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SHORT COMMUNICATION

SCANNING ELECTRON MICROSCOPY OF LYMPHOID CELL LINES AND LYMPHOCYTES FROM THYMUS, BURSA AND BLOOD OF CHICKENS

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In the mouse and rat thymus-derived (T) cells and thymus-independent (B) cells had unique functions (Playfair, 1971), specific surface receptors (Lay et al., 1971; Nussenzweig et al., 1971; Basten et al., 1972) and surface antigens (Raff, 1969; Raff et al., 1971), and can be separated from each other (Wioland et al., 1972). Recently, Lin et al. (1973a) and Lin et al. (1973b) examined the possibility that the unique functions of these cells are related to unique morphological features by scanning electron microscopy (SEM). They found that thymocytes differ strikingly from peripheral lymphocytes and leukemia cells in surface morphology: thymocytes have few microvilli while the latter cells are studded with numerous, long microvilli. From further studies, they suggested that the microvilli were important for primary contact between lymphocytes and the environment. These studies suggested that using SEM it might be possible to compare T-cell and B-cell surfaces in detail. In chickens, lymphocytes from the bursa of Fabricius and from the thymus have been distinguished with antisera directed against a thymus (T)-specific and a bursa (B)-specific antigen, respectively (Forget et al., 1970). It was observed that the activities of the anti-B and anti-T sera were not restricted to bursal and thymic cells but were also seen with peripheral bursa-dependent and thymus-dependent cells. We decided to determine the origin of cultured lymphoid cells derived from chickens with Marek's disease and leukemia using SEM. For comparison lymphocytes derived from the thymus, bursa of Fabricius and blood were also examined.

Two lymphoid cell lines derived from Marek's disease lymphomas, MOB-1 and MSB-1 (Akiyama and Kato, 1974) were maintained in suspension culture with RPMI 1640 nutrient mixture supplemented with 10% fetal calf serum, 10% tryptose phosphate broth and antibiotics in a CO_2 incubator at 41 C. Two leukemia cells, 1104B and X5 (Hihara et al., in press) were gifts from Dr. H. Hihara and were also maintained in the medium described above in a CO_2 incubator at 38.5 C. 1104B and X5 were originally derived from a bursal tumor and subcultured for over 2 years after cloning. X5 line cells grow loosely attached to culture plates, so the cells

were pipetted gently before centrifugation. Culture media containing lymphoid cells were centrifuged at 1,000 rpm for 10 min, washed twice with Hanks' solution and then fixed with 1% glutaraldehyde in Sørensen's phosphate buffer, pH 7.3. Then they were dehydrated by passage through increasing ethanol concentrations of 50 to 100%, and washed twice with pure amyl acetate. They were dried by Anderson's critical point method, shadowed with carbon-gold and viewed with a JSM 50A scanning electron microscope (JEOL Ltd., Tokyo). Fifty ml of heparinized blood of a 3 month old chicken were centrifuged at 1,500 rpm for 10 min and the buffy coat was collected. After washing twice with Hanks' solution, blood lymphocytes were prepared by the method described above for observation by SEM. Lymphocytes were isolated from the thymus and bursa of Fabricius of a 3 month old chicken. Immediately after sacrifice the organs were removed and minced with surgical knives. Tissue clumps were gently pipetted in Hanks' solution and filtered through six layers of gauze to remove clumps. After centrifugation at 1,500 rpm for 5 min, cells were washed twice with Hanks' solution and prepared for SEM by the above method.

Most thymocytes were spherical, having an average diameter of 3.3 μ. Their surface was essentially smooth and no microvilli were seen at this level of resolution (Fig. 1). However, a very few cells had shallow undulations. Cells of the bursa usually differed clearly from thymocytes. They were 3.5 to 4.4 μ in diameter and had stud-like microvilli (Fig. 2). Although these morphological differences between the two cell types are distinct, it is not known whether these differences are related to the functions of the two cell types. Peripheral lymphocytes had an average diameter of 4.0 μ and about 50% of them had

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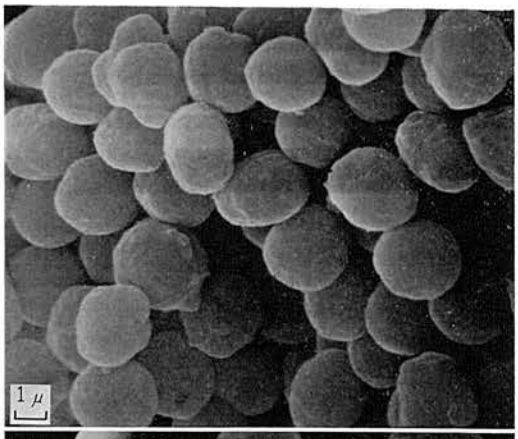

