Jpn. J. Cancer Res. **90**, 523–529, May 1999

Differentiation of a Cell Line of Human Cervical Argyrophil Small Cell Carcinoma to Macrophage Lineage Cells

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To investigate the origin of argyrophil small cell carcinoma (ASCC) of the uterine cervix, we examined the influence of dibutyryl cyclic adenosine 3′,5′-monophosphate (dB-cAMP), a known differentiation inducer, on the characteristics of an ASCC cell line, TC-YIK, which has been shown to be a useful *in vitro* experimental model of ASCC. In TC-YIK cells after treatment with dB-cAMP, two specific antigenic markers of macrophages, CD14 and human leukocyte antigen-DR, were detected by flow cytometric analysis. In addition, interferon-γ mRNA was detected by reverse transcription-polymerase chain reaction and interferon-γ protein was detected by ELISA. More than 90% of the cells stained positive for α-naphthyl butyrate esterase, 1% of the cells showed phagocytotic activity against *Micrococcus lysodeikticus*, and 22% of the cells had *M. lysodeikticus* adsorbed on their surface. Furthermore, granulocyte-macrophage colony stimulating factor accelerated the proliferation of TC-YIK cells. These results indicate that dB-cAMP promotes differentiation of ASCC cells to macrophages. In contrast, less than 10% of the cells showed stellate morphology, suggesting differentiation to neuronal cells after treatment with dB-cAMP, as reported previously. Thus, TC-YIK cells have been shown to differentiate both into macrophage lineage cells and neuronal cells, suggesting that ASCC originates from undifferentiated stem cells.

Key words: Argyrophil small cell carcinoma — Differentiation — Macrophage lineage — Neuronal cells — Dibutyryl cyclic AMP

Argyrophil small cell carcinoma (ASCC) of the uterine cervix, first described by Albores-Saavedra *et al.*,[1)] is characterized morphologically by the presence of argyrophil granules in the cytoplasm of the undifferentiated small cells and clinically by rapid metastasis and a poor prognosis.[1–7)] Because ASCC is a rare cervical tumor, accounting for only 1.5 to 5% of all cervical tumors,[1–7)] most reports on ASCC have been concerned with one or a few cases. Therefore, development of *in vivo* and *in vitro* experimental systems is desirable not only for examining the biological behavior of ASCC, but also for establishing an effective clinical treatment.

For this purpose we previously established and characterized an ASCC tumor line in nude mice and a cell line from the tumor, designated TC-YIK.[8–10)] In our previous studies, we found that TC-YIK cells retained the histochemical characteristics of ASCC cells and could be used as an *in vitro* experimental model of ASCC, and that the TC-YIK cells were possibly of neuroendocrine origin, based on electron microscopic evidence of small, electron-dense, membrane-bound neurosecretory-type granules and immunohistochemical evidence of neuron-specific enolase, chromogranin, serotonin and gastrin.[8, 9, 11)]

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In terms of the origin of ASCC, however, there have been three theories: ASCC originates from (i) amine precursor uptake and decarboxylation (APUD) cells,[2, 3, 12)] (ii) undifferentiated epithelial cells with multi-differentiation ability,[13)] or (iii) stem cells.[7)]

In this study, to investigate the origin of ASCC of the uterine cervix and the feasibility of treatment of the tumor with a differentiation inducer, we examined the influence of dibutyryl cyclic adenosine 3′,5′-monophosphate (dB-cAMP), a known differentiation inducer, on the characteristics of an ASCC cell line, TC-YIK.

MATERIALS AND METHODS

Cell culture TC-YIK cells were grown in RPMI-1640 medium (Nikken Biomedical Lab., Kyoto) supplemented with 10% bovine calf serum (FBS; Cam Sera, Ontario, Canada) under conditions of 5% CO_2 and saturated humidity at 37°C. Isolation and characterization of TC-YIK cells are described elsewhere.[9, 10)] TC-YIK cells were cloned twice after 60 cell generations from the start of culture using the limiting dilution method. The clone that showed the most stable growth was used for the current study.

Influence of dB-cAMP on cell proliferation of TC-YIK Adenosine dB-cAMP (Sigma, St. Louis, MO) was dissolved in distilled water at a concentration of 100 m*M*,

filtered through a 0.45-μm Millipore filter, and stored at –20°C until used.

The monolayer-cultured TC-YIK cells were dissociated by treatment with 0.4% (w/v) trypsin and 0.02% (w/v) EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS). The cells were then seeded in 96-well flat-bottomed tissue culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ) at a density of 1.5×10^4 cells/well and cultured at 37°C in the presence of 0, 1.25, 2.5, 5 and 10 m*M* dB-cAMP. After 4 days, 10 μl of Alamar Blue (Alamar Bio-sciences, Sacramento, CA) was added to each well. After 4 h of incubation at 37°C, the optical density of each well at 570 nm (A_{570}) was measured. The percent inhibition of the growth rate of the cells was calculated as ($1-A_{570}$ of dB-cAMP-treated group/A_{570} of dB-cAMP-untreated group) $\times$ 100.

