Ultrastructural Localization of Acid Phosphatase in Rosetted T and B Lymphocytes of Normal Human Blood

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Using electron microcopy and cytochemical techniques, the authors determined the distribution of acid phosphatase (AcPase) within the organelles of lymphocytes from blood rosetted with either neuraminidase-treated sheep erythrocytes (E_n) or sheep erythrocytes coated with antibody and complement (EACs). Subsequently, the various reactive organelles of the rosetted lymphocytes were counted, affording a comparison of T and B cells. It was found that AcPase was present in ~80% of T cells and 45% of B cells and was most frequently observed in secondary lysosomes of varying size and content. Although more T cells than B cells were reactive for AcPase, the extent of reaction in some B cells clearly precludes the use of AcPase for differentiating the two cell lines. It should be recognized that while the E_n rosetting procedure detects T cells in a nonselective manner, the EAC rosette is a marker of a major subpopulation of B lymphocytes, ie, those bearing complement receptors. We believe that the distribution of lysosomal enzymes in B and T lymphocytes probably reflects the functional state of individual cells rather than being a reliable indicator of cell lineage. A surprising finding (which could be established only by a fine-structural study) was the fact that 20% of circulating "resting" T cells contained reaction product for AcPase within endoplasmic reticulum and the perinuclear cisterna indicating that these cells are actively synthesizing AcPase, probably due to a foregoing inductive event. Such stimulus could be the result of recent endocytosis of surface receptors in combination with antigen, antibody, or immune complexes and/or recent mitosis, or possibly some unrelated autophagic incident. (Am J Pathol 1981, 102:72-83)

LYSOSOMES act as the intracellular digestive tract of virtually all eukaryotic cells, with the exception of mature red blood cells. Although their presence in mitogen-activated lymphocytes has been established biochemically[1-3] and cytochemically,[1,2,4] very little is known about the lysosomes of normal circulating human lymphocytes. Furthermore, in recent years, lymphocyte lysosomes have attracted the attention of pathologists because of reports that some neoplasms of T-cell origin contain large quantities of acid phosphatase (AcPase),[1] as detected cytochemically, whereas lymphoid neoplasms of non-T-cell origin are characterized by relatively low levels of AcPase.[5-11] Whether these cytochemical differences are characteristic of normal T and B cells is not known. Investigators have considered this question in recent histochemical and biochemical studies of normal lymphoid cells but have reached conflicting conclusions.[12-19]

Although the lysosomes of other blood leukocytes have been studied in detail (see review,[20]), to date there has been no thorough study to document the fine-structural localization of lysosomal enzymes within B and T lymphocytes of normal human blood. With this in mind, we undertook the present study. We first reacted blood leukocytes from five normal adults with neuraminidase-treated sheep erythrocytes (E_n) and sheep erthrocytes coated with antibody and complement (EACs) to produce E_n and EAC rosettes, which are formed by normal T and B lymphocytes, respectively. We then determined the ultrastructural localization of the lysosomal enzyme AcPase in these

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rosetted lymphocytes. More limited fine-structural studies were performed for another lysosomal enzyme, arylsulfatase B. We found that AcPase reactivity was detected more frequently in T cells than B cells; however, the extent of reactivity in some B cells precludes using AcPase for reliably differentiating normal T and B cells. Furthermore, a minor population of T cells was discovered to be actively synthesizing AcPase. A preliminary report of this study has appeared in abstract form.[21]

Materials and Methods

Isolation of Normal Lymphocytes

Peripheral blood lymphocytes were isolated from five healthy adult human donors, three male and two female. Blood (30–35 ml) from each of the donors was mixed with heparin (10 units/ml), and the mononuclear cells were separated by a Hypaque-Ficoll gradient according to the method of Böyum.[22] The isolated mononuclear cells were adjusted to a concentration of 5×10^6 cells/ml in RPMI-1640 tissue culture medium containing 10% fetal calf serum. The distribution of stain with α-naphthyl butyrate esterase[23] showed that 75–90% of the cells were lymphocytes and 10–25% were monocytes. The cells were then processed for the presence of either T or B cell surface receptors as described below.

Detection of T Lymphocytes by Rosette Assay with Sheep Erythrocytes (SRBCs)

SRBCs stored in Alsever's solution at 4 C were washed and then treated with neuraminidase by a modification[24] of the method of Weiner et al.[25] Equal volumes of E_n (1×10^8/ml) and isolated mononuclear cells ($20–50 \times 10^6$/ml) were incubated at 37 C for 15 minutes. The mixture was then centrifuged for 10 minutes at 200*g* and kept in an ice bath for 4 hours. Preliminary experiments showed that 65–70% of the lymphocytes formed rosettes within 4 hours; overnight incubation at 4 C did not increase this value significantly. More than 90% of the cells were viable as assessed by trypan blue dye exclusion. Rosetted cells were fixed in glutaraldehyde as described below.

Detection of B Lymphocytes by Rosette Assay for Complement Receptors

The presence of complement (C) receptors was detected by rosette formation with EAC by the method of Bianco et al.[26] The EAC reagent consisted of SRBCs that had been sensitized with rabbit IgM antibodies to SRBCs (Cordis Corp., Miami, Fla) and whole mouse serum as the C source (Colorado Serum Co., Denver, Colo). In preparing the EAC reagent, we incubated sensitized SRBCs at 37 C with whole mouse serum for 30 minutes. This technique produces SRBCs coated with C3d.[26,27] Equal volumes of the mononuclear cell suspension ($20–30 \times 10^6$/ml) and freshly prepared EACs ($1–2.5 \times 10^8$/ml) were mixed and gently rotated at 37 C for 30 minutes. The percentage of rosette-forming cells was then determined. A rosette was defined as a mononuclear cell with at least three attached SRBCs.