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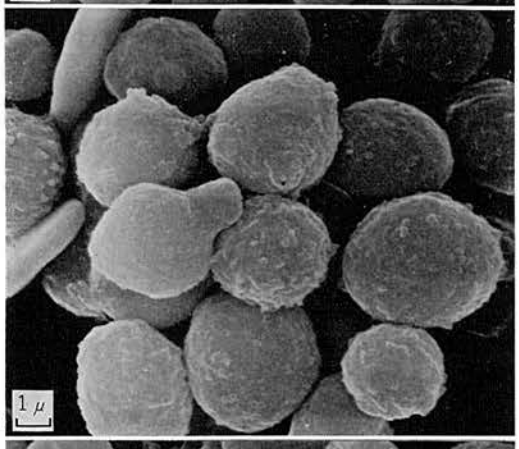

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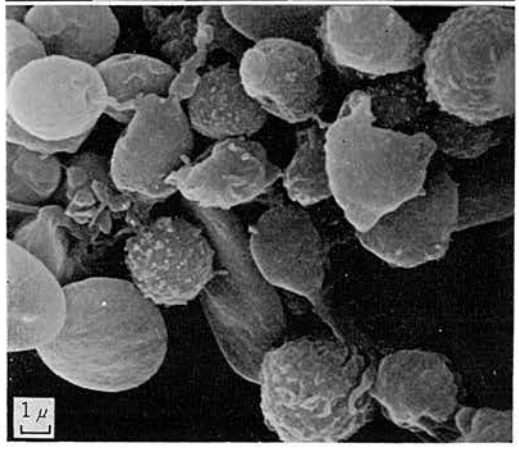

FIGURE 1. *Scanning electron micrograph of chicken thymocytes showing smooth surface.*

FIGURE 2. *Lymphocytes from the bursa. Most cells have stud-like microvilli.*

FIGURE 3. *Peripheral lymphocytes from a chicken. About half the lymphoid cells have many, long microvilli.*

many, long microvilli (Fig. 3). Their morphology was more like that of bursa cells than thymocytes. Cells resembling bursa cells are not likely to be cells other than lymphocytes because more than 90% of our preparation of white blood cells was lymphocytes. However, it is not known whether in chickens these cells are derived from bursa cells. Leukemia cells, 1104B and X5 had numerous microvilli. These cells were 5.0 to 7.0 μ in diameter and appeared just like peripheral lymphocytes with numerous microvilli (Fig. 4, 5). X5 line cells occasionally had an irregular shape. The two lymphoid cell lines derived from Marek's

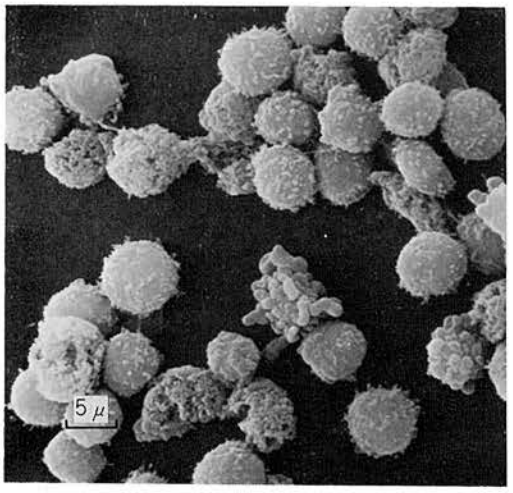

FIGURE 4. *Leukemia cells, 1104B. Cells are just like peripheral microvillous lymphocytes.*

