Parallel Tubular Arrays in Severe Combined Immunodeficiency Disease: An Ultrastructural Study of Peripheral Blood Lymphocytes

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The ultrastructure of the lymphocytes from three children with severe combined immunodeficiency disease (SCID) is described. Parallel tubular arrays (PTA) were found in a large percentage of circulating lymphocytes (53%, 41%, and 13%) in three SCID patients when compared to age-matched controls. The size of these inclusions was quite variable, with some attaining a length of 1.7 μm. They contained a tubular substructure with a diameter of 36–44 nm. The PTA were mostly located in the centriolar and Golgi regions of the cytoplasm, and were sometimes membrane bound. A centriolar origin of the inclusion was suggested. A second inclusion, the tubuloreticular structure, was found in only 1.4% of the circulating lymphocytes from one SCID patient. The origin of the PTA and its occurrence in severe combined immunodeficiency disease are discussed.

SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID) is characterized by a deficiency in both B-cell and T-cell function. Although this disorder has been extensively studied from both an immunologic and histopathologic standpoint, very few electron microscopic studies have been performed on the circulating lymphocytes. Criswell et al. have found the lymphocytes from a patient with sex-linked SCID to have an increased ratio of cytoplasmic-to-nuclear area compared to normal lymphocytes. Willms-Kretschmer et al. have found the medium-sized lymphocytes from two patients with SCID (one sex-linked and one autosomal recessive) to be twice as numerous as the small lymphocytes. Lawton et al. have not found typical lymphocytes in the peripheral blood of a patient with the autosomal recessive type of SCID. The predominant type of mononuclear cell present has had numerous granules in the cytoplasm, which the authors consider to be of monocytic origin.

We have recently had the opportunity to study three female children with SCID—one with the autosomal recessive type and two with no family history of immunoincompetence. In addition to finding a high percentage of lymphocytes with abundant cytoplasm, the lymphocytes of all three children contained inclusions called parallel tubular arrays (PTA). The fact that they were present with such high frequency in all three patients prompted this report.

MATERIALS AND METHODS

Patient Selection

Three infant girls with SCID were admitted to the Arizona Health Sciences Center in the winter of 1975–1976.
Patient (1) was hospitalized at 3 mo of age with pneumonia and oral and skin ulcers which had failed to heal. She developed diarrhea and is currently being maintained in a laminar flow unit with parenteral nutrition. She has had one episode of sepsis and has chronic oral and diaper-area candidiasis. There was no family history of immunoincompetence.

Patient (2) was placed in a horizontal laminar flow unit within hours of birth because of lymphopenia and absence of a thymic shadow on chest roentgenograms. She has thrived in this environment without serious infections. Two siblings had had this disease proven at autopsy.

Patient (3) was referred at 3 mo of age because of recurrent episodes of pneumonia, rhinitis, thrush, and diarrhea. She expired at 4½ mo of age with sepsis, bone marrow failure, and malnutrition. There was no family history of immunoincompetence.

All three patients were lymphopenic. Evaluation of T-cell function yielded decreased E rosetting, lack of specific and nonspecific mitogen stimulation in vitro, lack of clearance of bacteriophage ΦX-174, and negative skin test results. Evaluation of B-cell markers and function yielded a decreased number of EAC rosettes, lack of membrane immunoglobulin staining, lack of immunoglobulin production, and lack of specific antibody production.

**E and EAC Rosette Preparation**

Venous blood was anticoagulated with 10 µg/ml of preservative-free heparin (Panheparin). Mononuclear cells were isolated on Ficoll Hypaque density gradients, washed three times in Hank’s balanced salt solution (HBSS), and resuspended at a concentration of 1 × 10⁶ cells/ml.

E and EAC rosetting assays were based on the method of Stjernswärd et al. Sheep red blood cells (SRBC) were obtained from Micro Tech Diagnostics, Tucson, Ariz., washed with sterile saline, and resuspended at 10⁶ concentration in HBSS. E rosetting was performed by mixing 0.25 ml of 1%, SRBC with 0.25 ml mononuclear cells and incubating at 37°C in 5% CO₂ for 5 min. Preparations were then centrifuged at 300 g for 5 min and were incubated overnight at 4°C. The percentage of cells with three or more adherent SRBC per 200 lymphocytes was determined.

EAC rosetting was performed utilizing mononuclear cells and an SRBC preparation specifically prepared in the following manner. A mixture of 2.5 ml of 10% SRBC, 7 ml HBSS, and 0.5 ml of a 1:100 dilution of sheep cell hemolysin (Hyland) was incubated at 37°C for 30 min. This complex (EA) was washed three times in HBSS and centrifuged at room temperature at 500 g for 5 min. Equal volumes of 5%, EA and a 1:20 dilution of fresh human AB, Rh(−) serum were incubated for 30 min at 37°C. The EAC preparation was washed three times in HBSS and adjusted to a 10⁶ concentration; 0.25 ml of 1%, EAC was mixed with 0.25 ml mononuclear cells and incubated for 30 min at 37°C. The percentage of rosette-forming cells was then determined.