Washed SRBCs and SRBCs sensitized with IgM (EAs) were used as control reagents for the EAC rosetting procedure. A small percentage of lymphocytes (0–5%) formed rosettes with either the SRBCs or EAs under conditions used for EAC rosetting. Most of these rosettes were composed of only three erythrocytes attached to a lymphocyte, in contrast to the numerous attached erythrocytes when EACs were used.

Cytochemistry

Rosetted cell preparations were immediately fixed by incubation in 1.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 containing 1% sucrose for 10–15 minutes at 4 C. They were then washed in the

Table 1—Percentage of T Lymphocytes Containing AcPase Reaction Product at Indicated Sites

	Normal donors				
Organelle	1 (N = 68)	2 (N = 54)	3 (N = 82)	4 (N = 94)	Mean %*
Perinuclear cisternae	18	11	0	6	9
Endoplasmic reticulum	25	6	12	19	16
Golgi cisternae	12	17	8	5	10
Small lysosomes (<200 nm)	31	35	50	46	40
Large lysosomes (>200 nm)	78	44	46	67	59
Reaction at any site	94	65	71	83	79

This analysis was based on 298 micrographs of thin sections of rosetted T cells from four normal donors.
N = number of micrographs of rosetted T cells; one micrograph for each T cell.
* Average of the four preceding values.

same buffer and incubated for AcPase (pH 5.0) or arylsulfatase (pH 5.5) and subsequently processed as previously described.[29] The substrates used for these procedures were B-glycerophosphate, Grade I, and para-nitrocatechol sulfate, respectively; both substrates were obtained from Sigma Chemical Co., St. Louios, Missouri. Control experiments involved either omission of the substrate or addition of the inhibitor NaF (0.01 M).

Electron-Miroscopic Study and Analysis of Data

Thin sections of rosetted lymphocytes were photographed, regardless of the intracytoplasmic content of the cells. Micrographs were taken at a magnification of 4500× unless otherwise indicated. Three observers (EP, SB, MK) independently scored the presence of rosetted cells. A rosette at the ultrastructural level was defined as a lymphocyte whose plasma membrane was in contact with three or more separate SRBCs. Point attachments with transected microvilli were considered valid contacts; an example of such an attachment is shown in Figure 2d at the 9 o'clock position (see Results). The localization of AcPase within organelles was analyzed by one observer (DB) and confirmed and counted by another (EP). It should be emphasized that we sectioned all of the tissue blocks and obtained the maximal number of micrographs which conformed to our original criteria from each category. It is for this reason that we have unequal numbers of micrographs, as indicated in Tables 1 and 2. The cells from Donors 1–4 were processed for the presence of T cell surface receptors, and those from donors 1, 2, 3, and 5 were processed for the presence of B cell surface receptors.

Controls

Since it was possible that the procedures used for lymphocyte isolation or rosetting might stimulate synthesis or enhance detection of acid hydrolases, lymphocytes were isolated by three different procedures (Ficoll-Hypaque centrifugation, dextran sedimentation, and buffy coat preparation) and incubated for AcPase activity without prior rosetting. The proportion of AcPase-positive cells and the ultrastructural distribution of the reaction product were similar in the unrosetted lymphocytes, regardless of the method of separation.

Results

Localization of AcPase in T Lymphocytes

T lymphocytes were identified in thin sections by the presence of three or more attached E_n (the rosette, shown in Figure 1). Typically, sites of contact between T lymphocytes and E_n were narrow point attachments similar to those previously described for E rosettes.[30] In many cases, the SRBC membrane was deformed toward the rosetted lymphocyte.

AcPase activity indicated by the presence of dense reaction product, lead phosphate, was evident at some intracellular site in ~ 80% of rosetted T lymphocytes. Table 1 summarizes an analysis of the sites of AcPase activity in the rosetted T cells. Figures 1–3 illustrate organelle reactivity. Among the 4 samples of T cells studied, ~ 20% of cells contained reaction product in the perinuclear cisterna (PN) (Figure 1), the endoplasmic reticulum (ER) (Figure 2a and b) or both (Figure 3c). Reaction product was present in both the ER and the PN in only a small proportion of the rosetted cells (~ 3%), whereas ~ 10% were reactive in the PN and 16% in the ER separately.

Reaction product was detected in one or more Golgi cisternae in 10% of the T lymphocytes (Table 1). Examples of Golgi cisterna containing lead phosphate are shown in Figure 3. In order to minimize the loss of enzyme activity, a brief fixation time was used. As a result, the Golgi cisternae sometimes were poorly preserved; however, they were usually

Table 2—Percentage of B Lymphocytes Containing AcPase Reaction Product at Indicated Sites

	Normal donors				
Organelle	1 (N = 59)	2 (N = 100)	3 (N = 31)	4 (N = 81)	Mean %*
Perinuclear cisternae	0	0	0	0	0
Endoplasmic reticulum	3	0	0	0	<1
Golgi cisternae	17	8	10	7	10
Small lysosomes (<200 nm)	22	39	19	10	22
Large lysosomes (>200 nm)	39	29	32	5	26
Reaction at any site	56	56	42	22	45

This analysis was based on 271 micrographs of thin sections of rosetted B cells from normal donors.
N = number of micrographs of rosetted B cells; one micrograph for each B cell.
* Average of the four preceding values.

preserved well enough to permit detection of intracisternal reaction product. In some cells, reaction product appeared limited to only one Golgi cisterna (Figue 3b). More limited studies were carried out with the use of a weaker fixative (.25% glutaraldehyde), and the results were similar.