Expression of cell surface markers Expression of two antigenic markers for monocytes and macrophages on TC-YIK cells (CD14 and human leukocyte antigen (HLA)-DR), and expression of an antigenic marker for NK cells (CD16) were determined by flow cytometric analyses. After 24-h culture, TC-YIK cells were further cultured for 48 h in the presence of 5 m*M* dB-cAMP, then dissociated by treatment with 0.02% EDTA-PBS and washed three times with PBS containing 2% FBS (PBS/2% FBS). The cells were incubated with 20 μg/ml of fluorescein isothiocyanate (FITC)-labeled anti-CD14, anti-CD16, and HLA-DR (DAKO, Kyoto) for 1 h on ice. After the incubation, the cells were washed three times with PBS/2% FBS and analyzed by flow cytometry (FACScan, Becton Dickinson, San Jose, CA).

Reverse transcription-polymerase chain reaction (RT-PCR) for cytokine mRNA TC-YIK cells were cultured for 0, 1, 3, 6, 9, 12, 24 and 48 h in the presence of 5 m*M* dB-cAMP. After the culture, total RNA was extracted by the acid guanidinium-phenol-chloroform (AGPC)

Table I. Sequences of Primer Sets for Human Cytokine Genes

Cytokine		Sequence	Predicted size(bp)	
			cDNA	DNA
IL-1β	sense	ATAAGCCCACTCTACAGCT	443	1164
	antisense	ATTGGCCCTGAAAGGAGAGA		
IL-2	sense	CAACGTAATAGTTCTGGAAC	299	2160
	antisense	TAGGGCTTACAAAAAGAATC		
IL-3	sense	AATCTCCTGCCATGTCTGCC	371	473
	antisense	TGAGAACACAACCGCACAAG		
IL-4	sense	CAACTGAGAAGGAAACCTTC	278	2865
	antisense	TTTTCCAACGTACTCTGGTT		
IL-5	sense	CACCAACTGTGCACTGAAGA	379	485
	antisense	GCAAAGTGTCAGTATGCCTG		
IL-6	sense	GTACCCCCAGGAGAAGATTC	819	4313
	antisense	CAAACTGCATAGCCACTTTC		
IL-7	sense	GAATTCCTCTGGTCCTCATC	425	6900
	antisense	AGGAAGTCCAAAGATATACC		
IL-8	sense	GCTTTCTGATGGAAGAGAGC	585	1000
	antisense	GGCACAGTGGAACAAGGACT		
IL-9	sense	TGTCAAGATGCTTCTGGCCA	238	1741
	antisense	ATATCTTGCCTCTCATCCCT		
IL-10	sense	ATGCCCCAAGCTGAGAACCAAGACCCA	353	
	antisense	TCTCAAGGGGCTGGGTCAGCTATCCCA		
IFN-α	sense	CACCTTGATGCTCCTGGC	439	439
	antisense	TTCTTGCAAGTTTGTTGA		
IFN-β	sense	TAGGCGACACTGTTCGTG	614	614
	antisense	CCAGAGGCACAGGCTAGG		
IFN-γ	sense	ATAATGCAGAGCCAAATTGTCTC	300	1514
	antisense	CTGGGATGCTCTTCGACCTC		
IFN-γR	sense	GAATCCTATAACATGAACCCTA	508	
	antisense	ATACTGGAATCGCTAACTGGCA		
TNF-α	sense	TCGGGCCAATGCCCTCCTGGCCAA	468	769
	antisense	GTAGACCTGCCCAGACTCGGCAAA		
β-actin	sense	GTGGGGCGCCCCAGGCACCA	541	
	antisense	CTCCTTAATGTCACGCACGATTTC		

