Insulin requirement of human leukemic cell lines[1]

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Summary. The growth characteristics of human leukemic cell lines in serum supplemented medium and in serum free medium with and without the addition of insulin were investigated. No relation was found between the insulin binding capacity of the cells and their hormone-dependence for growth.

Key words. Leukemic cell lines, human insulin-binding capacity; hormone dependence; growth pattern; synthetic medium; FCS medium.

Leukemic cell lines established in vitro are useful models for studying the mechanisms regulating the proliferation and differentiation of neoplastic cells. Some human leukemic cell lines can be grown in synthetic media indefinitely[3,4]. Synthetic, i.e. serum-free media, can thus be used to evaluate the effects of identified substances on the growth and metabolic functions of leukemic cells. Studies on human and animal cell lines indicate that transferrin and insulin are the serum substitutes most frequently required to sustain cell growth[3-6].

In the present study, we evaluated the possibility of growing human leukemic cell lines in synthetic media, and compared their growth patterns in the presence and in the absence of serum.

Moreover, we investigated the effects of insulin on their proliferation, and related the insulin binding activity of each line to its hormone dependence for growth.

Materials and methods. Human promyelocytic cell lines HL-60 and ML-3[7,8], myeloblastic KG-1 cell line[9], histiocytic U-937 cell line[10] and erythroleukemia K 562 cell line[11] were kindly obtained from Dr Giovanni Rovera (Wistar Institute, Philadelphia, Pa) and pre-B NALM-1 cell line[12] from Dr Jun Minowada (Roswell Park Memorial Institute, Buffalo, NY).

Philadelphia positive undifferentiated BV173 cell line[13] was established in our laboratory.

Three types of medium were used: 1) fetal calf serum (FCS) supplemented medium, consisting of RPMI 1640 (GIBCO Laboratories, Grand Island, NY), 10% FCS, 2 mM glutamine, 50 units penicillin and 50 µg streptomycin per ml, and 0.2% sodium bicarbonate; 2) insulin-containing synthetic medium (complete synthetic medium) consisting of RPMI 1640 supplemented with glutamine, antibiotics and sodium bicarbonate as medium 1 and with 3×10^{-8} M selenium dioxide (J.T. Baker Chemical Co., Phillisburg, NJ), 5 µg transferrin per ml (Sigma Chemical Co., St Louis, Mo) and 5 µg bovine insulin per ml (chromatography purified, Hoechst AG, Frankfurt am Main, West Germany), and further buffered with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma) to pH 7.2; 3) insulin-free synthetic medium, i.e. medium 2 without insulin.

Cells from the different lines were seeded at the densities indicated in the table in T/25 tissue culture flasks (Corning, New York, NY), incubated in a 5% CO_2: 95% air humidified atmosphere at 37 °C, and re-fed every 3–4 days.

Insulin binding studies were performed on cells cultured in FCS-containing medium, as previously described[14].

Results. All leukemic cell lines were routinely grown in FCS supplemented medium. Their doubling times are shown in the table. Complete synthetic medium was unable to sustain the growth of ML-3 und U-937 cells. By contrast HL-60, KG-1, K562, NALM-1 and BV173 grew indefinitely, though their doubling times were longer than in medium containing serum. When seeded in insulin-free medium HL-60 cells were unable to grow, whereas the other cell lines showed no dependence on insulin for growth. However, while the doubling time of K562 was not affected by the hormone that of KG-1, NALM-1 and BV173 cell lines increased.

The insulin binding capacity of all seven lines is also shown in the table. Each line clearly possesses insulin receptors and a different binding capacity. Analysis of the competition curves, which show the percentage of total radioactive insulin bound specifically to the cell receptors, plotted as a function of the logarithm of total insulin concentration[14], indicate that receptors are specific for insulin (data not shown). These results suggest that no relation can be established between the insulin dependence of each line and the number of insulin receptors on the cell membrane.

Discussion. Breitman et al.[3] have shown that the HL-60 human promyelocytic cell line can be indefinitely grown and induced to differentiate in synthetic medium. Our results demonstrate that most human leukemic cell lines can grow in the absence of serum. This ability does not seem to be related to a given hemopoietic lineage, nor to the differentiation stage to which the cells are assigned. HL-60, for example, was able to grow in the absence of serum, whereas ML-3, the other promyelocytic line, was not.