Many rosetted T lymphocytes contained moderate numbers of membrane-bound organelles with morphologic and cytochemical characteristics of secondary lysosomes. These vacuoles contained whorls of membranes and other less well defined debris, as well as AcPase reaction product, lead phosphate (Figures 1–3). As many as 8–10 such vacuoles were present in some T cells. Another lysosomal enzyme, arylsulfatase B, was also present in these vacuoles (Figure 2d) but was not found in other organelles in 25 T cells so examined. All AcPase-positive lysosomes were segregated into two categories by size, using the lower limit of light-microscopic resolution (200 nm) as the dividing point. Analysis of the pooled data showed that T cells contained a mean of 0.73 "small" lysosomes/thin section (range: 0.51–0.88) and a mean of 1.06 "large" lysosomes/thin section (range: 0.66–1.58). Large lysosomes often contained membranous material arranged as myelin figures (see Figure 2c) or heterogeneous crystalloid debris (Figure 3c). Infrequent small reactive vesicles with a homogeneous content, which may be primary lysosomes, would have been counted in the "small" lysosome category. Typical multivesicular bodies (MVBs) were present in only 13 cells; of the 13, only 2 contained AcPase.

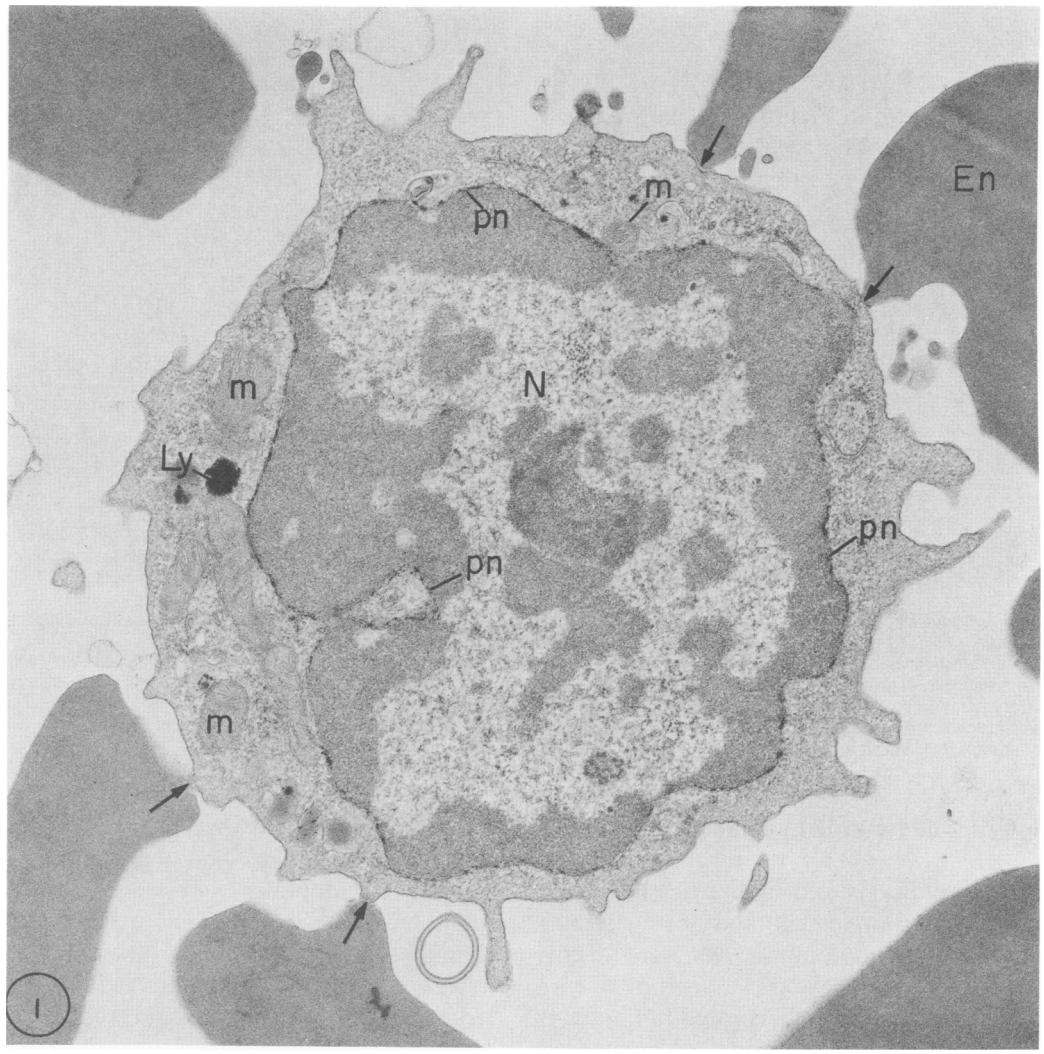

Figure 1—Localization of AcPase in a rosetted T lymphocyte. *Small arrows* indicate E_n-lymphocyte (*En*) point attachments. The electron-dense AcPase reaction product, lead phosphate, appears as black deposits throughout the PN (*pn*) and in a large lysosome (*Ly*). Note the mitochondria (*m*) and irregular cytoplasmic microvilli. (×20,000)

