Sézary Cell Morphology Induced in Peripheral Blood Lymphocytes: Re-evaluation

By A.C. Chu and J.F. Morris

In this study we examined the effect of mitogens and epidermal cells in inducing a Sézary cell morphology in normal peripheral blood lymphocytes. Peripheral blood mononuclear cells from six healthy volunteers were stimulated with the mitogens phytohemaglutinin and concanavalin A, and also cocultivated with human epidermal cell cultures. Incubation times with mitogens and epidermal cells were four days and stimulation of the lymphocytes by mitogens was confirmed by standard 3H-thymidine uptake. Standard transmission electron microscopy showed that in the mitogen-driven system 20% to 60% (33 ± 15%) and in the epidermal cell-driven system 5% to 15% (8 ± 4%) of the lymphoid cells exhibited mild to moderate indention of the nuclei with nuclear contour indices (NCI) of 4.6 to 6.5 but no Sézary cells were observed (cells with NCI > 6.5 and up to 19.2). In the mitogen-stimulated preparation 2% to 5% (3 ± 1%) of the lymphoid cells showed nuclear multilobulation resembling the cells seen in adult T cell lymphoma/leukemia. Incubation of mononuclear cells for longer periods of up to 4 weeks with mitogens and exogenous IL-2 resulted in no further morphologic changes. Using an indirect immunogold technique at the electron microscopic level, the cells showing nuclear indentation or lobulation were shown to bear both T helper (CD4) and T suppressor (CD8) cell phenotypes in a similar ratio to the total numbers of T helper and T suppressor cells present. Mitogens and epidermal cells are thus not able to induce a morphologic change to Sézary cells in normal peripheral blood lymphocytes.

The Sézary cell is a lymphoid cell with a unique micromorphology, showing a nucleus with a serpentine or cerebriform contour and a high nuclear to cytoplasmic ratio. The micromorphology of the Sézary cell was first described by Lutzner and Jordan in 1968 and was initially thought to be specific for the malignant cell in mycosis fungoides and Sézary syndrome. Since that time, the Sézary cell has been the center of considerable controversy as to its disease specificity and its relationship to the skin. The Sézary cell has now been described in the skin and blood of patients with a number of benign dermatoses including erythrodermic eczema, psoriasis, lichen planus, and actinic reticuloid. This diversity of dermatoses has led to one theory that the Sézary cell could be a skin-associated lymphocyte which, in the cases of mycosis fungoides and Sézary syndrome, has undergone neoplastic change.

Recent studies have shown that the Sézary cell in Sézary syndrome associated with cutaneous T cell lymphoma (CTCL) and chronic actinic dermatitis could exhibit different T cell phenotypes—being a T helper cell in CTCL and a suppressor T cell in chronic actinic dermatitis. These studies demonstrated that the Sézary cell morphology was not restricted to a particular subpopulation of T cells, but did not address the possible relationship between the Sézary cell and skin disease.

Two groups have now described Sézary-like cells in the blood of healthy individuals. Meijer et al. reported finding cerebriform mononuclear cells in normal adult blood (6.7% of peripheral blood mononuclear cells [PBMC]) and in cord blood (8.7% of PBMC). Matutes et al. found that 2% to 4% of PBMC had either a Sézary-like morphology or a convoluted or polynodular nuclei similar to that seen in adult T cell lymphoma/leukemia (ATLL). Other groups, however, have reported only very low levels of Sézary-like cells in normal blood representing only 0.9% to 1.2% of PBMC, and showing a maximum nuclear contour index (NCI) of 7.5.

Yecckley et al. in 1976 demonstrated that cells with a Sézary cell micromorphology could be induced in normal human peripheral blood lymphocytes after stimulation by mitogens. This study is widely quoted in the literature and suggests that the Sézary cell morphology was related to T-cell activation rather than specific response to the cutaneous microenvironment or skin disease.

In this report we have readdressed the question of the production of Sézary cells by mitogen stimulation. We have also investigated the induction of Sézary cells in normal human lymphocytes by human epidermal cell cultures.

MATERIALS AND METHODS

Mononuclear cells. Venous blood was taken from six healthy volunteers (age, 25 to 37 years) into heparinized syringes. Mononuclear cells were separated immediately by velocity sedimentation on ficoll hypaque. The cells were washed and resuspended in RPMI 1640 medium (GIBCO, Grand Island, NY) with 10% fetal bovine serum (Flow Laboratories, Rickmansworth, England) at a density of 3 × 10⁶ cells/mL.

Blood was also taken from four patients with documented Sézary syndrome secondary to CTCL. Mononuclear cells were separated as described for the healthy volunteers. These cells were only used for electron microscopic and immuno-electron microscopic studies.

