

A31 CELL AS A HOST OF MYCOBACTERIUM LEPRAEMURIUM AND  
MYCOBACTERIUM LEPRAE

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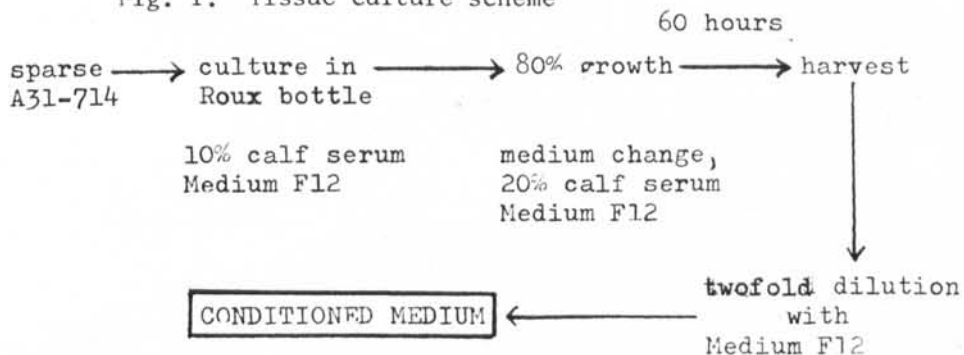
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A31 is a cloned strain cell of the 3T3 cell which is derived from a BALB/C mouse embryo. Todaro and his associates established the 3T3 cell in 1965 (1) and isolated the A31 cell from 3T3 by cloning in 1968 (2). Kakunaga isolated several subclones from A31 in 1973 (3) and one of these subclones, named A31-714, was used in this experiment. This cell is extremely sensitive to contact inhibition of cell division and stops growing at a very low saturation density.

#### MATERIALS AND METHODS

**Cell Culture.** Clone A31-714 was obtained from Dr. T. Kakunaga of the Department of Tumor Viruses, Research Institute for Microbial Diseases, Osaka University, and cells were grown in 37 mm Falcon plastic dishes containing 2 ml of F12 medium supplemented with 10% calf serum, and incubated in a CO<sub>2</sub>-incubator at 37°C. Conditioned medium for medium change was prepared as shown by Fig. 1.

Fig. 1. Tissue culture scheme



The medium change was carried out twice a week, and the cell monolayer could be maintained for more than three months in good condition.

Infection of M. lepraemurium and M. leprae. M. lepraemurium, strain Hawaii, was obtained from a subcutaneous murine leproma of a C3H mouse. M. leprae was obtained from the subcutaneous leproma of a relapsed case of Lepromatous leprosy. The bacillary suspension was prepared with the conditioned medium and 2 ml of the bacillary suspension was inoculated onto the cell monolayer. After 2 days of infection, a medium change was carried out with conditioned medium.

## RESULTS

Phagocytosis. Table 1 shows the phagocytic activity of A31-714 to M. lepraemurium and M. leprae.