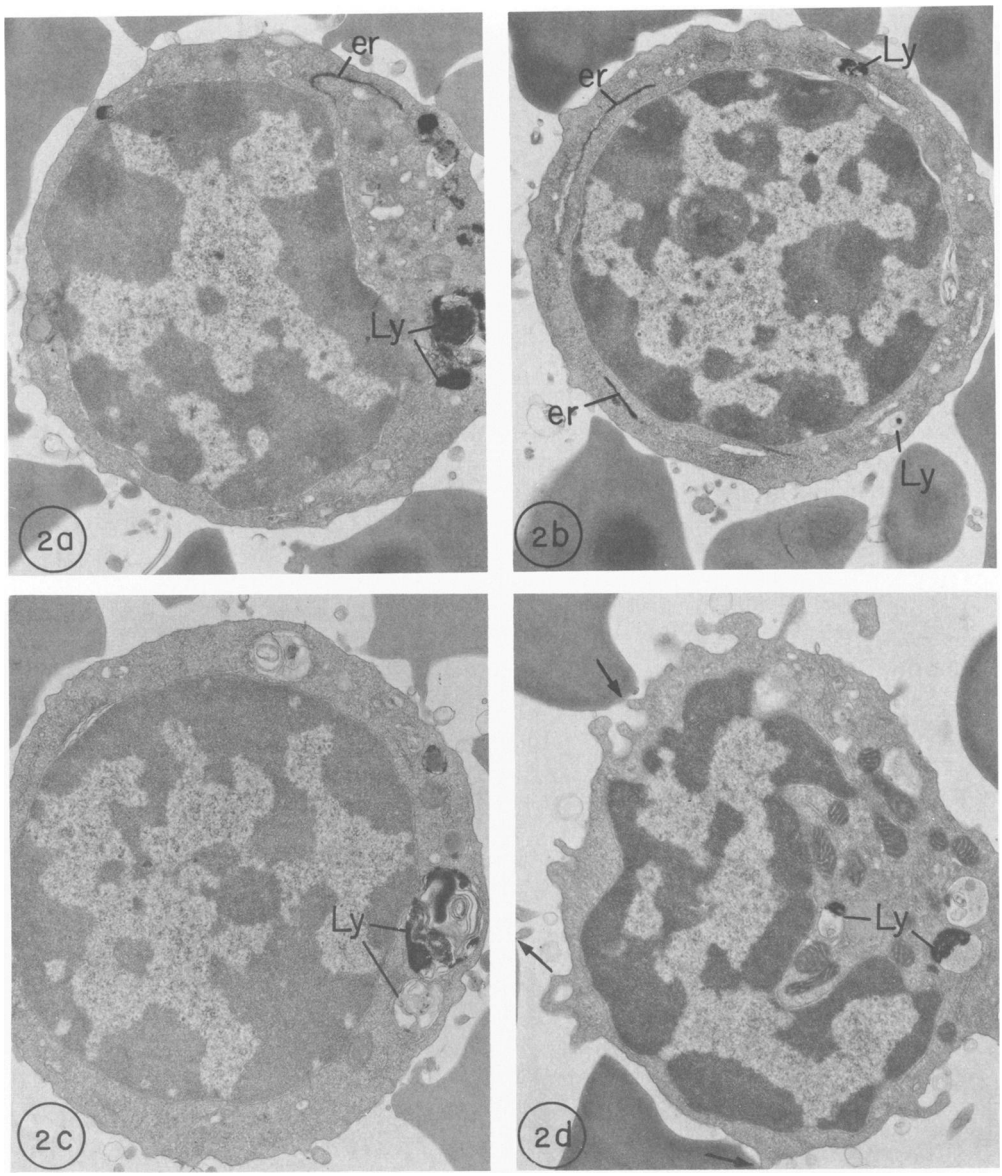

Figure 2—Localization of lysosomal enzymes in rosetted T lymphocyte. **A**—T lymphocytes surrounded by at least six adherent E_n. Dense deposits of AcPase reaction product are seen in several large secondary lysosomes (*Ly*), some of which also contain amorphous debris. A segment of the ER (*er*) also contains reaction product. (× 13,000) **B**—Five E_n are attached to the plasma membrane of this T cell. AcPase reaction product is present in segments of the ER (*er*) and in portions of two lysosomes (*Ly*). (× 12,000) **C**—AcPase reaction product is present in two secondary lysosomes (*Ly*), the contents of which are heterogeneous and include several prominent myelin figures. (× 14,000) **D**—Rosetted T lymphocyte processed for the presence of arylsulfatase activity. The reaction product, lead sulfide, is evident at the periphery of two large lysosomes (*Ly*). *Small arrows* indicate E_n-lymphocyte contact points. (× 13,500)

Localization of AcPase in B Lymphocytes

Normal B lymphocytes and monocytes have surface receptors for complement and form rosettes with EAC. In this study, monocytes were distinguished from B lymphocytes on the basis of one or more of four characteristics[29] illustrated in Figure 4. First, many monocytes contained phagocytosed EACs, whereas lymphocytes did not. Second, rosetted monocytes usually had long pseudopodia around adherent EACs even when phagocytosed EACs were absent. Pseudopodia were uncommon in rosetted lymphocytes and, if present, were generally shorter than those of rosetted monocytes. Third, some monocytes contained moderate numbers of small, homogeneous intracytoplasmic storage granules. Fourth, the larger cell size, typical reniform nucleus, and loose nuclear chromatin of monocytes were also helpful in

