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Human Lymphocytes: Similarity of B and T Cell Surface Morphology

Abstract. *When viewed by scanning electron microscopy, human lymphocytes fixed in suspension and processed with minimal cell loss appear uniformly covered with short microvilli. Contrary to previous reports, lymphocytes from subpopulations selectively enriched for T cells are villous and indistinguishable from B lymphocytes. Whereas lymphocyte surface architecture can change rapidly and substantially in response to environmental modifications, such as contact with an underlying surface, these alterations are similar for both B and T cells and do not serve to distinguish these subpopulations.*

Despite differences in function, surface receptors, and membrane properties B (bursa-equivalent, or thymus-independent) lymphocytes and T (thymus-dependent) lymphocytes have proved indistinguishable by light and transmission electron microscopy (*1*). With the recent application of scanning electron microscopy (SEM) to cellular immunology, attempts have been made to delineate differences in lymphocyte surface morphology which might provide a definitive means of identifying B and T cell prototypes. Previous investigators (*2–5*) have reported that human B and T lymphocytes can be distinguished by differences in their surface architecture. The B lymphocyte has been described as "villous," replete with microvilli, whereas the T lymphocyte has been represented as "relatively smooth" with few surface irregularities. However, in our experience, when human peripheral blood lymphocytes have been fixed in suspension and processed for SEM with minimal cell loss, no such dichotomy in cell surface morphology has been observed (*6*).

Mononuclear cells were obtained from the peripheral blood of seven normal human donors by Ficoll-Hypaque density gradient centrifugation (*7*). Lymphocyte yields by this procedure were greater than 80 percent. Subpopulations enriched for T lymphocytes were obtained by passing washed mononuclear cells over nylon columns (*8*). Nonadherent cells were eluted from the column, and adherent cells were displaced from the fibers by gentle mechanical agitation (*9*). Approximately 45 percent of the cells placed on the column were recovered in the nonadherent subpopulations and 10 percent in the adherent subpopulations. Surface markers were studied by standard methods (*10*). The percentage of cells with surface immunoglobulin and of phagocytic cells for each fraction is shown is Table 1. Cell viability, as estimated by the exclusion of trypan blue, was greater than 98 percent for all populations under consideration. After they were washed, cells from the Ficoll-Hypaque and column preparations were resuspended in Eagle's minimum essential medium with 10 percent fetal calf serum and fixed in suspension for 90 minutes at 23°C by the addition of an equal volume of 3.0 percent glutaraldehyde in 0.1*M* phosphate buffer (*p*H 7.2). Parallel samples were allowed to settle live onto silver filters for varying time periods before fixation. Fixed cells were sampled for SEM by trapping them on filters in a thin film of water, freezing them quickly, and vacuum-coating them with carbon and gold-palladium. By this procedure approximately 90 percent of fixed cells were retained for viewing by SEM (*6*). Duplicate samples were also processed by the critical point drying method (*11*). In some samples, individual cells examined by SEM were identified by their characteristic nuclear morphology as revealed by subsequent light microscopy. The samples were examined and micrographed in an Etec Autoscan microscope at 20 kv and 45° tilt.

As shown in Table 1, in nine experiments the surface architecture of 200 cells was analyzed in micrographs of each of three preparations—the unseparated Ficoll-Hypaque population and the non-

Table 1. Surface morphology of human lymphocytes. Values are means ± standard deviations. The total number of cells in each category = (200 per experiment) × (9 experiments) = 1800.