**Preparation of Cells for Electron Microscopy**

Venous blood samples were drawn from the three SCID patients at 4 mo of age and anticoagulated with 10 µg/ml of heparin. Control blood specimens were similarly obtained from two age-matched controls (control patients 4 and 5). A third control specimen (patient 6) was obtained from a term infant on the day after birth. A mononuclear cell fraction was obtained using a Ficoll Hypaque gradient. The cells were centrifuged in conical plastic centrifuge tubes at 1100 g for 10 min in a Sorvall GLC-1 swinging bucket clinical centrifuge. The cells were fixed for 2 hr at room temperature in 3% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.2). The cells were then transferred to 0.1 M phosphate buffer (containing sucrose) and postfixed for 1½ hr in cold 1%, osmium tetroxide buffered with 0.1 M phosphate. The cells were dehydrated through a graded series of alcohol and embedded in Spurr’s low-viscosity epoxy. Then 1 µm sections were cut and stained with toluidine blue and examined with the light microscope. Ultrathin sections of the selected blocks were cut on a Sorvall MT2-B ultramicrotome and mounted on uncoated 200-mesh copper grids. Increased contrast was obtained by staining in 5% aqueous uranyl acetate followed by lead citrate. The grids were lightly carbon-coated before examination with a Hitachi HU-12 electron microscope. E and EAC rosettes were fixed for electron microscopy in a similar manner as that given for the mononuclear cell fraction.

**RESULTS**

PTA have been identified in a large percentage of lymphocytes from three patients with SCID (Figs. 1-4). Patient (1) had PTA in 53% of her lymphocytes,
while patients (2) and (3) had 41% and 13%, PTA, respectively (Table 1). This incidence was in marked contrast to age-related control patients (4 and 5) who had PTA in 7% and 0% of their lymphocytes, respectively. The lymphocytes from a term infant (control 6) on the day after birth also contained no parallel tubular arrays. The highest percentage of lymphocytes containing PTA occurred in the two infants with the highest number of PTA recorded in a single lymphocyte section (Table 1).

The lymphocytes containing the PTA had an abundant cytoplasm and con-
Fig. 2. Higher magnification of Fig. 1 identifying the inclusions as parallel tubular arrays (PTA). The tubular nature of the PTA is evident at this magnification. Numerous vesicles are found in the vicinity of the PTA. × 35,400.

tained numerous ribosomes and polyribosomes (Fig. 1). Several strands of rough endoplasmic reticulum were usually present and some of the lymphocytes contained an abundance of large mitochondria.

The PTA were quite variable in overall size, some being as large as 1.3 μm in diameter or 1.7 μm in length (Fig. 3). Lymphocytes contained either large PTA, small PTA, or a mixture of both. All of the PTA contained a tubular sub-
structure (Figs. 2–4) whose diameter ranged from 36–44 nm, with a mean of 41 nm. The tubules making up the PTA were usually packed in wall-to-wall contact. The PTA were located in the hof region of the nucleus in favorable sections and were frequently found near the centriole (Fig. 3) and the Golgi apparatus (Fig. 4). Figure 3 illustrates the close association of the PTA with the centriole. The microtubules of the centriole radiated out toward and between
the PTA but did not extend beyond the region of the PTA. The diameter of the centriolar microtubules ranged from 24 to 31 nm, with a mean of 26 nm. Some of the PTA were surrounded by a membrane (Fig. 3), but in most profiles distinct membranes were difficult to identify.

Some lymphocytes contained a mixture of parallel tubular arrays and homogeneous electron-dense granules (Fig. 4). In some sections hybrid granules could be identified which were partly tubular and partly homogeneously electron dense. SCID patient (3) was different from patients (1 and 2) in that...
Table 1. Frequency of TRS and PTA in Infants With Severe Combined Immunodeficiency Disease (SCID) and Age-matched Controls

<table>
<thead>
<tr>
<th>Patients</th>
<th>Percent Lymphocytes* Containing TRS</th>
<th>Percent Lymphocytes* Containing PTA</th>
<th>Maximum Number of PTA in a Lymphocyte Section</th>
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<tbody>
<tr>
<td>SCID</td>
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<tr>
<td>1</td>
<td>1.4</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>41</td>
<td>20</td>
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<td>3</td>
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<td>4</td>
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<td>7</td>
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*At least 200 lymphocytes were counted in each case.

her lymphocytes contained predominantly a mixture of PTA and electron-dense granules. No lymphocyte sections were observed that were comparable to the large PTA seen in patient (1) (Fig. 1) or the large number of smaller PTA seen in both patients (1 and 2). Many lymphocyte sections from patient (3) contained only electron-dense granules and no PTA.

PTA were identified in lymphocytes forming both E and EAC rosettes from patient 2. This child had 15\(^\circ\), E-rosette--forming cells and 22\(^\circ\), EAC-rosette--forming cells, while normal ranges were 55\(^\circ\)–75\(^\circ\), and 25\(^\circ\)–35\(^\circ\), respectively.

