formation or mitochondrial changes at either age. Degenerative changes, including abundant lysosome formation and pyknosis, were first observed at 14.25 days.

If an epitheliomesenchymal interaction initiates changes leading to cell lysis, it is likely to take place shortly before the time when degenerative changes become apparent. The ECPs represent a potential pathway for communication and may play a crucial role in the epitheliomesenchymal interaction involved in normal palatogenesis.

The relationship between the terminal part of the ECP and any subjacent mesenchymal cell is of interest. 1 example was found of an ECP crossing the basal lamina and terminating close to the plasmalemma of a mesenchymal cell in the same plane of section (figure 2). It would be worthwhile following the progress of an ECP in serial sections to determine if such contacts commonly occur and the nature of junctions, if any, between ECP and mesenchymal plasmalemma.

The observation of cell processes uniting cells involved in embryogenetic interaction does not establish whether such processes are or are not necessary for such an interaction to take place, However, the fact that cell processes similar to ECPs have been described during the formation of the tooth[12] and the amphibian limb bud[13] suggests that ECPs could play a part in many epitheliomesenchymal interactions.

1 M. Pourtois, J. dent. Res. *48*, 1135 (1969), abstr.
2 M.S. Tyler and W.E. Koch J. dent. Res. *53*, 64 (1974), abstr.
3 M.S. Tyler and W.E. Koch, Anat. Rec. *182*, 297 (1975).
4 V. De Angelis and J. Nalbandian, Archs oral Biol. *13*, 601 (1968).
5 B.L. Shapiro and L. Sweney, J. dent. Res. *48*, 652 (1969).
6 G.R. Smiley and W.E. Koch, J. dent. Res. *50*, 1671 (1971).
7 G.R. Smiley and W.E. Koch, Anat. Rec. *173*, 405 (1972).
8 A.I. Farbman, Devl Biol. *18*, 93 (1968).
9 G.R. Smiley and A.D. Dixon, Anat. Rec. *161*, 293 (1968).
10 G.R. Smiley, Archs oral Biol. *15*, 287 (1970).
11 A.N. Goss and J.K. Avery, Aust. dent. J. *20*, 152 (1975).
12 E. Pannese, J. Ultrastruct. Res. *6*, 186 (1962).
13 M. Lauthier, J. Embryol. exp. Morph. *38*, 1 (1977).

## Enhancement of the antitumor effect of illudin S by including it into liposomes

S. Shinozawa, K. Tsutsui and T. Oda[1]

*Department of Biochemistry, Cancer Institute, and Department of Hospital Pharmacy, Okayama University Medical School, Okayama 700 (Japan), 17 October 1978*

*Summary.* Entrapping illudin S in liposome markedly enhanced life prolonging effect in ddN mice bearing Ehrlich ascites tumors, presumably by decreasing the side effects.

Chemical composition of illudin S, the toxic principle of *Lampteromyces japonicus,* was clarified by Nakanishi and others[2,3] in 1963, and its antitumor effects has been noted from early times[4]. However, illudin S showed fairly strong side effects, such as tissue damage in the liver, and hemorrhagic changes in digestive organs, lung and kidney[5]. Therefore, the problem was how to decrease this side effect without the loss of its antitumor effect. Based on such a concept, we examined the in vivo antitumor effect of illudin S in mice implanted with Ehrlich ascites tumor cells, using positively charged liposomes to decrease the side effects and to increase the antitumor effect of illudin S further.

*Materials and methods.* Illudin S was purified from *Lampteromyces japonicus* by the method of Nakanishi et al.[2]. Positively charged and sonicated liposomes entrapping illudin S were prepared by the method of Kimelberg[6], and the composition was lecithin (purified from chicken egg yolk by the method of Rhodes and Lea[7]), cholesterol (Nakarai Kagaku Co., Tokyo, Japan) and stearylamine (Tokyo Kasei Kogyo Co., Japan) in 3.2:2.2:1 molar ratio. Illudin S entrapped in liposomes and non-entrapped illudin S were separated by the method previously described[8]. Animals used for therapeutic experiments were male ddN mice (6 weeks old, weighing 20 g), divided into groups of 10 each. Mice in each group were inoculated i.p. with a suspension of Ehrlich ascites carcinoma cells ($2\times 10^7$ cells in 0.2 ml per animal) and, 24 h later, administered with aqueous solution of free illudin S or liposome-entrapped illudin S. The controls were given physiological saline. Survival time of the mice was then observed.