method,[14] and 1 μg of RNA was used for cDNA synthesis with a reverse transcriptase (Superscript II RNAse H; GIBCO-BRL, Grand Island, NY) and an oligo$(dT)_{16}$ primer. One-tenth of this reaction mixture was used as a template for PCR amplification with *Taq* DNA polymerase ("Expand" High Fidelity PCR System; Boehringer Mannheim GmbH, Mannheim, Germany). The PCR reaction was run for 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min.[15] The primer pairs used in this study are shown in Table I. These oligonucleotide primers were synthesized using a DNA synthesizer (Gene Assembler Plus; Pharmacia LKB Biotechnology AB, Uppsala, Sweden). Primers for interleukin (IL)-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, tumor necrosis factor (TNF)-α, interferon (IFN)-α, IFN-β, IFN-γ and IFN-γ receptor (IFN-γR), were designed on the basis of previously published references, and were checked against the GenBank database.[15–17] To exclude amplification of contaminating genomic DNA, whenever possible, the primers were designed to include one or more introns. Primers for β-actin (a control) were constructed on the basis of previously published oligonucleotide sequences.[18] A 10-μl aliquot of the reaction mixture was then electrophoresed on a 1.5% agarose gel in TBE buffer (45 m*M* Tris-borate and 1 m*M* EDTA, pH 8.0), stained with 0.5 μg/ml ethidium bromide and visualized under UV light to evaluate the fragment size.

ELISA IFN-γ was measured in the culture medium and in the cytoplasm of TC-YIK cells by using an ELISA kit (Endogen, Cambridge, MA) in accordance with the manufacturer's instructions. After 24- and 48-h culture in the presence of 5 m*M* dB-cAMP, the culture supernatant was collected and stored at −80°C until used. The cells were dissociated with trypsin/EDTA, resuspended in 1 ml of PBS containing 1% FBS, ruptured by rapidly freezing and thawing 3 times, and centrifuged at 10,000*g* for 15 min to remove the cell debris. The supernatant thus obtained was stored at −80°C until used.

Esterase stain α-Naphthyl butyrate-esterase staining (Li method) was performed as previously described.[19] Briefly, after 48-h culture in the presence of 5 m*M* dB-cAMP, TC-YIK cells were dissociated with trypsin/EDTA and smeared on a slide glass. The smear was fixed for 30 s at 4°C with formalin/acetate (pH 6.6), stained with hematoxylin, and mounted with glycerin/gelatin. Cells containing dark-red granules were considered to be positively stained.

Phagocytosis test Phagocytotic ability was tested by using the *Micrococcus lysodeikticus* (*M. lyso*) method.[20, 21] Briefly, after 48-h culture in the presence of 5 m*M* dB-cAMP, cells were dissociated with trypsin-PBS, washed once with PBS and resuspended in RPMI-1640 containing 1% FBS at a concentration of 4×10^4 cells/ml. An equal volume of *M. lyso* (0.15 mg/ml in PBS) was added to the cell suspension and the mixture was incubated for 37°C for 30 min. After the incubation, the cells were washed twice with RPMI-1640/1% FBS to remove the *M. lyso* that was not adsorbed or phagocytosed. A cell smear was then prepared and stained with Wright-Giemsa solution.

Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on the growth of TC-YIK GM-CSF (ICN Biomedicals, Cleveland, OH) was dissolved in distilled water at a concentration of 1×10^7 units/ml and stored at −20°C until used. TC-YIK cells were inoculated into 24-well tissue culture plates (Falcon) at a density of 60,000 cells/well, cultured for 24 h, and then cultured for an additional 2, 3 or 4 days in the presence of GM-CSF at a concentration of 0, 250, 500 or 1000 units/ml.

RESULTS

Influence of dB-cAMP on the growth and morphology of TC-YIK As shown in Fig. 1, the growth of TC-YIK cells was inhibited by treatment with dB-cAMP in a dose-dependent manner. After treatment with dB-cAMP, some of the TC-YIK cells showed a stellate morphology (Fig. 2). The frequency of the stellate morphology was 0, 1.4, 6.7 and 9.7% on days 0, 1, 2 and 3 after dB-cAMP treatment, respectively.

Expression of cell surface markers Flow cytometric analyses of the expression of cell surface markers were carried out in TC-YIK cells before and after treatment with dB-cAMP. dB-cAMP induced the expression of two

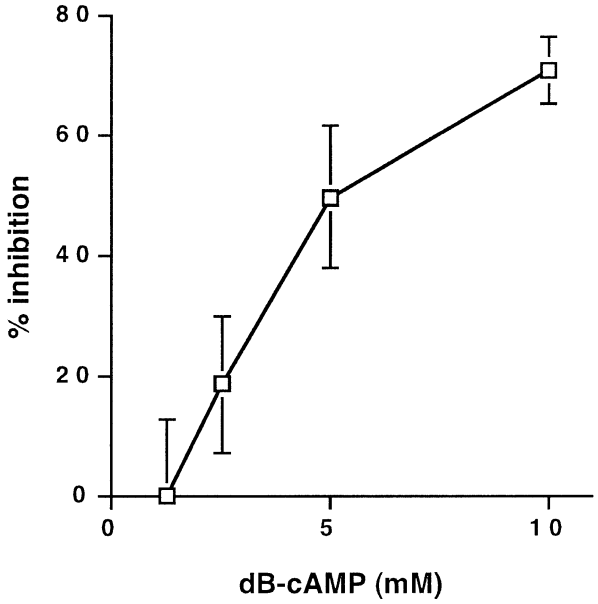

Fig. 1. Inhibition of the growth of TC-YIK cells by dB-cAMP. Each point represents the mean value±SD.

specific antigenic markers of macrophages, CD14[22] and HLA-DR,[23] but it did not induce the expression of a marker of NK cells, CD16[22] (Fig. 3).