Property	Percentage of total cells in		
	Unseparated Ficoll-Hypaque population	Nonadherent population	Adherent population
Cell marker			
Latex ingestion	24.17 ± 9.29	1.12 ± 0.76	3.03 ± 2.42
Surface immunoglobulin	13.56 ± 6.40	1.72 ± 1.35	51.51 ± 21.82
SEM category			
(0) Devoid of microvilli; smooth or with slight surface irregularity	0	0	0
(1) Relatively smooth with occasional short microvilli	0.17 ± 0.50	0.28 ± 0.57	0
(2) Moderate number of microvilli	5.33 ± 2.06	5.06 ± 2.05	5.90 ± 1.41
(3) Large number of microvilli	82.83 ± 3.96	93.56 ± 1.93	88.11 ± 3.21
(4) Extensive number of surface microvilli or long, tortuous microvilli, or both	2.28 ± 2.17	0.56 ± 1.21	1.83 ± 2.03
(5) Ruffled cells	9.39 ± 4.24	0.56 ± 0.47	4.06 ± 3.05

adherent and adherent subpopulations from the nylon column. The number of microvilli per cell was estimated subjectively on a scale of 0 to 4 with ruffled cells (monocytes) considered separately. These numerical categories correspond to descriptions by Polliack *et al.* (*2*) of the surface topography of lymphocytes as follows: 0 to 1, "smooth"; 2, "intermediate"; 3 to 4, "villous." As illustrated in Fig. 1, a to c, the surfaces of virtually all nonruffled cells are villous. Even in samples rich in presumed T lymphocytes, such as the unseparated Ficoll-Hypaque population (approximately 60 percent of the cells lack surface immunoglobulin or fail to ingest latex beads) and the nonadherent column subpopulation (more than 90 percent of the cells lack surface immunoglobulin and fail to ingest latex beads), the cells display villous surfaces (*12*). The nonruffled cells in the heterogeneous adherent subpopulations are also villous and thus indistinguishable from cells in the unseparated and nonadherent samples. Subpopulations highly enriched for T lymphocytes by other methods also do not contain a substantial portion of smooth cells. Among lymphocytes stained with fluorescein-conjugated antiserum to human immunoglobulin and processed by the fluorescence-activated cell sorter (*13*), both deflected (B) and nondeflected (T) cells are equally villous. Likewise, lymphocytes which do not attach to plastic coated with antigen and antibody (T cells) are villous (*14*). More than 95 percent of the cells from both of these T-enriched subpopulations lack surface immunoglobulin or fail to bind aggregated human immunoglobulin, and more than 90 percent form E-rosettes.

The appearance of nylon-separated nonadherent and adherent lymphocytes settled live onto filters for varying time intervals was also examined. Brief settling (less than 5 minutes) can induce the development of numerous long microvilli and delicate ruffles (Fig. 1e) unlike the short microvilli displayed by the same subpopulations fixed in suspension. Similar surface changes are observed with both nonadherent (presumptive T) cells and adherent cells. Longer periods (30 minutes) of contact with silver filters before fixation affect cells variably, and increasing numbers of smooth-surfaced cells are seen in both subpopulations. Thus, the variation in surface morphology between lymphocytes within a particular B or T subpopulation which are fixed under different conditions (Fig. 1, b and e) may greatly exceed the variation between different subpopulations examined under identical conditions (Fig. 1, b and c).

Transmission electron microscopy (TEM) of human lymphocytes fixed in suspension corroborates the villous nature of lymphocytes previously described by others with TEM (*1*) and in this study by SEM. In particular, lymphocytes from T-enriched subpopulations—cells which adhere neither to nylon columns (Fig. 1d) nor to plastic coated with antigen and antibody—are villous. In addition, there are no readily discernible differences in the type, number, and distribution of intracellular organelles between lymphocytes from B- and T-enriched subpopulations examined under the conditions specified above.

To exclude the possibility that changes in surface topography were induced by preparative conditions, we examined mononuclear cells before and immediately after Ficoll-Hypaque density gradient centrifugation and contrasted their appearance to that of washed cells. When cells from each of these preparative steps are fixed in suspension, their surface morphology resembles that described for the washed Ficoll-Hypaque and column preparations. Thus, neither the Ficoll-Hypaque density gradient centrifugation nor the specified wash conditions detectably alter the villous surface morphology of lymphocytes fixed in suspension. The same villous surface anatomy has also been observed