*Results and discussion.* In the group given free illudin S, average survival period of the mice with Ehrlich ascites tumor was longest in the group given 166 μg/kg of free illudin S in aqueous solution, and its percentage relative to the control (T over C) was 170%. In the group given liposome-entrapped illudin S, a more marked antitumor effect was observed, T over C being 225% in the group given 166 μg/kg, and 287% in the group given 333 μg/kg. More than one-half of the mice given 333 μg/kg of liposome-entrapped illudin S showed survival of over 25 days (table).

Komatsu et al.[4] reported that illudin S showed an antitumor effect against Ehrlich ascites carcinoma cells and mouse Sarcoma 180 cells. Illudin S in submicromolar concentrations inhibits the growth of cultured chicken

Chemotherapeutic effect of illudin S free or entrapped in liposomes

| | Dose of illudin S (μg/kg) | Mean survival time (days ± SD) | Number of 25-day survivors per 10 mice |
|---|---|---|---|
| Free illudin S | 83.0 | 10.1 ± 3.3 | 0 |
| | 166.0 | 15.8 ± 8.4 | 1 |
| | 333.0 | 14.5 ± 6.6 | 0 |
| | 800.0 | 8.5 ± 2.3 | 0 |
| Illudin S entrapped in liposome | 16.5 | 9.0 ± 2.8 | 0 |
| | 83.0 | 14.7 ± 3.3 | 0 |
| | 166.0 | 20.9 ± 7.4 | 2 |
| | 333.0 | 26.7 ± 11.1 | 6 |
| | 800.0 | 11.2 ± 4.0 | 0 |
| Illudin S + liposome (mixture) | 333.0 | 13.2 ± 1.3 | 0 |
| Liposome only | 0 | 10.7 ± 4.3 | 0 |
| Control | 0 | 9.3 ± 1.2 | 0 |

Mice were inoculated with $2\times 10^7$ Ehrlich ascites carcinoma cells i.p. on day 0 and treated i.p. 24 h later with a single dose of the drug.

embryo cells and L929 cells. Although the precise growth inhibition mechanism of the drug is yet unclear, the rate of RNA synthesis in the illudin S-treated cells markedly decreases without any effect on protein synthesis[9]. Inspite of such an antitumor effect, illudin S has not been taken up as an antitumor agent because of its effect. Our present series of experiments with illudin S has shown that enclosure of this compound into liposomes markedly enhanced its effect on survival time in Ehrlich ascites tumor, apparently by decreasing the side effects without diminishing its antitumor effect. As for the action mechanism of this increased antitumor effect by liposome entrapment, it was considered, as was reported for the action of actinomycin-D[10] and 1-$\beta$-D-arabinofuranocylcytosine[11], that the slow liberation of illudin S from liposome alleviated tissue damage and that increased contact between liposomes and tumor cells increased the affinity of illudin S to tumor cells, thus potentiating the antitumor effect of illudin S further.