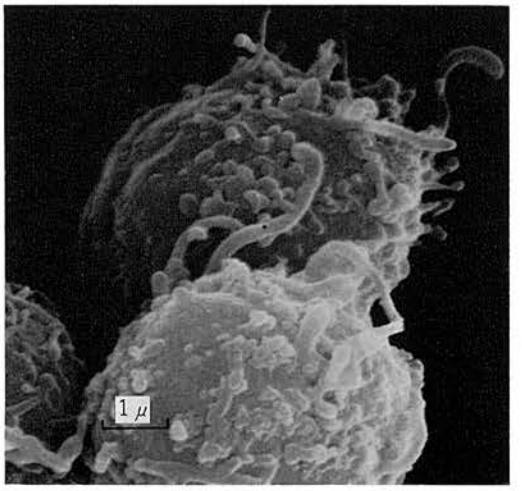

FIGURE 5. *Leukemia cells, X5. Cells have many, long microvilli.*

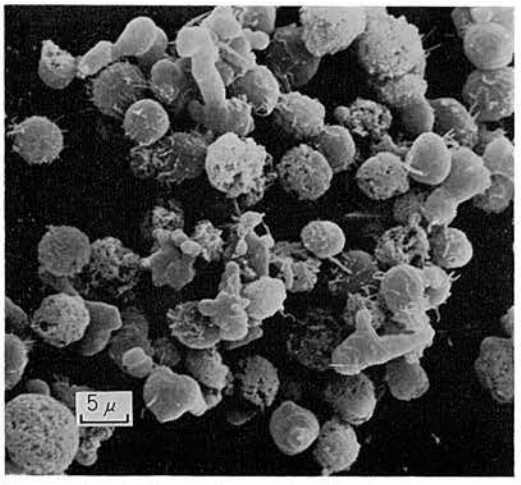

FIGURE 6. *MOB-1, lymphoid cells derived from a chicken with Marek's disease lymphoma. Cells have a few, long microvilli. Some cells have a relatively smooth surface.*

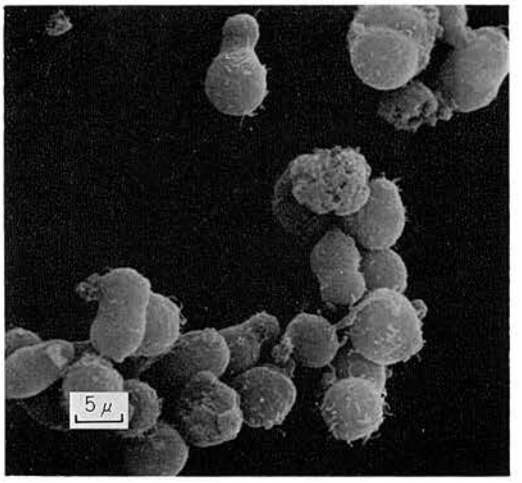

FIGURE 7. *MSB-1, Marek's disease lymphoma cells. Most cells have fewer microvilli than leukemia cells.*

disease lymphomas, MOB-1 and MSB-1, had fewer microvilli than leukemia cells and were 5.0 to 8.0 μ in diameter (Fig. 6, 7). About one third of the MOB-1 line cells and some of the MSB-1 line cells had a smooth surface. However, some cells of the MOB-1 line were attached by long microvilli to other degenerated cells.

The four lymphoid cell lines examined may be considered to be bursa dependent cells (B-cell) from the presence of microvilli on them. The two lymphoid cells derived from Marek's disease lymphoma may contain T-cells because these two lines were not cloned. However, the microvilli of these cells differed from those of bursa cells in being much longer and frequently much more numerous, although they resembled the microvilli of peripheral lymphocytes. To clarify this point, T antigen on these cell lines were examined. Rabbit anti-chicken thymocyte globulin (Microbiological Associates Inc., Maryland) was conjugated with fluorescein isothiocyanate as described elsewhere (Akiyama et al., 1973).

As shown on Table 1 this serum gave a positive reaction with most viable thymocytes but with only 5 to 10% of the spleen cells and bursa cells. Neither MOB-1 nor MSB-1 cells stained with this serum. This result supports the results obtained with SEM. However, it has been observed that T or B antigen is attenuated or lost during aging (Potworowski, 1971) so MOB-1 and MSB-1 cells may have very low concentrations of T antigen because they were derived from about 50 day old chickens. At any rate, MOB-1 and MSB-1 do not have the characteristics of typical T-cells. To determine the origin of lymphoid cells, the functions of microvilli must be clarified.

TABLE 1. *Distribution of cells having thymus-specific antigen*

Treatment[a]	Source of cells[b]	Fluorescence positive cells (%)
Anti T serum	MOB-1	0
	MSB-1	0
	1104B	0
	thymus 1	98
	thymus 2	86
	bursa 1	4
	bursa 2	9
	spleen 1	8
	spleen 2	4

[a] FITC conjugated anti T serum was added to cell suspensions in Hanks' solution and kept at 37 C for 15 min. After staining cell suspensions were washed twice with Hanks' solution.

[b] Organs were prepared from 35 day old chickens.

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