Table 1. Phagocytic Index of A31-714 Cell for M. lepraemurium and M. leprae

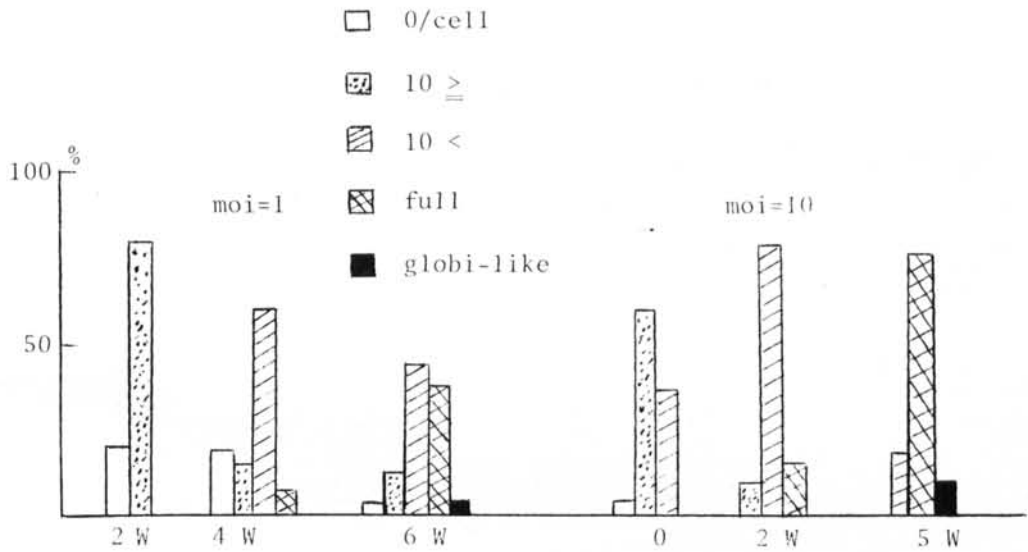
<u>M. lepraemurium</u>		<u>M. leprae</u>	
moi <sup>a</sup> =1	moi=10	moi=10	moi=20
80.0%	94.0%	52.5%	86.0%

<sup>a</sup> moi=multiplicity of infection

Two hundred cells were randomly observed after 2 days of infection, and the number of cells that had phagocytized bacilli was scored. Eighty percent of cells phagocytized M. lepraemurium when the multiplicity of infection was 1; 94% of cells showed phagocytosis when the moi was 10, and 100% of cells showed phagocytosis when moi was 100. This means that the phagocytic activity of this cell for M. lepraemurium is very high. The phagocytic activity for M. leprae seems to be a little low, but the bacillary suspension of M. leprae contains bacillary clumps in no small numbers, so considering this fact we should say that phagocytic activity of A31 cell to M. leprae is fairly good.

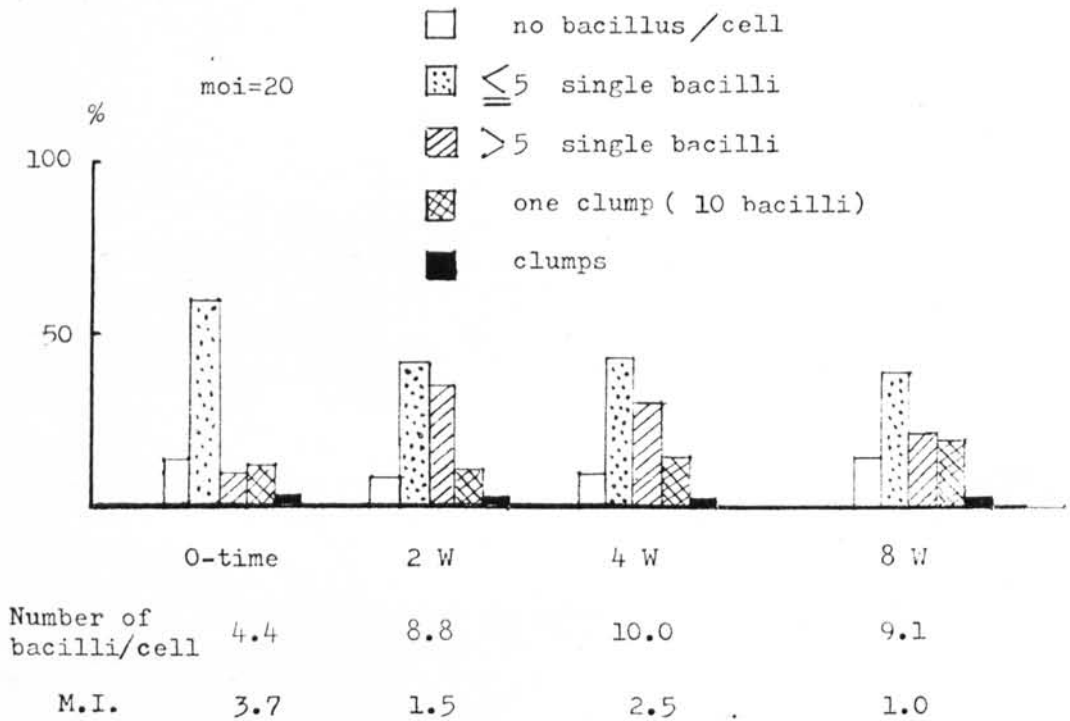
Growth of M. lepraemurium in A31-714. Fig. 2 shows the appearance of growth of M. lepraemurium. One hundred cells of each stage were randomly observed and scored. Remarkable elongation of bacilli was observed at the second week.