Another inclusion, called the tubuloreticular structure (TRS), was observed in the cytoplasm of 1.4\(^\circ\), of the lymphocytes from one patient (Table 1). The TRS, like the PTA, has a tubular substructure (Fig. 5). The tubules of the TRS are nonparallel and branching and are always surrounded by smooth membrane. The diameter of the tubules of the TRS ranged from 18 to 28 nm, with a mean of 22 nm; the mean diameter of the PTA was 41 nm. Only 0.5\(^\circ\), of the lymphocytes of control patient (4) contained the TRS. The PTA and the TRS coexisted in the cytoplasm of one lymphocyte from patient 1.

DISCUSSION

PTA are an unusual cell organelle apparently found only in the cytoplasm of lymphocytes. They were first described in 1968 by Hovig et al.\(^{14}\) in a 70-yr-old female suffering from chronic rheumatoid arthritis. In the same year, Huhn\(^{15}\) reported finding them in 12 healthy people. Since then they have been reported with varying degrees of frequency in disorders including chronic lymphocytic leukemia,\(^{13}\) infectious mononucleosis,\(^{11}\) systemic lupus erythematosus,\(^{16}\) Hodgkin disease,\(^{17}\) Chediak-Higashi syndrome,\(^{18}\) and late-onset amaurotic idiocy.\(^{19}\)

The present report describes their occurrence in three patients with SCID.

The overall dimensions of the PTA described in the SCID patients were variable and fell within the size range previously reported.\(^ {11,14,15}\) The substructure of the PTA consisted of parallel tubular structures with a mean diameter of 41 nm packed in wall-to-wall contact. Previous reports have indicated a range of 15–42 nm.\(^ {11,14,18,20}\) The average diameter of the tubules making up the PTA is approximately 15 nm larger than individual microtubules lying free in the cytoplasm or those directly attached to the centriole. Notwithstanding the difference in diameter between the tubules making up the PTA (41 nm) and those of
Fig. 5. Electron micrograph of an area of lymphocyte cytoplasm from patient 1 containing a number of tubuloreticular structures (TRS). These structures can be identified by their nonparallel, branching tubules. The TRS is surrounded by smooth membrane. × 48,900

the centriole (26 nm), it is possible that the PTA originate from the centriole, as has been previously suggested by Brunning and Parkin. Figure 3 illustrates the positional relationship of the PTA to the centriole. We have no comparable electron micrographs to support a mitochondrial or an endoplasmic reticular origin for the PTA.

To determine the significance of PTA in disease states, the frequency of these structures in circulating lymphocytes must be evaluated in normal individuals
as well as in specific pathologic conditions. The frequency of PTA in circulating lymphocytes of normal individuals ranges from 0 to 9%. Certain disease states have a similar low frequency of PTA. Conditions with greater than normal numbers of lymphocytes containing PTA are chronic lymphocytic leukemia (T-cell origin) and Hodgkin disease—90% and 67%, respectively. The percentage of PTA-laden lymphocytes in our infants with SCID (13%, 41%, 53%) is intermediate. The maximum number of PTA observed in a lymphocyte section appears to increase as the percentage of lymphocytes containing the PTA increases. This observation is consistent with those of Halie et al.

Previous ultrastructural studies in SCID patients did not report the presence of PTA in peripheral lymphocytes. One of these reports described another inclusion, the TRS, in several lymphocytes. This inclusion was seen in low frequency among our infants with SCID (Table 1).

A second inclusion was observed in our patients in cells which also contained PTA. This inclusion corresponded morphologically to the homogeneous electron-dense granules reported in some mature lymphocytes. Because of this dual occurrence, we believe that some PTA may be forerunners of homogeneous electron-dense granules. The finding of hybrid granules which had PTA in one portion and a homogeneous electron-dense material in another supported this assumption. Witzleben initially described dense, apparently homogeneous, osmiophilic inclusions among the tubular inclusions, although no hybrid granules were described.

PTA may represent normal organelles of precursor or immature cells seen infrequently in normal individuals. The presence of large numbers of PTA in Hodgkin disease, T-cell leukemia, and now SCID, all states with impaired cellular immunity, may indicate the presence of a large number of immature, immunoincompetent cells in the peripheral circulation. PTA have also been described in individuals with viral infections. Under such infectious conditions the finding of increased numbers of PTA in the peripheral blood may represent a "shift to the left" in the lymphoid series.

The report by Brunning and Parkin suggested that PTA are found in cells of T-cell origin. Our observations indicate that PTA are found in both E and EAC-rosette forming cells. Thus cells with both B and T surface markers appear to contain PTA. Lymphocytes containing these inclusions may therefore represent a developmental stage in lymphocyte differentiation.

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REFERENCES

3. Gupta S: Cell surface markers of human T
and B lymphocytes. Their profile in primary immunodeficiencies. NY State J Med 24-31, 1976
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