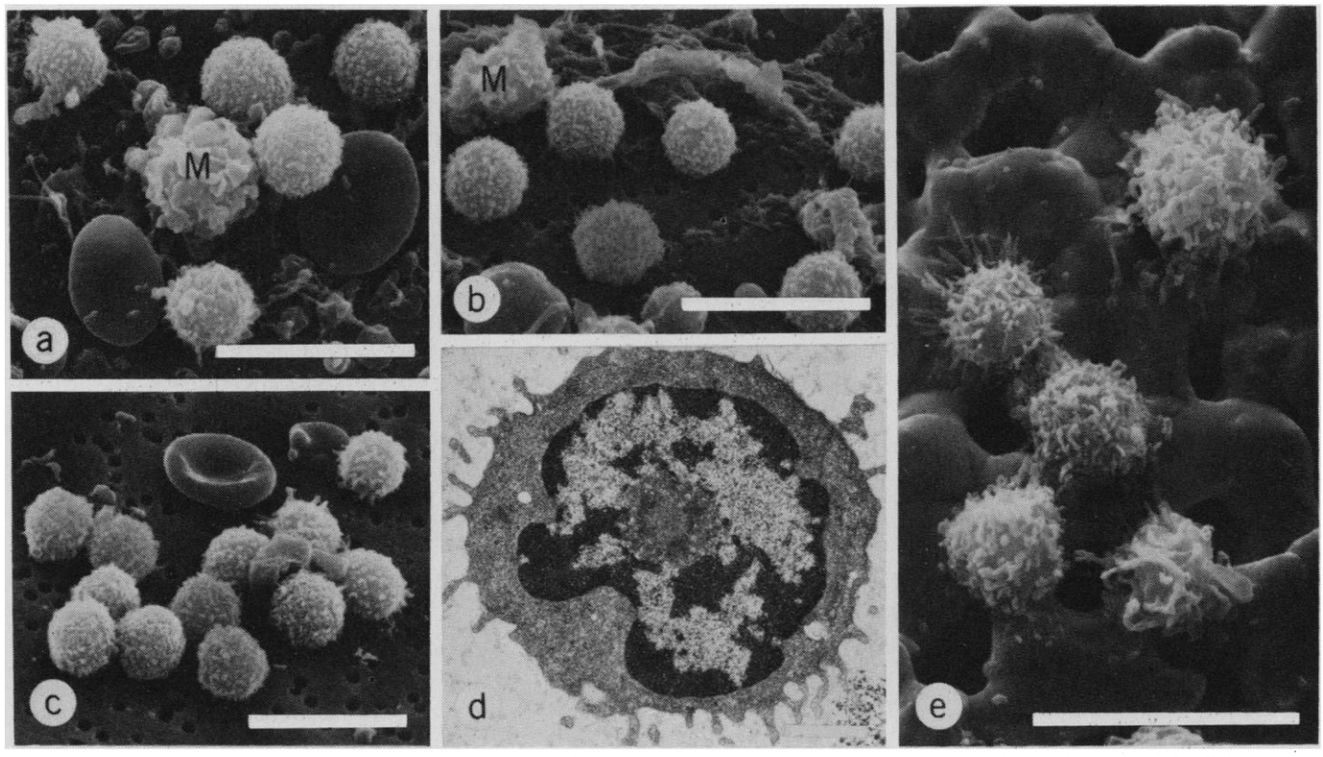

Fig. 1. The cells in (a) to (c) were fixed in suspension, processed for SEM on Nuclepore filters, and individually identified by correlative light microscopy (*6*). All cells are lymphocytes except when identified as monocytes (*M*). Scale bar represents 1 μm in (d) and 10 μm elsewhere. (a) Leukocytes from normal human peripheral blood after Ficoll-Hypaque density gradient centrifugation and before application to nylon column ($\times$ 2480). (b) Column nonadherent cells ($\times$ 2540). (c) Column adherent cells ($\times$ 2620). (d) Transmission electron micrograph of column nonadherent lymphocyte fixed in suspension ($\times$ 10,200). (e) Column nonadherent cells which settled live onto silver filters for 5 minutes before fixation ($\times$ 3250).

with lymphocytes from non-anticoagulated whole blood fixed directly after venipuncture (*6*).