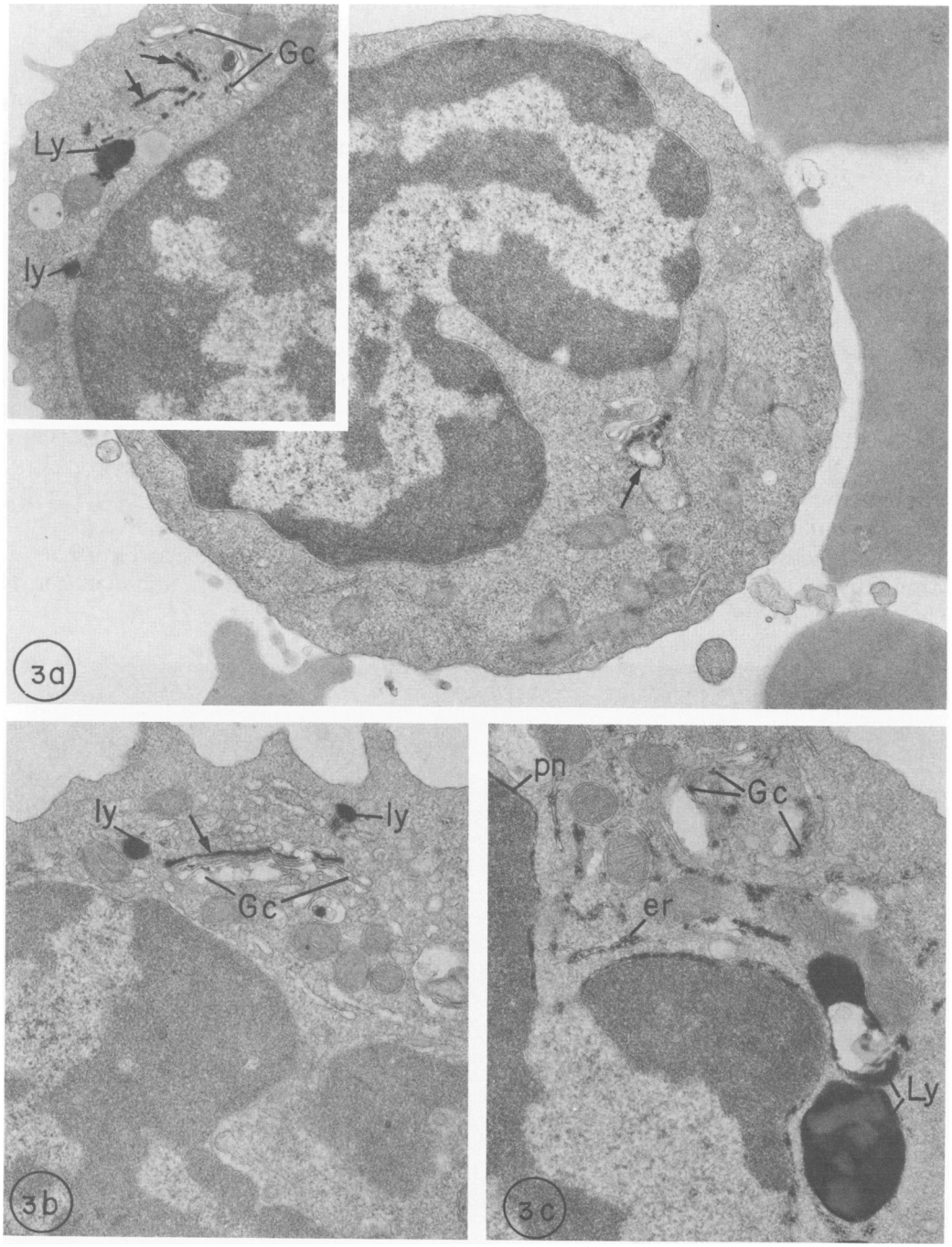

Figure 3—Distribution of AcPase in the Golgi region of rosetted T lymphocytes. **A**—Rosetted T cell containing AcPase reaction product in at least one, and possibly two, Golgi cisternae (*small arrow*). The remaining cisternae lack reaction product and are artefactually dilated. (×20,000) **Inset**—Unrosetted lymphocyte isolated Ficoll-Hypaque centrifugation. Heavy deposits of AcPase are evident in two adjacent cisternal elements (*arrows*), whose organization suggests GERL (see Discussion). Scant reaction product is present in the stacked Golgi cisternae (*Gc*). One large lysosome (*Ly*) and one small lysosome (*ly*) also contain reaction product. (×17,000) **B**—One Golgi cisterna of this rosetted T lymphocyte (E_n not shown) contains a dense lead phosphate deposit (*arrow*). Lead phosphate is not present in the adjacent cisternae (*Gc*). Several small lysosomes (*ly*) also contain reaction product. In this figure and **C**, portions of the Golgi cisternae and ER appear dilated and distorted, perhaps due to inadequate fixation. (×22,000) **C**—In this high magnification of a rosetted T cell (E_n not shown), reaction product is seen in several segments of the ER (*er*) and PN (*pn*) and in several large secondary lysosomes (*Ly*) containing heterogeneous crystalloid material. Smaller amounts of reaction product are also present in several Golgi cisternae (*Gc*). (×16,000)

distinguishing monocytes from B cells. Any rosetted cells with equivocal morphologic characteristics were excluded from the analysis.

Forty-five percent of EAC-rosetted B lymphocytes from 4 donors contained AcPase reaction product in some intracytoplasmic organelle (Table 2), typically secondary lysosomes. Two B cells with numerous reactive secondary lysosomes are shown in Figure 5a and b, and a very large lysosome is illustrated in Figure 5c. Table 2 summarizes the results of an analysis of all reaction sites in rosetted B lymphocytes. Reaction product was not present in the PN in any B cells and was present in the ER of only three. B cells contained a mean of 0.35 "small" lysosomes/thin section (range: 0.14–0.70) and 0.36 "large" lysosomes/thin section (range: 0.05–0.51). MVBs were seen more frequently than in T cells, and were observed in 28 cells. They were usually negative for AcPase; only 3 out of the 28 contained reaction product. There was only scanty reaction product for AcPase in the Golgi cisternae of 10% of B cells. Figure 5d shows the typically small amount of Golgi reaction product in a B cell when compared with that of T cells, Figure 3.