We should say that M. lepraemurium can grow uniformly and steadily in A31 cell.

Fig. 2. Growth of *M. lepraemurium* in A31-714.

The growth of *M. lepraemurium* was suppressed by addition of 100  $\mu$ g of streptomycin in the medium, but the *M. lepraemurium* began to grow after a medium change to streptomycin-free medium. Growth of *M. lepraemurium* was completely inhibited by heat treatment at 56°C for 30 minutes, and considerably inhibited by storage in a deep freeze at -70°C, but no suppression was seen by treatment at 37°C for 30 minutes.

Change of *M. leprae* in A31-714. Fig. 3 shows the change of *M. leprae* in A31-714. Two hundred cells were randomly observed and scored.

Fig. 3. Change of *M. leprae* in A31-714

The morphologic index (MI) of the *M. leprae* in the bacillary suspension used for infection was 3.7. The MI of bacilli in A31 cells was 1.5 at the second week, 2.5 at the fourth week, and 1.0 at the eighth week. The number of bacilli per cell increased slightly at the fourth week, and the number of cells containing a bacillary clump also increased slightly at the eighth week, but we should not say that *M. leprae* can grow in A31 cell at present.

#### DISCUSSION

Successful growth of *M. lepraemurium* by established strain cell culture was reported by Rees and Garbutt (4) in 1962, and by Matsuo (5) in 1970. But the intracellular growth of *M. lepraemurium* in these two cell culture systems seems not to be uniform in comparison with that observed in mouse peritoneal macrophage cultures by Chang and his associates (6), probably for the reason that the macrophages are not cloning strains.

In the present experiments, M. lepraemurium seemed to be phagocytized and to grow uniformly in A31 cells. The generation time and the subculture of M. lepraemurium in A31 cell cultures will be examined in future experiments.

Multiplication of M. leprae in A31 cells at 37°C has not been recognized yet, so the low temperature culture of this cell line should be adopted for cultivation of M. leprae.

#### ACKNOWLEDGMENT

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#### REFERENCES

1. TODARO, G. J., LAZAR, G. K. and GREEN, H., The initiation of cell division in a contact-inhibited mammalian cell line. *Cell. and Comp. Physiol.*, 66, 325-334, 1965.
2. AARONSON, S. A. and TODARO, G. J., Development of 3T3-like lines from BALB/C mouse embryo cultures. Transformation susceptibility to SV-40. *J. Cell Physiol.*, 72, 141-148, 1968.
3. KAKUNAGA, T., A quantitative system for assay of malignant transformation by chemical carcinogens using clone derived from BALB/3T3., *Int. J. Cancer*, 12, 463-473, 1973.
4. REES, R. J. W. and GARBUTT, E. W., Studies on Mycobacterium lepraemurium in tissue culture. I. Multiplication and growth characteristics in cultures of rat fibroblasts. *Brit. J. Exp. Pathol.*, 43, 221-235, 1962.
5. MATSUO, Y., Multiplication of Mycobacterium lepraemurium in mouse foot-pad cell culture. *Japan. J. Microbiol.*, 14, 233-240, 1970.
6. CHANG, Y. T. and NEIKIRK, R. L., Mycobacterium lepraemurium and Mycobacterium leprae in cultures of mouse peritoneal macrophages. *Internat. J. Leprosy*, 33, 586-598, 1965.