Thus we find, in agreement with preliminary reports (*6, 15*), that human lymphocytes can appear villous when either fixed in suspension or settled live onto substrate. It is not possible to distinguish T and B lymphocytes on the basis of surface morphology. Similar observations have been made with spleen cells in this (*16*) and other (*17*) laboratories. These findings are in contrast to those of Polliack *et al.* (*2, 3, 5*), who reported that 80 percent of human peripheral blood lymphocytes are smooth and 20 percent are villous and suggested that these differences in surface morphology can serve to distinguish T and B lymphocytes.

Of several differences in preparative techniques that might account for these divergent observations, perhaps the most important is the method of cell collection for SEM. Pollack *et al.* (*2, 3, 5*) aspirated live cells onto silver membrane filters for variable time periods [1 to 15 minutes (*18*)] before fixing them in 1 percent glutaraldehyde. It has been demonstrated (*19*) that this procedure nonspecifically smooths the surfaces of cells. Degenerating cells with phycnotic nuclei also can have smooth surfaces (*20*). In the previous investigations by Polliack *et al.* (*2, 3, 5*), observations of surface morphology were made with samples which had been subjected to preparative procedures for SEM involving large (up to 80 percent), potentially selective cell losses.

From the findings reported here, we conclude that it is not possible to identify reliably T and B lymphocytes by their surface morphology. In addition, monocytes (*6*), polymorphonuclear leukocytes (*21*), and basophils (*19, 21*) may all appear covered with short microvilli and, under certain conditions, are indistinguishable from lymphocytes. It is therefore necessary to delineate the nuclear morphology of individual cells in order to identify them in heterogeneous populations studied by SEM (*6, 21*). Furthermore we, as others (*22*), find that cell surface form can change quickly and dramatically in response to a variety of environmental modifications. Thus, rigorous monitoring of experimental parameters and preparative techniques for SEM is essential if SEM is to be meaningfully employed in cell biology and clinical immunology.

ELAINE L. ALEXANDER
Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014
BRUCE WETZEL
Dermatology Branch, National Cancer Institute

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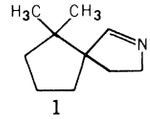
Polyzonimine: A Novel Terpenoid Insect Repellent Produced by a Milliped

Abstract. *A nitrogen-containing terpene 6,6-dimethyl-2-azaspiro[4.4]non-1-ene* (polyzonimine) *was isolated from the defensive secretion of the milliped* Polyzonium rosalbum. *Polyzonimine, which is repellent to such natural enemies of the milliped as ants, acts as a topical irritant to insects (10^{-4} M induces scratching in cockroaches). Its structure was confirmed by a five-step synthesis starting from 2,2-dimethyl-7-oxabicyclo[4.1.0]heptane.*

Years ago (*1*) the presence of "camphor" was reported from the defensive secretion of the milliped *Polyzonium rosalbum*. The purported utilization of a well-known plant secondary metabolite by an arthropod was intriguing, but since the claim was not based on definitive chemical evidence, a reinvestigation of the secretion was in order. We here report on the isolation from *P. rosalbum* of a highly volatile substance, which proved not to be camphor, but rather the novel nitrogenous terpene 6,6-dimethyl-2-azaspiro[4.4]non-1-ene (**1**), for which we propose the name *polyzonimine*.



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The *Polyzonium* were collected in New York State (Tompkins and Albany counties), in dead and decaying logs, mostly in beech-hemlock forest. Their defensive glands are serially arranged, one pair per each of most body segments, with openings that are visible as small pores along the margins of the body (Fig. 1, A and B). Seizing or pinching the animals with forceps causes them to discharge their sticky whitish secretion, which can be readily wiped from their bodies with small pieces of filter paper. Several thousand *Polyzonium* (body lengths 5 to 18 mm) were "milked" in this fashion to provide secretion for chemical analysis. Most of the animals were returned to their original location after milking.

Polyzonimine, $[\alpha]_D^{20}$, + 3.26° (chloroform), was obtained in pure form by preparative gas-liquid chromatography