Mitogen stimulation. Parallel experiments were set up in 96-well flat-bottomed plates (Costar, Badhoevedorp, The Netherlands) and 5-mm flat-bottomed tubes (Eklay, Shrewsbury, MA). In the 96-well plates, 100 μL of mononuclear cells were pipetted into each well and 100 μL of mitogen (phytohemaglutinin, Sigma Chemical, St Louis) and 0.065 μg/mL of concanavalin A (Sigma, 0.25 μg/mL) were added to the wells in triplicate. Control wells were prepared in triplicate, in which 100 μL of RPMI 1640 medium were substituted for the mitogen.

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Plates were incubated at 37°C in 5% CO₂ for three days and were then pulsed with [³H]-thymidine and incubated for a further six hours before harvesting onto filter paper and counting on a β counter.

The stimulation was calculated using the formula:

\[
\text{Stimulation} = \frac{\text{mean count of test wells}}{\text{mean count of control wells}}\]

In the 5-mL tubes, 1-mL volumes of cells and mitogen were added in triplicate; the control tube consisted of 1-mL of cells with 1-mL RPMI 1640 medium with 10% fetal bovine serum (Flow). Cells were harvested on day 4; half the cells were used for immunocytochemistry and immunoelectron microscopy and half were used for standard transmission electron microscopy.

Further experiments using bulk cultures were conducted. The cells were incubated for up to 4 weeks with continual stimulation by mitogen and exogenous IL2 (Genzyme, Boston). Cells were harvested at weekly intervals and subjected to immunoelectron microscopy.

**Epidermal cell cultures.** Epidermal cell cultures were established using the method described by Rheinwald and Green. In brief, normal human skin from mastectomy or breast reduction mammoplasty operations were cut into strips and trypsinized overnight at 4°C. Single cell suspensions of epidermal cells were then produced after separation of the epidermis from the dermis. Cells were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 50 mg/mL of cholera toxin, and 5 µg/mL of hydrocortisone at a density of 5 × 10⁶ cells/mL and were plated onto high-density irradiated 3T3 fibroblast feeder cell layers. Cultures were fed twice weekly and a confluent monolayer of cells was established after seven days. Cell cultures were used in cocultivation studies after 14 days.

**Cocultivation of peripheral blood mononuclear cells with epidermal cell cultures.** Mononuclear cells separated from the blood of the six volunteers were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum at a density of 10⁶ cells/mL and were placed onto epidermal cell cultures at 5 mL/25 cm² plate. The cells were incubated for four days then harvested. Half the cells were used for immunocytochemistry and immunoelectron microscopy and half were used for standard transmission electron microscopy.

**Indirect immunogold electron microscopy.** Cells from patients with Sézary syndrome and cells harvested after mitogen stimulation and cocultivation with epidermal cell cultures were used in an indirect immunogold technique, as previously described. In brief, cells were resuspended in RPMI 1640 medium with 10% fetal calf serum at a density of 10⁷ cells/mL. The cell suspensions (100 µL) were pipetted into the wells in a microtiter plate, and 100 µL of OKT4, OKT8, OKT3 (Ortho), a pan T cell monoclonal antibody, anti-CD25, or RPMI 1640 as a negative control were added. After a further three washes, the cells were resuspended in 20 mm of 50% glycine in PBS, mounted under a cover slip, and examined under a Leitz Ortholux II fluorescence microscope. Two hundred cells were counted in each preparation and the percentage of positively labeled cells was calculated.

**RESULTS**

**Mitogen stimulation.** Mononuclear cells from all six volunteers gave good responses to both mitogens (Table 1).

![Fig. 1. Lymphocyte with cleaved nucleus found in control specimen. (Original magnification × 6,000, NCI = 5.9.]

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<th>Table 1. Mitogen Response of PBMS</th>
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<td>1</td>
<td>21,035</td>
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<td>15,440</td>
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When examined under an electron microscope, no Sézary cells were observed in either the control or test samples. Large numbers of grids were scanned in all samples, and several thousand cells were examined. Many of the lymphoid cells showed some morphologic change. Very few cells in the control samples were observed showing significant nuclear irregularity. Only 0.5 ± 1% of the cells showed minor indentations with NCI of 4.1-5.9 (Fig 1), which would correspond to the intermediate lymphocytes described by Stolz et al. In the mitogen-stimulated cells, as well as large blastic cells, many of the lymphocytes present exhibited varying degrees of nuclear indentation, varying between 20% and 60% (33% ± 15%) of the lymphocytes present with NCI of 4.6 to 6.5. None of the cells had acquired a cerebriform nucleus and none had NCI >6.5, which would classify it as a Sézary cell. A small percentage, 2% to 5% (3% ± 1%) of the lymphocytes had also acquired a multi-lobulated nuclear morphology, resembling the cells seen in ATLL. The mononuclear cells from the Sézary syndrome patient showed a wide variation in the number of Sézary cells present, but these cells had NCI's ranging from 8.6 to 19.2.