Most of the human and animal neoplastic cell lines adapted to grow in synthetic media so far share a common requirement for transferrin and insulin[5]. Pessano et al.[4], however, have demonstrated that the human K562 erythroleukemia and the mouse erythroleukemia Friend cell lines do not need insulin. Our study has suggested that these are not the only exceptions, since the human leukemic cell lines KG-1 and NALM-1 also grew indefinitely in the absence of insulin with no significant change in their doubling time. The BV173 line which, like the NALM-1 line, is

Doubling time of human leukemic cell lines in serum-supplemented medium and synthetic medium with and without insulin, and their specific insulin binding

Cell lines	Doubling time (h) Cell density at seeding ($\times 10^6$/ml)	Serum supplemented medium	Synthetic medium with insulin	Synthetic medium without insulin	Specific insulin binding (%)
HL-60	0.2	63 ± 8.0*	90 ± 11.0	No growth	18.9 ± 0.9**
ML-3	0.2	55 ± 9.5	No growth	No growth	4.3 ± 1.5
KG-1	0.5	40 ± 5.5	77 ± 14.5	91 ± 17.0	3.3 ± 1.6
U-937	0.2	27 ± 9.0	No growth	No growth	16.8 ± 6.0
K562	0.2	43 ± 8.0	53 ± 11.5	52 ± 10.5	4.8 ± 1.3
BV173	1.0	23 ± 5.5	28 ± 10.0	53 ± 12.0	5.6 ± 2.1
NALM-1	0.5	30 ± 4.5	36 ± 9.0	44 ± 10.0	12.3 ± 2.7

*Mean values and SD of the doubling time (in h and decimal fractions of h) during the first five passages after seeding. Each experiment was performed in triplicate. **Specific insulin binding: average of three experiments ± SD.

composed of undifferentiated blast cells, was also able to grow in the absence of insulin, though its growth rate was much slower. The conclusion may be drawn that insulin is not an essential growth factor for most human leukemic cell lines.

The observation that some of these lines are more sensitive to the growth stimulation effects of insulin prompted us to investigate whether this was related to their insulin binding capacity. We and other groups had previously found by competition curve analysis that HL-60 cells possess specific insulin receptors[14-16]. In the present study we demonstrated that all the human leukemic cell lines we tested bind insulin specifically. However, the degree of this activity could not be related to the cell phenotype of the various lines or with their insulin requirement for growth.

It has been shown in other experimental systems that high concentrations of insulin can promote growth in the absence of biologically active insulin[17]. It has also been suggested that insulin's growth promoting activity is distinct from its metabolic activity, and that these activities are mediated by different cell membrane receptors[18].

With the exception of the HL-60 cell line, our study demonstrates that insulin, whether acting as a metabolic agent or as a growth promoter, is not essential for the maintenance of human leukemic cell lines. The presence of specific receptors for insulin on the membranes of cells which are able to grow indefinitely in the absence of the hormone can be regarded as an expression of deranged growth regulatory mechanisms in neoplastic cells.

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Injection of a soluble sperm fraction into sea-urchin eggs triggers the cortical reaction

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Summary. Fertilization membranes form around unfertilized sea-urchin eggs after microinjection of a soluble spermatozoa fraction isosmotic with seawater. This demonstrates that the spermatozoon contains a chemical that triggers an increase in cytosolic calcium, leading to exocytosis of cortical granules. It also demonstrates that the triggering mechanism does not require an externally-activated egg-membrane process. Further experiments show that the chemical trigger is not calcium.
Key words. Sea-urchin eggs; *Lytechinus pictus;* fertilization membrane; spermatozoa fraction; triggering mechanism.

One of the first events in the normal activation of sea-urchin eggs is exocytosis of the cortical granules leading to formation of the fertilization membrane[1]. Although it has been well established that an increased concentration of cytosolic calcium is necessary for this event[2-5], an important question is how the spermatozoon triggers the calcium increase. We report here that fertilization membranes form around unfertilized sea-urchin eggs after microinjection of a soluble spermatazoa fraction isosmotic with seawater. This indicates that the spermatozoon contains a chemical that triggers an increase in cytosolic calcium, leading to exocytosis of cortical granules. It also demonstrates that the triggering mechanism does not require an externally-activated egg-membrane process.

Injection of live spermatozoa directly into the cytoplasm of sea-urchin eggs does not lead to formation of fertilization membranes[6], indicating that interaction of the spermatozoon with the egg membrane is required to trigger the calcium increase. There are two general types of processes that might be associated with this interaction. One is the entrance of the contents of the spermatozoon into the egg cytoplasm. Another involves egg-membrane-mediated activity, such as the activation that follows binding of a hormone to its receptor. We have addressed the question as to which of these two types of processes are required for cortical granule exocytosis by pressure-injecting a soluble fraction of spermatozoa directly into the cytoplasm of unfertilized eggs and observing whether fertilization membranes form.

All experiments were carried out at room temperature, using gametes of the sea urchin *Lytechinus pictus* (Pacific Biomarine, Venice, California). Egg jelly was removed by washing the eggs several times in natural seawater. To prepare the sperm fractions, the testes of several animals were washed several times in calcium-free seawater, pH 7.8. Dry sperm was collected by pipette, diluted into about three times its volume of deionized water, and homogenized with a teflon rod spinning at 1700 rpm for 2 min. The resulting suspension was centrifuged for 1 h at 110,000 × g at 4 °C and the supernatant collected. Over a period of two months, 9 such fractions were prepared. The osmolarity of these fractions was usually about 0.25 osmoles. The major cations, as determined by atomic absorption spec-