Antibody Production in Cultured Lymphoblastoid Cell Line

A human lymphoblastoid cell line P3-J[1], which had been exposed to coliphages, produced a factor capable of neutralizing phage, most likely an antibody[2,3]. In a supplementary study, P3-J cells apparently produced antibody-like activity against *E. coli* and a human cell line RPMI No. 41[4] derived from an osteosarcoma, after the P3-J cells had been exposed to *E. coli* or RPMI No. 41 cells, respectively.

Cell wall-free *E. coli* lysate prepared from 10^{10} cells was incubated with 10^8 P3-J cells for 2 h and then washed 3 times to remove unadsorbed bacterial lysate. The P3-J cells were then cultured in spinner flasks[2,3]. 10^8 of the cultured P3-J cells were mixed and incubated with 10^7 of RPMI No. 41 cells for 3 h; then the cell mixture was cultured in stationary flasks for 12 h. The floating cells, which mostly consisted of P3-J cells, were collected and incubated in second stationary culture bottles for 12 h. The floating P3-J cells were collected and finally placed in spinner flasks for further culturing. Cell-free culture media were collected 6 days after exposure of the cells to the antigen, and were prepared for the globulin concentrates. The methods of culture and the preparation of globulins were described in previous papers[2,3].

To assay *E. coli* colony formation, 1 ml portions of protein concentrate (containing 5 g/100 ml) from cultured medium of P3-J which had been exposed to *E. coli* lysate, were mixed with 1 ml of *E. coli* containing approximately 10^5 cells, and the mixture was kept at room temperature. A control test was set up from the culture media of P3-J which was not exposed to *E. coli* lysate. From 0 time to 120 min, 0.2 ml of the mixture were sampled and diluted in normal saline. 2 ml of the diluted sample was mixed with 2 ml of 1% agar, and 1.8 ml of this mixture was poured on each petri dish containing nutritional agar basal layer. The colony count was made after overnight incubation at 37 °C.

Similarly, the protein concentrates (containing 2 g/100 ml) from the cultured media of P3-J which had been previously exposed to RPMI No. 41 cells were incubated with packed RPMI No. 41 cells for 2 h at 37 °C. Then the cells were washed and cultured in petri dishes (2×10^4 cells) with 10 ml of fresh medium at 37 °C in a CO_2 incubator. The total cell count in petri dishes was counted at appropriate intervals from 0 time to 5 days. Controls were set up with similar protein preparations obtained from the medium in which P3-J cells were not exposed to RPMI No. 41 cells.

Although the number of *E. coli* colonies showed slight increase at room temperature from 0 time to 60 min in the control group, the colony count of the experimental samples decreased to approximately 50% of the control at 60 min in all of 3 experiments (Figure 1). The growth of RPMI No. 41 cells treated with preparation from spent media of P3-J as the control was the same as the growth of RPMI No. 41 similarly treated by only fresh medium or protein concentrates from spent media of RPMI No. 41 cells. However, the growth of RPMI cells treated with protein preparation from spent media of P3-J, which had been exposed to RPMI No. 41 cells, was significantly inhibited in 3 experiments tested (Figure 2). The growth of the human melanoma cell line RPMI No. 4177 was not inhibited by the above preparations. There was no detectable cytolytic activity by the addition of guinea-pig complement in the protein concentrates. The growth of *E. coli* was not inhibited by the protein preparation from culture media of P3-J which had been exposed to RPMI No. 41. Similarly, the growth of RPMI No. 41 cells was not affected specifically by the protein concentrates of cultured media of P3-J which had been exposed to *E. coli* lysate.

By electronmicroscopic study it was observed that P3-J cells which were exposed to purified coliphage T2 showed characteristic ribosome arrangement after the fifth day of T2 exposure (personal communication from Dr. I. SUZUKI). These ribosomal changes were also found in P3-J cells after exposure to *E. coli* lysate, RPMI No. 41 cells, or phytohemagglutinin, respectively. Although it is not known whether the ribosomal changes were related to an antibody production, this finding and the above results suggest that the P3-J cells are capable of producing antibodies against multiple kinds of antigens introduced in vitro. P3-J cultures always contain a mixed population of lymphoblasts in various degrees of maturation, a few plasmablast-like cells, and rare macrophage-like cells. The present study cannot clarify whether the formation of antibodies observed represents a primary or secondary response. Furthermore, it is not known whether the cells which are potent to produce T2 neutralizing substance