Expression of cytokine mRNA The expression of cytokine mRNA was examined by RT-PCR in TC-YIK cells before and after treatment with dB-cAMP. As shown in Table II, only IFN-γ showed significant changes; namely, IFN-γ mRNA expression became detectable 6 h after treatment with dB-cAMP, reached a peak at 24–48 h after treatment, and disappeared 72 h after treatment, while it was not detected before the treatment (Fig. 4). Amplification of contaminating genomic DNA, whose predicted size was 1514 base pairs, was not observed. The expression of IFN-γ in the cells was further confirmed by ELISA at the protein level, though the level was low (Table III).

The expression of IFN-γ mRNA was also detected by RT-PCR in TC-YIK cells after treatment with 1 ng/ml of phorbol 12-myristate 13-acetate (PMA) for 24 or 48 h (data not shown). PMA is known to induce IFN-γ in hematopoietic cells.[24]

Regardless of the treatment with dB-cAMP, mRNAs of IL-3, IL-6, IFN-α, IFN-β and IFN-γR were detected in TC-YIK cells, while those of IL-2, IL-4, IL-5, IL-8, IL-9, IL-10 and TNF-α were not (Table II).

Non-specific esterase stain and phagocytosis test To investigate whether TC-YIK cells have some of the characteristics of macrophages after treatment with dB-cAMP, the cells were examined for α-naphthyl butyrate-esterase stainability and phagocytosis activity. Of 1011 TC-YIK cells stained for non-specific esterase, most (64.2%) showed a strong positive reaction, with dark red granules in their cytoplasm (Fig. 5a). The frequencies of weak positive and negative reactions for the stain were 28.5 and 7.3%, respectively. None of the TC-YIK cells was positive for the stain without the treatment (Fig. 5b). Of 1384

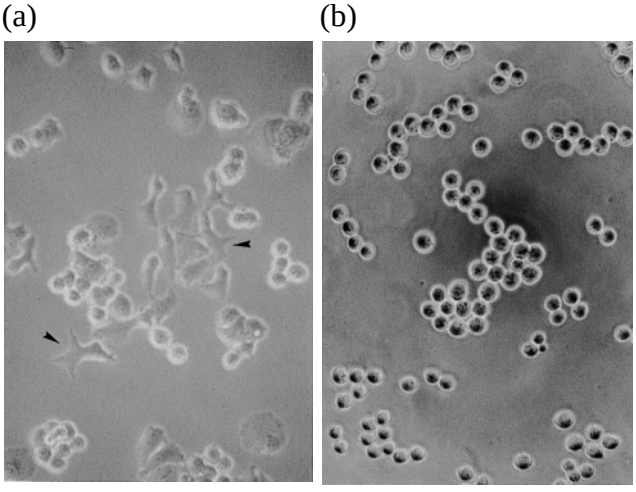

Fig. 2. TC-YIK cells after 48-h treatment with (a) and without (b) dB-cAMP. Arrows represent a stellate morphological change.

Table II. Induction of Cytokine mRNA in TC-YIK Cells by dB-cAMP

	Time after dB-cAMP treatment (h)				
	0	6	12	24	48
IL-1β	−	−	−	−	+
IL-2	−	−	−	−	−
IL-3	±	+	+	+	−
IL-4	−	−	−	−	−
IL-5	−	−	−	−	−
IL-6	+	+	+	+	+
IL-7	±	−	−	+	+
IL-8	−	−	−	−	−
IL-9	−	−	−	−	−
IL-10	−	−	−	−	−
TNF-α	−	−	−	−	−
IFN-α	+	+	+	+	+
IFN-β	+	+	+	+	+
IFN-γ	−	±	+	+	+
IFN-γR	+	+	+	+	+
β-actin	+	+	+	+	+

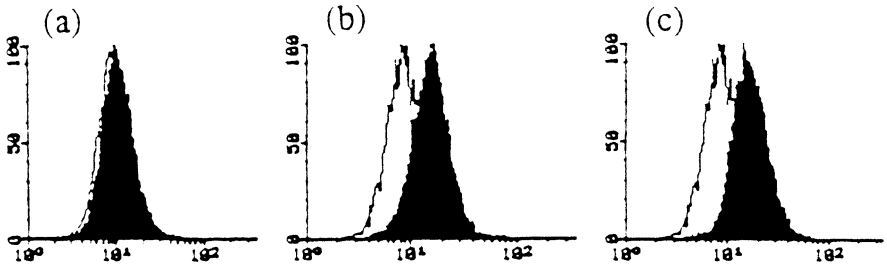

Fig. 3. Flow cytometric analysis of surface markers of TC-YIK cells with (black) and without (white) dB-cAMP treatment. (a) CD16, (b) HLA-DR and (c) CD14. CD16 is a marker of NK cells and CD14 and HLA-DR are markers of macrophages.

