-independent amino acid transport system (1-aminocyclopentane-1-carboxylate) was unaffected.

INTRODUCTION

We have previously shown [1] that leukemic human T-type lymphocytes (Molt-4 line) develop multiple, long surface microvilli during contact with sheep erythrocytes in a rosetting reaction. It is conceivable that their change of surface topology represents a more general contact response of lymphocytes and that it is accompanied by functional modifications. We have therefore examined the surface topology, amino acid transport and cyclic AMP content of rat thymocytes before and after passage through columns of nylon wool. Such columns have been shown to be effective in separating T-type lymphocytes in murine spleens or lymph nodes [2, 3] from B-type cells bearing surface immunoglobulins and/or possessing antibody-forming potential [3].

EXPERIMENTAL

Thymocytes. Thymuses were removed from anesthesized 6- to 8-week-old Wistar-Lewis rats (Charles River Breeding Laboratory). Suspensions of viable thymocytes were obtained as in [4].

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Nylon wool columns. We followed the procedure of Julius et al. [2] with Leukopak nylon wool obtained from Fenwal laboratories. After the cells had been equilibrated in the columns for 45 min at 37 °C, they were eluted at 37 °C. For scanning electron microscopy, portions of the cell suspension applied to the column and portions of the column effluent were mixed with 10 volumes of 2 % glutaraldehyde, pH 7.4 [1]. For functional studies aliquots of the cell suspension were applied to the column and portions of the column effluents were centrifuged at 4000 $g \cdot$ min (20 °C) prior to further processing. The cell density was adjusted to 3–4 $\cdot 10^7$ cells/ml in both cases. Cell viability, judged by Trypan blue exclusion [4], exceeded 90 % both before and after the column step.

Morphological and biochemical measurements. Scanning electron microscopy [1] and measurements of amino acid uptake and efflux [4] were carried out exactly as before. Cyclic adenosine monophosphate, cyclic AMP, was assayed essentially as in ref. 5.

RESULTS

Morphology. The thymocytes applied to the column exhibited the "bald" surface topology we have previously described [6]. Their average diameter, estimated by scanning electron microscopy at 5000 × (Fig. 1) was 4.52 μm (range 4–5.6 μm). Their surfaces bear only a few stud-like microvilli. We have measured the dimensions of the five longest villi on the surface of all cells (Table I) and found an average length of 0.22 μm with only 3 % of the villi longer than 0.4 μm.

The cells eluted from the columns differ strikingly in surface topology (Fig. 2),

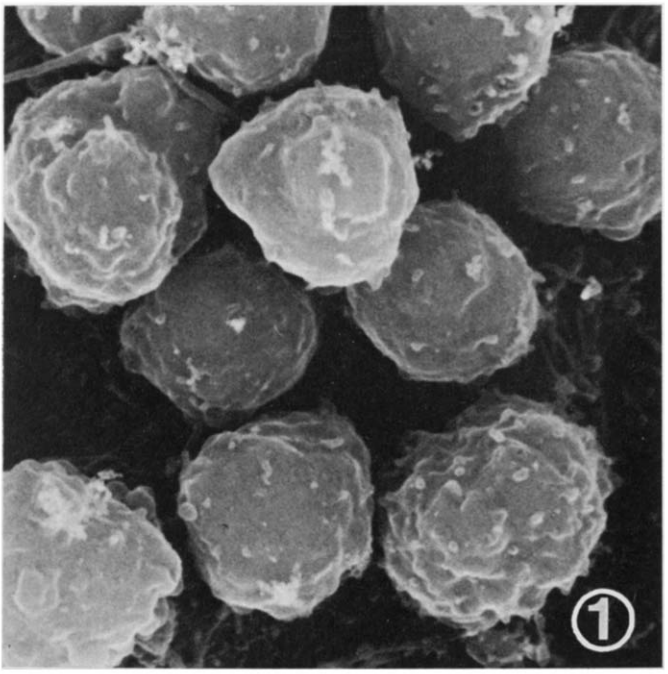

Fig. 1. Thymocytes before nylon wool column treatment. (Magnification 6200 ×.)

TABLE I

MICROVILLI ON THYMOCYTES BEFORE AND AFTER EQUILIBRATION WITH NYLON WOOL*

	Number of cells evaluated	Number of microvilli evaluated*	Average length of microvilli (μm)*	% distribution of microvillus length (μm)*								
				$\leq$ 0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	> 2.0
Before column	72	360	0.22	97	2.8	0.3	0	0	0	0	0	0
Eluted from column	69	345	1.55	10	9	20	17	14	8	9	4	9

* Only the five longest microvilli per cell were evaluated. More than 90 % of the nylon-treated cells observed in the scanning electron microscope fields chosen at random were found to have extended microvilli. The table lists the microvillus lengths of control (72) and nylon-treated (69) cells chosen at random from these scanning electron microscopy fields. Most control cells bore only 5–6 visible microvilli, compared to 10–20 after column treatment.