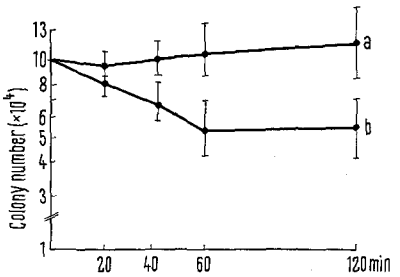

Fig. 1. *E. coli* growth kinetics in protein concentrates from cultured media of (a) non-treated P3-J; (b) P3-J which had been exposed to *E. coli* lysate. Each point shows mean count of 4 samples.

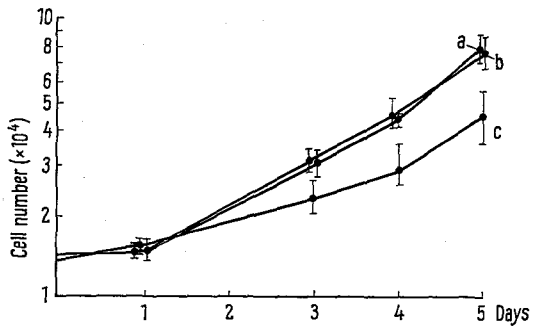

Fig. 2. Growth curve of RPMI No. 41 in petri dishes after the cells were treated with protein concentrates from cultured media of (a) RPMI No. 41; (b) non-treated P3-J; (c) P3-J which had been cultured after exposure to RPMI No. 41. Each point shows mean count of 4 dishes.

[1] R. J. V. PULVERTAFT, J. clin. Path. *18*, 261 (1965).
[2] H. KAMEI and G. E. MOORE, Nature *215*, 860 (1967).
[3] H. KAMEI and G. E. MOORE, J. Immun. *101*, 587 (1968).
[4] G. E. MOORE and A. KOIKE, Cancer *17*, 11 (1964).

are also capable of synthesizing a protein to inhibit the growth of *E. coli* or RPMI No. 41 cells. Further studies are in progress to clarify the above questions[5].

Zusammenfassung. Ein humaner Lymphoblastoiden-Zellstamm P3-J einer an Burkitt-Lymphoma Erkrankten wurde mit entsprechenden *E.-coli*-Bakterien eines Stammes RPMI Nr. 41 zusammen exponiert. Es muss angenommen werden, dass die Zellen vom Stamm P3-J Antikörper gegen *E. coli* oder RPMI Nr. 41 gebildet haben.

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[5] We wish to thank Drs. Y. YAGI and J. MINOWADA for their advice.

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Hormonale Beeinflussung der menschlichen Tubenmotilität[1]

Unsere Fragestellung lautete: Besteht bei der Frau eine Abhängigkeit der motorischen Tubenaktivität von der hormonalen Ovarialfunktion und welcher Art ist diese Beeinflussung, falls sich eine solche nachweisen lässt? Mit Hilfe kombinierter In-vivo-/In-vitro-Teste suchten wir darauf eine Antwort.

Von über 300 nichtgraviden Frauen wurde bisher die Spontanmotilität der Eileiter in vitro untersucht[2]. Die Einteilung der Testpersonen erfolgte aufgrund der Zyklusanamnese, Vaginalzytologie und Endometriumsbiopsie. Zudem wurde von allen Untersuchten unmittelbar präoperativ die 24-h-Urinausscheidung der Östrogene (Östron-Östradiol, Östriol), des Pregnandiols und der Gesamtgonadotropine bestimmt.

Die Figur 1 gibt Aufschluss über die Tubenspontanmotilität von Frauen, die sich im Zeitpunkt der Operation in der Proliferationsphase, in der Sekretionsphase oder in einem anovulatorischen Zyklus befanden. Der statistische Vergleich der durchschnittlichen Kontraktionsfrequenzen ergibt, dass die Intensität der Spontanmotilität der Tube in vitro in der Sekretionsphase gegenüber der Proliferationsphase signifikant herabgesetzt ist ($p < 0{,}025$). Bei Tuben anovulatorisch menstruierender Frauen besteht hingegen während der ganzen Zyklusdauer die gleiche motorische Aktivität wie in der ersten Hälfte des biphasischen Zyklus ($p > 0{,}6$).