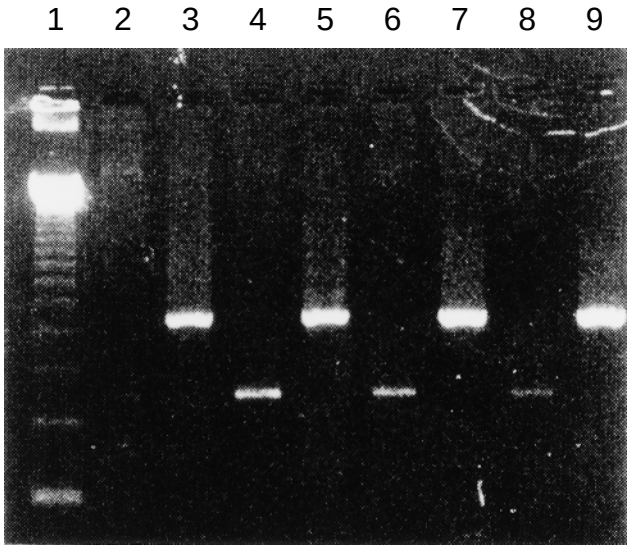

Fig. 4. RT-PCR analysis of the expression of IFN-γ mRNA in TC-YIK cells before and after treatment with dB-cAMP. Lane 1: 123-base-pair DNA ladder. Lanes 2, 4, 6 and 8: IFN-γ on days 0, 1, 2 and 3 after dB-cAMP treatment, respectively. Lanes 3, 5, 7 and 9: β-actin (a control) on days 0, 1, 2 and 3 after dB-cAMP treatment, respectively.

Table III. Expression of IFN-γ in TC-YIK Cells after Treatment with dB-cAMP

Time after treatment with 5m*M* dB-cAMP (h)	Intracellular (IU/10^6 cells)	Supernatant (IU/ml)
0	<1	<1
24	2.1	1.3
48	3.7	<1

TC-YIK cells tested for phagocytosis, 22.3% showed adsorption of *M. lyso* and 1.0% showed phagocytosis of *M. lyso*, while no cells showed these activities without the treatment (Fig. 6).

Influence of GM-CSF on the growth of TC-YIK As shown in Fig. 7, the growth of TC-YIK cells was increased in a dose-dependent manner when GM-CSF was added to the medium (RPMI-1640 containing 0.5% FBS). However, this was not the case when GM-CSF was added to RPMI-1640 containing 10% FBS, even after 48-h treatment with dB-cAMP (data not shown).

DISCUSSION

In the present study, we examined the influence of dB-cAMP, a differentiation inducer, on the characteristics of an ASCC cell line, TC-YIK, to investigate the origin of ASCC of the uterine cervix.

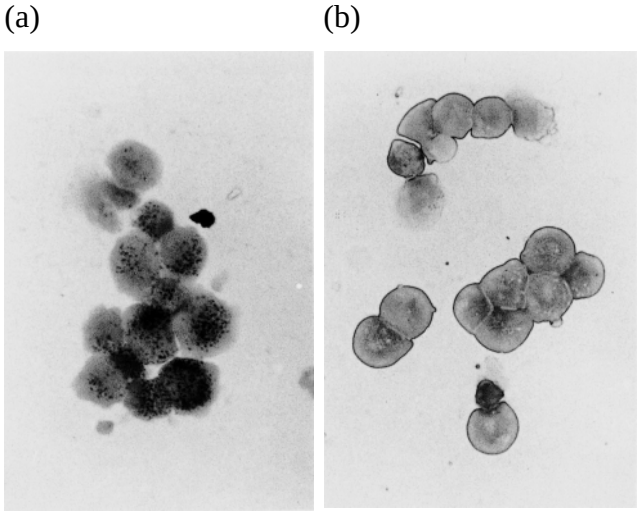

Fig. 5. α-Naphthyl butyrate esterase stain of TC-YIK cells with (a) and without (b) dB-cAMP treatment.