Additional Observations on Rosetted Cells

Rosetted T and B lymphocytes were morphologically similar. Both had round, occasionally eccentric and/or indented nuclei. (Figure 1-3, 5). A single nucleolus was identified in 37% of thin sections of normal T cells and 26% of thin sections of normal B cells. Multiple nucleoli were rarely seen in either T or B cells. Characteristically, the nucleus was surrounded by a thin rim of cytoplasm containing scattered mitochondria, sparse endoplasmic reticulum, occasional groups of 10-nm filaments, and several other membrane-bound organelles. Only some of these organelles proved to be lysosomes, as shown by their content of AcPase. Lysosomes of T and B lymphocytes did not differ morphologically. They were ovoid to elliptical in shape and irregular in size, measuring from 100 to 600 nm in diameter. No definite

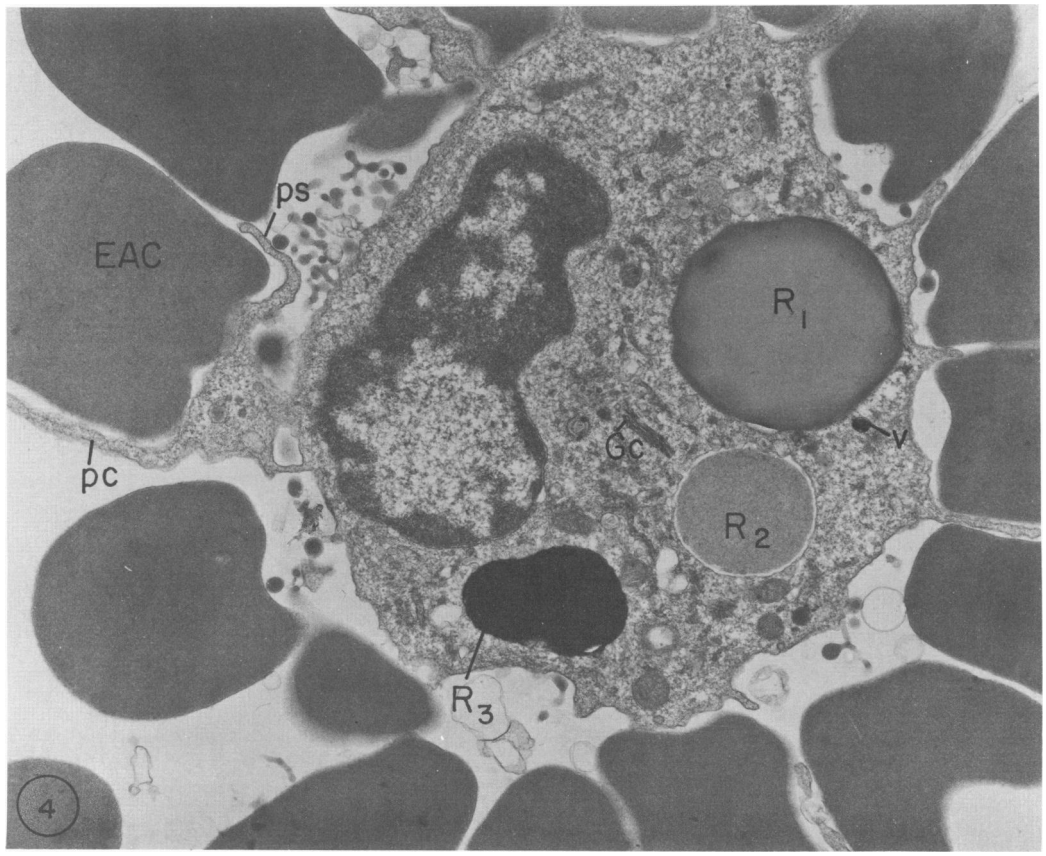

Figure 4—Monocyte rosetted with EAC and incubated for the presence of AcPase activity. Long cytoplasmic pseudopodia (*ps*) project around an attached EAC. Three ingested EACs are evident within phagocytic vacuoles (R_1, R_2, R_3). Note the intense AcPase activity in one of these vacuoles (R_3). Reaction product is also present in the Golgi cisternae (*Gc*) and a small vesicle (*v*). Because degranulation has probably occurred, cytoplasmic storage granules are depleted in this cell. (× 13,000)