Anhand von Dosiswirkungstesten versuchten wir festzustellen, ob diese postovulatorische Hemmung der Tubenmotilität durch eine veränderte Empfindlichkeit der Tube auf *neurotrope Wirkstoffe* zustande kommt[3,4].

Ein Vergleich der Dosiswirkungskurven des Adrenalins in der Proliferations- und Sekretionsphase zeigt, dass diese Kurven identisch liegen: Die ED_{50} beider Kurven beträgt 0,03 µg/ml. Die Adrenalinsensibilität der Tube bleibt demnach während des ovulatorischen Zyklus konstant.

Auch die Empfindlichkeit der Tuben auf Serotonin verändert sich im biphasischen Zyklus nicht, wie vergleichende Dosiswirkungsteste ergaben: Die ED_{50}-Werte in der Proliferations- und Sekretionsphase liegen bei 0,2 µg/ml.

Ganz anders verhält es sich mit der Sensibilität der Tubenmuskulatur auf Acetylcholin. Während in der Proliferationsphase die ED_{50} 0,95 µg beträgt, liegt dieser Wert in der Sekretionsphase bei 8,0 µg (Figur 2). Aus

[1] Vortrag an der 37. Versammlung der Deutschen Gesellschaft für Gynäkologie, Travemünde 24. bis 28. September 1968.

[2] H. ERB, *Zur hormonalen Regulation der Tubenmotilität*, Habilitationsschrift Bern 1968, Bibliotheca Gynaecologica, Nr. 52 (S. Karger, Basel–New York).

[3] H. ERB, Méd. Hyg. *26*, 597 (1968).

[4] F. SANDBERG, A. INGELMAN-SUNDBERG, L. LINDGREN und G. RYDEN, Acta obstet. gynaec. scand. *39*, 506 (1960).

Zyklen	Mittlere Frequenz pro 10'				Mittlere Amplitudenhöhe, mm			
	isth.	int.	amp.	total	isth.	int.	amp.	total
Anovulatorischer Zyklus	20,9	27,6	31,8	(27,6)	1,6	3,2	4,6	(3,3)
Proliferation	23,6	28,2	32,0	(28,3)	2,4	3,6	4,4	(3,6)
Ovulatorischer Zyklus — Sekretion	19,9	25,1	31,7	(26,0)	3,3	4,0	5,5	(4,3)

Fig. 1. Vergleich der Tubenspontanmotilität im anovulatorischen und ovulatorischen Zyklus.

Kein signifikanter Unterschied zwischen der mittleren Frequenz in anovulatorischem Zyklus und Proliferationsphase ($p > 0{,}6$) bei signifikanter Verschiedenheit dieser Werte gegenüber der Frequenz in der Sekretionsphase ($p < 0{,}025$). Geringe Unterschiedlichkeit der mittleren Amplitudenhöhe aus versuchstechnischen Gründen bedeutungslos.

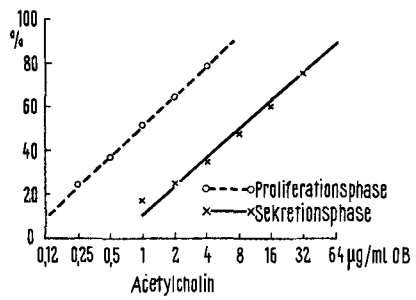

Fig. 2. Vergleich der Acetylcholin-Sensibilität von Tuben in der Proliferations- und Sekretionsphase.

Die erste Kurve basiert auf segmentären Dosiswirkungsmessungen an 41 Tuben von 22 Frauen in der Proliferationsphase, die zweite Sammelkurve auf Dosis-Wirkungs-Tests an 42 Tuben von 22 Frauen in der Sekretionsphase. ED_{50} des Acetylcholins in Proliferationsphase 0,95 µg, in Sekretionsphase 8 µg/ml. – Tubenmuskulatur in Proliferationsphase 9,23(6,82–12,48)mal empfindlicher auf Acetylcholin als in Sekretionsphase ($P = 0{,}05\%$).