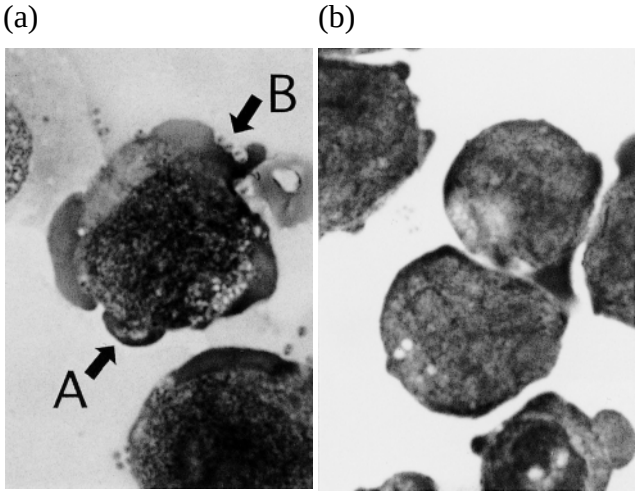

Fig. 6. Phagocytosis of TC-YIK cells with (a) and without (b) dB-cAMP treatment. In (a), A and B represent phagocytosis and adsorption of *M. lyso*, respectively.

The expression of two specific antigenic markers of monocytes and macrophages, CD14 and HLA-DR, was observed in TC-YIK cells after treatment with dB-cAMP. CD14 is mainly expressed on monocytes and macrophages,[22] and HLA-DR is only expressed on antigen-presenting cells such as B-cells, macrophages and dendritic cells.[23] However, the expression of CD16, a specific antigenic marker of NK cells[22] was not observed after treatment with dB-cAMP.

IFN-γ was induced in TC-YIK cells by treatment with not only dB-cAMP but also PMA, which induces IFN-γ in

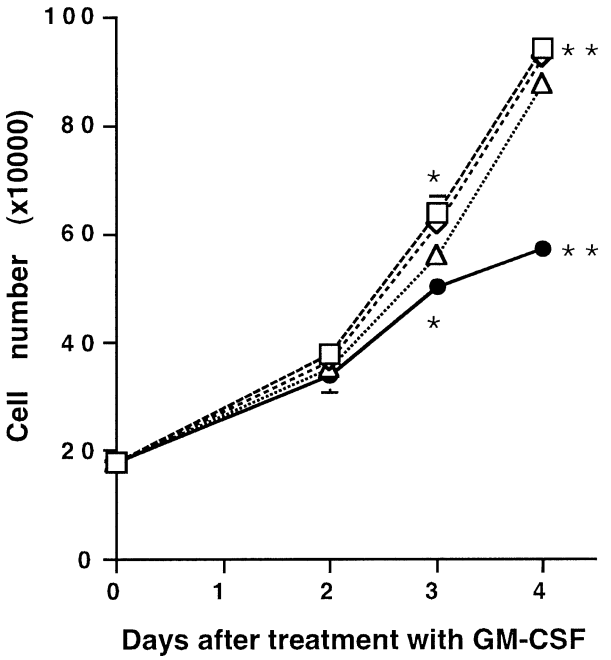

Fig. 7. Effect of GM-CSF on the growth of TC-YIK cells. GM-CSF: 0 unit/ml (△), 250 units/ml (○), 500 units/ml (◇), and 1000 units/ml (□). * $P<0.0025$, ** $P<0.0001$. Each point of GM-CSF 0 unit/ml and 1000 units/ml represents the mean value±SD.

hematopoietic cells. Only lymphocyte lineage cells such as helper T-cells and macrophage lineage cells have been reported to produce IFN-γ.[25]

TC-YIK cells stained positively for esterase (Li-method) after treatment with dB-cAMP. It has been shown that monocyte lineage cells are strongly positive for this stain, and megakaryocytes, lymphocytes, eosinophils, and erythroblasts are sometimes weakly positive.[19] After treatment with dB-cAMP, some of the TC-YIK cells also showed phagocytotic activity, which is one of the characteristics of macrophages.[20, 21] Furthermore, the growth of TC-YIK cells was accelerated by GM-CSF, which induces the growth of neutrophils, eosinophils, promacrophage lineage cells, CFU-GM, BFU-e, and CFU-Meg.[26, 27]

These results suggest that dB-cAMP induces differentiation of TC-YIK cells into hematopoietic cells, especially macrophage lineage cells.

In contrast, less than 10% of TC-YIK cells acquired a stellate morphology after treatment with dB-cAMP, suggesting that they differentiated into neuronal cells, as reported previously.[11] Development of this neuronal morphology seems inconsistent with the differentiation of TC-YIK cells into macrophages after treatment with dB-cAMP. However, the percentage of the cells that were negative for esterase stain (7.3%) was almost the same as the percentage of the cells that acquired a stellate morphology (6.7%). Thus, most of the cells differentiated into macrophages, while less than 10% of the cells became neuronal cells after treatment with dB-cAMP. Therefore, it appears that TC-YIK cells are undifferentiated cells and that ASCC originates from stem cells that have an ability to differentiate into many different cell types.