Occasional large cells with abundant cytoplasm were observed that had irregular and markedly indented nuclei. The nuclear chromatin in these cells was mainly euchromatin with some peripheral heterochromatin and conspicuous nucleoli, showing the macrophage nature of these cells.

Indirect immunofluorescence of the cells before and after mitogen stimulation showed no significant difference between the T-cell subpopulations before and after mitogenic stimulation (Table 2). At the electron microscopic level, Sézary cells from patients with Sézary syndrome invariably labelled with OKT4 (Fig 2). The mitogen-stimulated cells showing nuclear indentation or nuclear lobulation exhibited both helper and suppressor T-cell phenotypes (Figs 3 and 4) and numerically the ratio of helper to suppressor T cells exhibiting the change in nuclear morphology was not significantly different (1.3 to 1.8) to the ratio of total helper to suppressor T cells (1.5 to 1.73).

**Cocultivation with epidermal cells.** In the examination of these preparations at the electron microscopic level, several grids were examined in each volunteer and several thousand cells examined. No Sézary cells were observed. Some of the lymphoid cells present had acquired mild indentation of the nuclei, but this was less marked than in the mitogen-stimulated lymphocytes with NCI of 4.3 to 5.9 and accounted for only 5% to 15% (8% ± 4%) of the lymphocytes present. T-cell subpopulations as estimated by indirect immunofluorescence showed that only minor changes occurred following cocultivation of the mononuclear cells with the epidermal cells. The main change was a slight decrease in the number of phenotypic suppressor T cells present (Table 2).

Immunogold electron microscopy demonstrated that cells exhibiting mild indentation of the nucleus expressed both helper and suppressor T-cell phenotypes in similar proportions to the total numbers of helper and suppressor T cells present.

**DISCUSSION**

The Sézary cell has a unique micromorphology that has puzzled cell biologists since its first description in 1968. The nucleus is thrown into multiple convolutions so that the nucleus cut in cross section resembles the cross section of the brain. These cells are not, however, metabolically active cells, as the scant cytoplasm they possess contains few organelles and the cells exhibit little expression of the transferrin receptor, HLA DR or CD25 complex. In one study it was shown that the Sézary cells in CTCL were HLA Dr negative, but were reactive with a monoclonal antibody 4F2 which binds to activated, but not resting, peripheral blood lymphocytes. The discrepancy in the phenotypic labelling of the Sézary cells was attributed, by the authors, to the malignant nature of these cells. The purpose of the nuclear contour in these cells remains a mystery.

Sézary cells were initially thought to be specific for cutaneous T-cell lymphoma, but a large number of studies have now disproved this with the demonstration of Sézary cells in the skin and blood of a large number of benign dermatoses. The study by Yeckle et al. suggested that the Sézary cell morphology was simply related to activation of T cells as a similar morphology could be induced in peripheral blood lymphocytes after stimulation with the mitogen phyto-
hemagglutinin. Against this suggestion is the lack of activation antigens on the surface of these cells.\textsuperscript{15,16} Our study failed to confirm the report of Yeckley et al. In our study, no Sézary cells were generated by the mitogen phytohemagglutinin or concanavalin A, despite proliferative responses as detected by thymidine uptake and activation of the T cells shown by reactivity with anti-Tac antibodies. Some of the lymphoid cells did show mild indentation of their nuclei, but none could be identified as even vaguely Sézary-like, and none developed NCI > 6.5. To confirm that longer incubation with the mitogen could not induce further morphologic change in the cells, we incubated mononuclear cells for up to 28 days with the mitogens and exogenous IL-2. This longer incubation resulted in no further morphologic change.

Our study also failed to confirm previous reports of up to 6.7% Sézary-like cells in normal peripheral blood.\textsuperscript{6,7} We observed only 0.5% ± 1% cells with mild indentation of the nucleus in control specimens, and these cells could not be classed as Sézary-like. This may be due in part to our criteria for the recognition of Sézary cells. Stolz et al.\textsuperscript{8} used DNA morphometry to define Sézary cells; that is, as cells with a nuclear contour index of ~6.5. In our study we used Stolz's objective criteria to recognize Sézary cells. No cells with a nuclear contour index > 6.5 were observed.
SÉZARY CELL RE-EVALUATION

Apart from the study by Yeckley et al, no group has as yet been able to induce a Sézary morphology in lymphocytes in vitro. Certain forms of CTCL are due to retroviral infection with HTLV-I and recently a new retrovirus HTLV-V has been partially characterized in patients with classical CTCL. HTLV-I-induced CTCL is associated with a morphologic change in affected T cells but these cells develop nuclear multilobulatera rather than a Sézary morphology and the micromorphology of HTLV-V-infected T cells has not, as yet, been described.

The Sézary cell thus remains an enigmatic cell and the purpose of its complex micromorphology a mystery. Our study refutes previous studies suggesting that the Sézary morphology was related to cell activation, and we were unable to demonstrate the induction of this morphology in cells cocultivated with epidermal cells.

REFERENCES

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