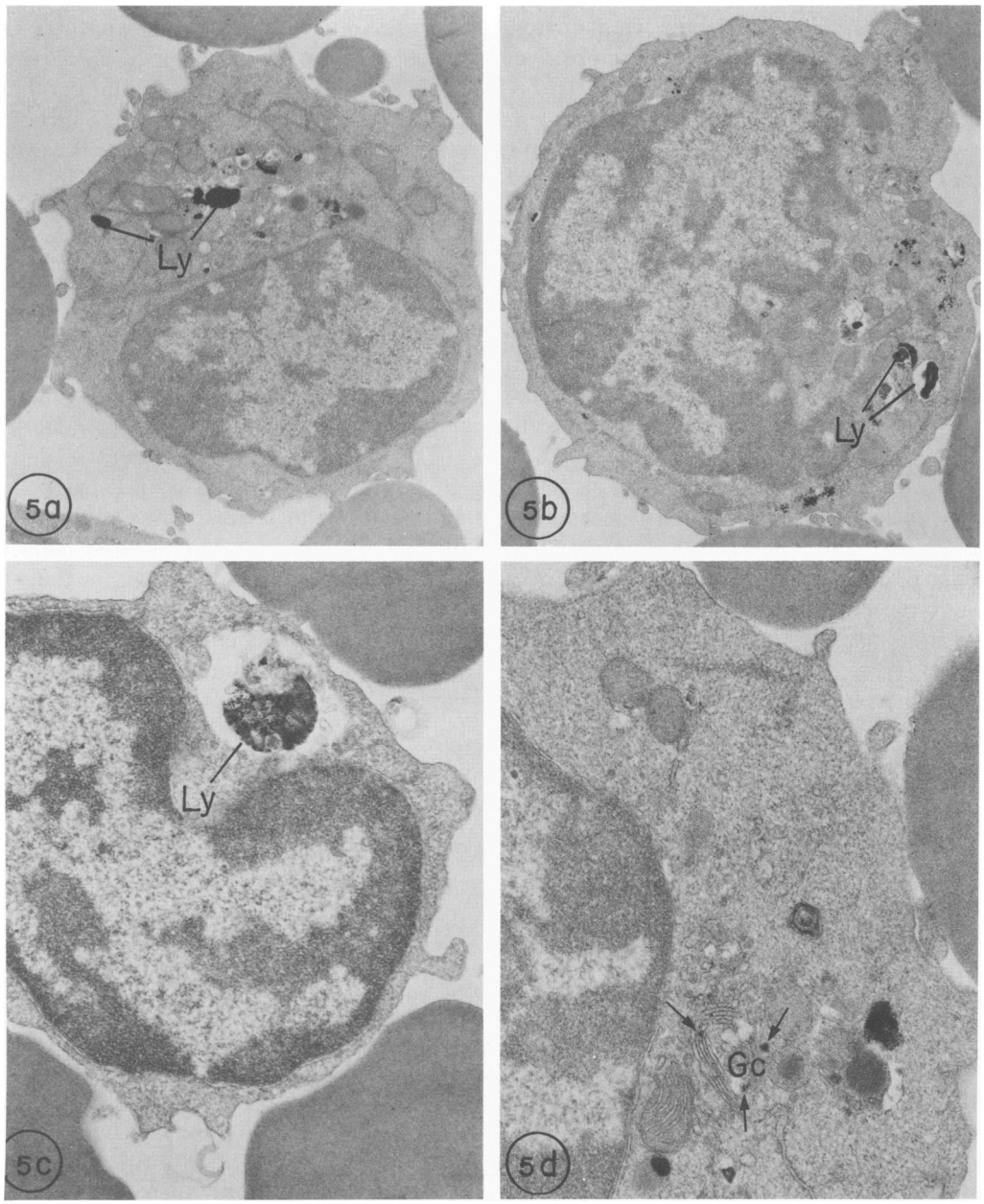

Figure 5—B lymphocytes rosetted with EACs and incubated for AcPase (**a–d**). Note the characteristic broad EAC–lymphocyte contact points, well demonstrated in **b**. Irregular deposits of AcPase reaction product are present in several large lysosomes (*Ly*), some of which appear to contain heterogeneous debris. An extremely large reactive secondary lysosome (*Ly*) can be seen in **c**. A portion of cytoplasm containing the Golgi complex (*Gc*) and a few lysosomes of varying size can be seen in **d**. Although the Golgi cisternae (*Gc*) of 10% of B cells contained reaction product for AcPase, it should be noted that this was only a few specks of lead phosphate and greatly differed quantitatively from the reactive cisternae in the Golgi region of T cells (compare with Figure 3). Microvilli are also apparent on the surfaces of both of these cells. (**a**, × 12,500; **b**, × 13,000; **c**, 17,000; **d**, 25,000)

Gall bodies[31] were encountered in rosetted cells. In addition, we occasionally observed membrane-bound organelles with heterogeneous content that morphologically resembled lysosomes but were AcPase-negative. No reaction product was seen within cells in which incubation for AcPase was carried out in the presence of NaF or in the absence of substrate.

Discussion

In comparison to other leukocytes, blood lymphocytes have been considered to be poor in lysosomes (32, 33). Lymphocytes do not contain easily recognizable storage granules corresponding to primary lysosomes—as do neutrophils, eosinophils, and to less an

extent, monocytes (20). However, some lymphocytes do contain moderate numbers of AcPase-positive organelles, many of which are large enough to be seen by the light microscope. (It has long been recognized that 5–10% of circulating human lymphocytes contain azurophilic inclusions measuring 0.3–0.6 nm. Bessis[29] states that these inclusions correspond, at least in part, to lysosomes. We concur with this interpretation.) To our knowledge, the present report is the first documenting the distribution of AcPase within the organelles in a large sample (more than 500 micrographs) of E_n and EAC-rosetted lymphocytes from normal human blood. It should be emphasized that while the E_n rosetting technique is a reliable and nonselective marker for T cells, the EAC rosette detects only a major subpopulation of B cells, ie, those that bear complement (C3d) receptors. (Some investigators have concluded that nearly all B cells bear surface receptors for complement.[27,28]) We cannot exclude the possibility that very small numbers of T_M lymphocytes may also be detected by this EAC technique.

Nonetheless, the results are of interest from both the quantitative and the qualitative points of view.

Quantitatively, this enzyme is present at some intracellular site in 45% of B cells and 79% of T cells. The main reactive organelles in both types of cells are largely secondary lysosomes. We further observed that although more T cells than B cells are reactive for AcPase, the extent of reaction in some B cells (Figure 5) clearly precludes using this method for distinguishing the two cell types.

As mentioned earlier, histochemical and biochemical studies evaluating the usefulness of AcPase as a discriminator of normal lymphocyte lineage have reached different conclusions,[12-19] although most suggest that AcPase is of limited value. To facilitate comparison of our fine-structural data with previous light-microscopic studies, we further subdivided the lysosomal category into reactive organelles larger or smaller than 200 nm, the level of resolution with the light microscope. Our data show that 59% and 26% of T and B cells, respectively, contain lysosomes larger than 200 nm. These organelles would be visible at the light level. These data are comparable to the results of several histochemical investigations of normal T and B or "non-T" cell populations in which AcPase was detected in 42–83% of normal T cells and 44–56% of B or "non-T" cell populations, respectively.[12-15] The higher proportion of reactive B or "non-T" cells in these reports, compared with our results (in the "large" lysosome category), may be due to contamination of their lymphocyte fractions by T cells, or monocytes; the latter are known to be rich in AcPase. In the present study we excluded monocytes, using specific morphologic criteria (see Results). Monocyte and/or platelet contamination may also account for the relatively high amounts of AcPase reported in non-T cell populations in two biochemical studies.[16,18] For example, in one of these, the investigators used a technique for quantitating the activity of the enzyme and concluded that AcPase was in fact a good marker of B cells.[16,17] As already mentioned, we have found that AcPase is not a reliable marker of lymphocyte lineage in normal circulating cells. (There is general agreement, however, that malignant T-cells contain high enzyme levels, whereas malignant B-cells contain low enzyme levels.[5-11])