IFN-γ expression was induced in TC-YIK cells after treatment with dB-cAMP, and IFN-γR mRNA was found to be constitutively expressed in the cells. IFN-γ is a cytokine that regulates the immune response and inflammatory reaction through activation of T-cells, B-cells, macrophages, NK cells, endothelial cells and fibroblasts, and induction of differentiation of these cells.[28, 29] Therefore, we expect that IFN-γ endogenously expressed in the cells plays some role in the differentiation of TC-YIK cells induced by dB-cAMP. However, external addition of IFN-γ did not induce a change in the stellate morphology or in the expression of cell surface markers in TC-YIK cells, though it inhibited the proliferation of the cells (data not shown). Therefore, we suggest that IFN-γ does not play an important role in the differentiation of TC-YIK cells by dB-cAMP.

GM-CSF accelerated the proliferation of TC-YIK cells when it was added to the medium containing 0.5% FBS, but not when it was added to the 10% FBS-containing medium (growth medium). This suggests that growth factor(s) in FBS masked the function of GM-CSF, or that a low concentration of FBS in the medium inhibited the proliferation of TC-YIK cells and induced the differentiation of the cells into macrophage lineage cells. Thus, we suggest that GM-CSF accelerates the proliferation of ASCC cells under certain conditions. However, GM-CSF is currently used as a chemotherapeutic agent to supplement *cis*-diaminedichloroplatinum and etoposide for ASCC. Therefore, the effect of GM-CSF on the growth of ASCC *in vivo* needs to be clarified. We are presently conducting such studies using our *in vivo* experimental system.

The results of the present study suggest that TC-YIK originates from stem cells that have a multi-differentiation ability. Further clarification of the mechanism of differentiation induction in TC-YIK cells should provide clues for developing new treatments, including a differentiation-induction therapy, for ASCC.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (09671707) from the Ministry of Education, Science, Sports and Culture of Japan.

(Received December 11, 1998/Revised February 13, 1999/Accepted February 20, 1999)