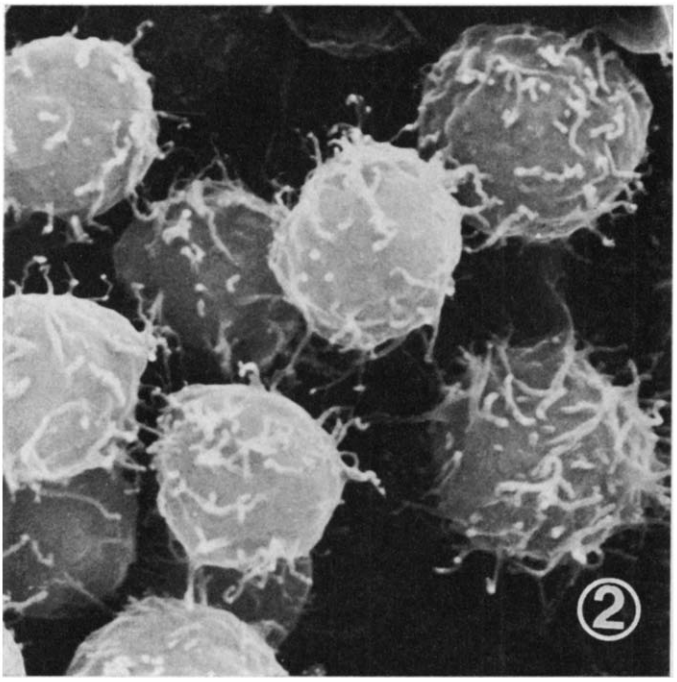

Fig. 2. Thymocytes after nylon wool column treatment. (Magnification 6200 ×.)

although their average diameter, 4.42 μm, was essentially identical to that of the control cells, the number of microvilli per cells appears increased (Fig. 2). In addition. the average length of the microvilli on the effluent cells is 1.15 μm greater (Table I) and they have a more undulating character (Fig. 2).

Amino acid uptake. Eluted cells exhibit a lower uptake (33 %–53 %) of 2-aminoisobutyrate than the applied cells (Table II) but there was no change in the efflux of 2-aminoisobutyrate or the uptake of 1-aminocylopentane-1-carboxylate. The differences in 2-aminoisobutyrate uptake by control cells and effluent cells remained stable for at least 3 h.

TABLE II

INFLUX AND EFFLUX OF 2-AMINOISOBUTYRATE, 1-AMINOCYCLOPENTANE 1-CARBOXYLATE UPTAKE AND CYCLIC AMP LEVELS BY RAT THYMOCYTES BEFORE AND AFTER EXPOSURE TO NYLON WOOL

Each experiment is an average of 5 replicates. Uptake is expressed as nmol amino acid/10^7 cells per min and the fractional efflux coefficient is expressed in min^{-1}. Cyclic AMP is in pmol/mg protein and is the average of 3 replicate determinations.

	2-Aminoisobutyrate		1-Aminocyclopentane 1-carboxylate	Cyclic AMP
	Uptake	Efflux		
Control	0.042 ± 0.002	0.021 ± 0.001	0.025 ± 0.001	164.36 ± 20.1
Column-treated	0.022 ± 0.001	0.020 ± 0.002	0.024 ± 0.001	84.15 ± 1.5

Cyclic AMP levels. The cells eluted from the nylon wool columns exhibited much lower cyclic AMP levels than the applied cells (Table II). Incubation at 37 °C per se did not alter cyclic AMP levels within the relevant time period.

DISCUSSION

Our observations show that contact of thymocytes with nylon wool increases the length of their microvilli, while decreasing intra-cellular cyclic AMP and also diminishing the uptake of 2-aminoisobutyrate. Similar changes of surface topology occur when human T-type lymphocytes contact sheep erythrocytes in rosetting processes [1], and preliminary studies (Lin, P.S. and Pohl, S., unpublished) as well as data from other investigators [7] indicate that in this case cyclic AMP also diminishes. Moreover, recent data on non-lymphoid cells [8] suggest a relationship between the appearance of extended microvilli and low intracellular cyclic AMP.

Circumstantial evidence suggests that the interaction of lymphoid cells leads to an integrated or concerted surface response, rather than plasma membrane impairment. (a) Thymocytes decrease the uptake of 2-aminoisobutyrate, but not of 1-aminocyclopentane 1-carboxylate, which is transported via a different pathway; moreover the efflux rate of 2-aminoisobutyrate remains unchanged. (b) Adhesion of lymphoid cells to nylon wool requires an active metabolism and does not occur at low temperatures or when respiration is blocked by azide [9, 10]. (c) The formation of T-cell rosettes, with its modifications of surface topology, also requires active energy metabolism [11].

While our observations fit the suggestion of Willingham and Pastan [8] of a correlation between intracellular cyclic AMP levels and surface topology, they do not support their contention that this correlation relates to the different surface properties of normal and neoplastic cells. On the contrary, our data suggests that we might be dealing with a process significant to normal lymphocyte physiology. For example, it is established [12] that T-cell precursors migrate from the bone marrow to the thymus and that T-cells move from the thymus into the blood stream, thereupon circulating through lymphoid organs such as lymph nodes and spleen. In lymph nodes the lymphocytes adhere to the cuboidal endothelium of the postcapillary venules and then pass through or between these endothelial cells. In the spleen, the lymphocytes pass through the marginal sinus surrounding the periarteriolar lymphoid sheath. All of these processes require cell surface contacts and implicitly involve cell surface responses. It is possible that the processes we have described relates to the physiological behavior of T-type lymphocytes.

Our results clearly indicate that one must apply caution to the use of columns to separate specific classes of lymphoid cells. Despite the fact that cells eluted from nylon wool columns retain some of their immunological functions [2, 3, 13], their surface morphology and amino acid transport are modified in the process, possibly through an alteration of cyclic AMP metabolism.

ADDENDUM

After we submitted our manuscript, van Ewijk et al. [14] demonstrated by scanning electron microscopy that lymphocytes passing through spleen or lymph node

postcapillary venules contact the endothelial walls by forming microvillus projections. Upon passing the endothelial lining, lymphocytes withdrew their microvilli and appeared smooth upon arrival in the lymphatic stroma.

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