To our surprise, AcPase was easily detected within the ER and PN in $\sim$ 20% of T cells, and moderate amounts were also seen in the Golgi cisternae of 10% of these lymphocytes. On the other hand, only rarely did B cells contain AcPase within their ER (<1%), and none was positive in the PN. About 10% of B cells, however, did contain trace amounts of lead phosphate within some Golgi cisternae. These organelles (PN, ER, and Golgi cisternae) are known to be the sites of lysosomal enzyme synthesis, segregation, and packaging in non-lymphoid cells (see review[34]). Thus, although there is little evidence for active lysosomal enzyme production in B cells, about 20% of normal circulating T cells are actively synthesizing AcPase. Indeed, the distribution of the reaction product in the Golgi region in some lymphocytes resembles GERL, Golgi endoplasmic reticulum–lysosome (inset, Figure 3, as described by Novikoff).[35] Inadequate morphologic preservation of the Golgi cisternae precluded a more detailed analysis of this issue. If analogy with other cell systems is valid, the finding of AcPase synthesis in some T cells implies that these circulating lymphocytes have been exposed to a recent stimulus sufficient to induce such synthesis. For example, phagocytosis or pinocytosis of digestible materials in macrophages leads to a tenfold increase in their intracellular content of lysosomal enzymes.[36]

The stimulus for induction of lysosomal enzyme synthesis in these circulating T cells is unknown; however, the possibility that lymphocyte isolation and rosetting procedures used in the present study might have stimulated AcPase synthesis was essentially excluded in separate experiments (see Methods). In these control studies, unrosetted leukocytes were processed for AcPase activity by the use of the same cytochemical procedure employed in later experiments with rosetted lymphocytes. A small portion of unrosetted lymphocytes contained AcPase reaction product in the same organelles as did the rosetted

lymphocytes, as observed earlier by Cawley and Hayhoe.[37] An example of the distribution of AcPase activity in unrosetted lymphocytes is shown in Figure 3 (inset).

In contrast to T cells, B cells of blood examined in the current investigation rarely showed evidence of active lysosomal enzyme synthesis. Yet we know that B cells must produce AcPase at some stage in their development, since many contain AcPase-rich lysosomes and trace amounts of the enzyme in Golgi cisternae (see Table 2). We presume that such synthesis occurs in B cells while they are in an extravascular site. In rodents, there is good evidence that the great majority of recirculating lymphocytes are T cells. Recent studies have established that B cells also recirculate, although B cell recirculation differs from that of T cells; i e, B cell recirculation is slower and takes place in follicular areas of the spleen, Peyer's patches, and lymph nodes.[38] Therefore, lysosomal enzyme synthesis and secondary lysosome formation could occur in B cells prior to release into the circulation. Cytochemical studies of lymph node or spleen ultrastructure after administration of an antigen to stimulate B cells might be of value in testing this hypothesis. In addition, it has been noted by others that T cells have a longer average life span than most B cells.[39] Hence, the accumulation of secondary lysosomes and autophagic debris could also be a reflection of cell age, and thus it would not be surprising that, in general, lysosomes would be more numerous in T cells. These are some of the questions that remain unanswered and represent potential subjects for further investigation.

What function do these digestive enzymes serve in normal lymphocytes? By analogy with the known role of lysosomes in other cells, it is likely that lysosomes in these cells serve the function of digesting material obtained from outside the cell (heterophagy) or the cell's own substance (autophagy). We have already mentioned the necessity of a certain degree of autophagy, particularly in long-lived lymphocytes. In terms of heterophagy, unlike other leukocytes, lymphocytes cannot phagocytize large particles but are capable of internalizing certain exogenous molecules,[40-44] such as antigens, antibodies, or lectins, and degrading them—presumably by delivery to lysosomes.[34] As mentioned in the introduction, lysosomes of human blood lymphocytes were first intensively studied in the mid 1960s[1-3] in cultured cells stimulated with the mitogen phytohemagglutinin (PHA). It was established that lysosome formation was a prominent feature of blast transformation in these cells, in that a marked increase in lysosomal enzymes occurred within 24 to 48 hours after mitogen stimulation. Furthermore, the presence of AcPase within the lysosomes of these stimulated cells was demonstrated by electron-microscopic examination, work which was significantly extended by Biberfeld.[4] Although he did not observe reaction product within the PN or ER, he was able to demonstrate that certain elements of the Golgi apparatus contained reaction product—particularly small vesicles, which he believed to be primary lysosomes. We concur with Biberfeld's conclusion that the small Golgi-associated vesicles labeled in his Figure 33, which appear to be "coated," are probably the equivalent of the primary lysosome in lymphocytes. In our study, we were disappointed that we were unable to document clearly their presence in unstimulated cells. In this regard, it should be recognized that to sample for this organelle in PHA-activated lymphocytes would be a definite advantage, because these cells are synthesizing large amounts of AcPase.

Finally, a few speculations may be pertinent concerning the meaning of the minor population (~ 20%) of T cells that are actively synthesizing AcPase within the circulation as follows: 1) Since AcPase synthesis is induced during blast transformation[1-4] and subsequent mitosis, is it possible that these T cells have recently undergone such events preceding their entry into the circulation? 2) Are some T cells undergoing an endocytic event—possibly receptor-mediated endocytosis[45]—due to an as yet undefined stimulus *in vivo*? 3) Could the synthetically active lymphocytes we observed correspond to any known T cell subtypes, or are these cells functionally unique? In this regard, the existence of various functionally defined subpopulations of T cells has recently been shown.[46-50] We cannot answer any of these questions on the basis of the present data. The still obscure relationship between these synthetically active T cells and known subpopulations of T cells continues to be a matter for future study.

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