REFERENCES

1) Albores-Saavedra, J., Poucell, S. and Rodriguez-Martinez, H. A. Primary carcinoid of the uterine cervix. *Sorbreitiro Pathol.*, **10**, 185–193 (1972).
2) Tateishi, R., Wada, A., Hayakawa, K., Hongo, J., Ishii, S. and Terakawa, N. Argyrophil cell carcinomas (apudomas) of the uterine cervix. *Virchows Arch.*, **366**, 257–274 (1975).
3) Albores-Saavedra, J., Larraza, O., Poucell, S. and Rodriguez-Martinez, H. A. Carcinoid of the uterine cervix: additional observation on a new tumor entity. *Cancer*, **38**, 2328–2342 (1976).
4) Yamasaki, M., Tateishi, R., Hongo, J., Ozaki, Y., Inoue, M. and Ueda, G. Argyrophil small cell carcinoma of the uterine cervix. *Int. J. Gynecol. Pathol.*, **3**, 146–152 (1984).
5) van Nagell, J. R., Powell, D. E., Gallion, H. H., Elliott, D. G., Donaldson, E. S. and Carpenter, A. E. Small cell carcinoma of the uterine cervix. *Cancer*, **62**, 1586–1593 (1988).
6) Abeler, V. M., Holm, R., Nesland, J. M. and Kjorstad, K. E. Small cell carcinoma of the cervix. *Cancer*, **73**, 672–677 (1994).
7) Gersell, D. J., Mazoujian, G., Mutch, D. G. and Rudloff, M. A. Small-cell undifferentiated carcinoma of the cervix. *Am. J. Surg. Pathol.*, **12**, 684–698 (1988).
8) Ichimura, H., Yamasaki, M., Yamane, T., Shimizu, H., Katsumoto, T., Kurimura, O. and Kurimura, T. Heterotransplantation of argyrophil small cell carcinoma of the uterine cervix integrating HPV16 DNA into nude mice. *Jpn. J. Cancer. Res.* (*Gann*), **79**, 1255–1258 (1988).
9) Ichimura, H., Yamasaki, M., Tamura, I., Katsumoto, T., Sawada, M., Kurimura, O. and Kurimura, T. Establishment and characterization of a new cell line TC-YIK originating from argyrophil small cell carcinoma of the uterine cervix integrating HPV16 DNA. *Cancer*, **67**, 2327–2332 (1991).
10) Hori, T., Ichimura, H., Minamihisamatsu, M., Takahashi, E., Yamauchi, M., Hama, Y., Kurimura, O., Yamasaki, M. and Kurimura, T. Chromosomal insertion and amplification of human papillomavirus 16 DNA sequences in a cell line of argyrophil small cell carcinoma of the uterine cervix. *Jpn. J. Cancer Res.*, **82**, 371–375 (1991).
11) Shimizu, H., Sawada, M., Tohyama, M., Takemura, K., Akedo, H., Ichimura, H., Ueda, G. and Tanizawa, O. Effect of dibutyryl cyclic AMP on morphologic features and marker production of human cervical argyrophil small cell carcinoma cell line. *Int. J. Cancer*, **56**, 766–771 (1994).
12) Inoue, M., Ueda, G. and Nakajima, T. Immunohistochemical demonstration of neuron-specific enolase in gynecologic malignant tumors. *Cancer*, **12**, 684–698 (1988).
13) Matsuyama, M., Inoue, T., Ariyoshi, Y., Doi, M., Suchi, T., Sato, T., Tashiro, K. and Chihara, T. Argyrophil cell carcinoma of the uterine cervix with ectopic production of ACTH, β-MSH, serotonin, histamine, and amylase. *Cancer*, **44**, 1813–1823 (1979).
14) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159 (1987).
15) Kita, M., Hayashi, T., Yamagishi, H., Oka, T. and Imanishi, J. Expression de l'ARN messanger des cytokines dans la rate humaine. *C. R. Soc. Biol.*, **188**, 277–282 (1994).
16) Kaashoek, J. G. J., Mout, R., Falkenburg, J. H. F., Willemze, R., Fibbe, W. E. and Landegent, J. E. Cytokine production by the bladder carcinoma cell line 5637: rapid analysis of mRNA expression levels using a cDNA-PCR procedure. *Lymphokine Cytokine Res.*, **10**, 231–235 (1991).
17) Benjamin, D., Knobloch, T. J. and Dayton, M. A. Human B-cell interleukin-10: B-cell lines derived from patients with acquired immunodeficiency syndrome and Burkitt's lymphoma constitutively secrete large quantities of interleukin-10. *Blood*, **80**, 1289–1298 (1992).
18) Maraskovsky, E., Troutt, A. B. and Kelso, A. Engagement of CD3 with LAF-1 or ICAM-1 adhesion molecules enhances the frequency of activation of single murine $CD4^+$ and $CD8^+$ T cells and induces synthesis of IL-3 and IFN-γ but not IL-4 or IL-6. *Int. Immunol.*, **4**, 475–485 (1992).
19) Li, C.Y., Lam, K.W. and Yam, L.T. Esterase in human leukocytes. *J. Histochem. Cytochem.*, **21**, 1–12 (1973).
20) Nagata, T., Hamagami, K., Isemura, T. and Shimada, N. Quantitative measurement of phagocytic activity by leukocytes using *Micrococcus lysodeikticus* as a particle. *J. Med. Technol.*, **27**, 1045–1048 (1983).
21) Nagata, T., Kitani, T. and Shimada, N. Simultaneous measurement of phagocytic and lysozyme activities in leukemic cells. *Jpn. J. Clin. Pathol.*, **32**, 890–894 (1984).
22) Stockinger, H., Majdic, O. and Knapp, W. 1995 directory for the human leukocyte clusters of differentiation. *Transfusion*, **36**, 268–285 (1996).
23) Auffray, C. and Strominger, J. L. Molecular genetics of the human histocompatibility complex. *Adv. Hum. Genet.*, **15**, 197–247 (1987).
24) Sandy, D. der and Lau, A. S. Involvement of the double-stranded-RNA dependent kinase PKR in interferon expression and interferon mediated antiviral activity. *Med. Sci.*, **92**, 8841–8845 (1995).
25) Callard, R. and Gearing, A. "The Cytokine Facts Book," pp. 157–162 (1994). Academic Press Inc., San Diego.
26) Nimer, S. D., Champlin, R. E. and Golden, D. W. Serum cholesterol-lowering activity of granulocyte-macrophage colony-stimulating factor. *JAMA*, **260**, 3297–3300 (1988).
27) Gasson, J. C. Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood*, **77**, 1131–1145 (1991).
28) Vilcek, J., Gray, P. W., Rinderknecht, E. and Sevastopoulos, C. G. Interferon γ: a lymphokine for all seasons. *Lymphokines*, **11**, 1–32 (1985).
29) Farrar, M. A. and Schreiber, R. D. The molecular cell biology of interferon-γ and its receptor. *Annu. Rev. Immunol.*, **11**, 571–611 (1993).