1 We thank Mr M. Yabe for various technical assistance.
2 K. Nakanishi, M. Tada, Y. Yamada, M. Ohashi, N. Komatsu, and H. Terakawa, Nature *197*, 292 (1963).
3 T.C. McMorris and M. Anchel, J. Am. chem. Soc. *85*, 831 (1963).
4 N. Komatsu, M. Hamada, S. Nakazawa, S. Ogata, A. Yamamoto, H. Terakawa and T. Yamamoto, J. Ferment. Ass. *19*, 464 (1961) (in Japanese).
5 K. Nakai, Med. Biol. *48*, 33 (1958) (in Japanese).
6 H.K. Kimelberg and D. Papahadjopoulos, J. biol. Chem. *246*, 1142 (1972).
7 D.N. Rhodes and C.H. Lea, Biochem. J. *65*, 526 (1957).
8 S. Shinozawa, K. Tsutsui, M. Yabe, Y. Araki and T. Oda, Igaku no Ayumi *108*, 414 (1979) (in Japanese).
9 O. Hatase, personal communication.
10 Y.E. Rahman, E.A. Cerny, S.L. Tollaksen, B.J. Wright, S.L. Nance and J.F. Thomson, Proc. Soc. exp. Biol. Med. *146*, 1173 (1974).
11 T. Kataoka and T. Kobayashi, Annls N. Y. Acad. Sci. *308*, 387 (1978).

## Estrogen receptor in uteri of mice of different *H-2* genotypes[1]

Deborah A. Palumbo and A.O. Vladutiu

*State University of New York at Buffalo, Laboratory of Immunopathology at The Buffalo General Hospital, Buffalo (N.Y. 14203, USA), 11 December 1978*

*Summary.* A relationship between the amount of available estradiol receptors in uteri of inbred mice and their *H-2* genotype is suggested by study in congenic animals.

The *H-2* gene complex contains the major histocompatibility system of the mouse as well as genes which control various physiological processes e.g., immune responses to autoantigens[2], the level of cyclic AMP in the liver[3], the levels of serum hemolytic complement[4], testosterone and testosterone binding capacity[5], the 'mating preference'[6], and the level of fetal palatal glucocorticoid receptor[7]. In target tissues, estrogens bind to cytoplasmic receptors of high affinity and specificity; the steroid-receptor complexes are then taken up by the cell nucleus where the physiological effects of estrogens are initiated. We investigated whether the amount of estrogen receptors in the uteri of mature, virgin mice is genetically controlled and related to the *H-2* locus.

*Materials and methods.* Female mice, 8 weeks old, of different inbred strains, were obtained from Jackson Laboratories, Bar Harbor, Maine. Groups of 10–15 animals were housed in the same cage in air-conditioned quarters with 12-h lighting cycle and maintained for a week on a dried pellet diet and tap water ad libitum. A group of random bred male Swiss mice were kept in a separate cage in close proximity to the female mice. Vaginal smears and visual inspection of the vaginas[8] were used to determine the estrus cycle of the mice. Animals in the diestrous phase were killed by cervical dislocation and the uteri were quickly dissected out. Both uterine horns, from just below the entry of the oviduct to above the cervical junction, were separated from the remains of the uterus and used in the experiments. Because of the small size of the mouse uterus, 10–15 uteri from mice of the same strain were pooled. They were snapped frozen in liquid nitrogen or a mixture of acetone and dry ice, and stored in liquid nitrogen or at −85 °C. The estradiol receptor was assayed by a standard charcoal technique[9]. Briefly, the tissue was homogenized in ice cold Tris buffer, pH 7.4, with a steel mechanical homogenizer (Tekmar Company, Cincinnati, Ohio) and centrifuged for 30 min at 105,000 × g (Spinco model L5-40 ultracentrifuge, Beckman Instruments, Palo Alto, Cal.). The protein was determined by the method of Lowry et al.[10]. Increasing amounts of [2,4,6,7(n)-$^3$H]-estradiol (Amersham Corp., England) were added and plots were constructed from 6 points, with the bound estradiol, as described by Scatchard[11]. From these plots the dissociation constants ($K_d$) for the binding reactions were obtained. The estradiol receptor was expressed in fmoles/mg of cytosol protein. The analysis of the slopes of the Scatchard plots showed that the test was reliable with respect to variability within the assay and between assays.

*Results.* Table 1 shows a wide range of values for estrogen receptor in various inbred strains of mice. Some strains had

Table 1. Estrogen receptor in uteri of inbred mice

| Strain | *H-2* | Estrogen receptor* | $K_d$** |
|---|---|---|---|
| C57BL/6J | b | 68 | 1.2 |
| C57BL/10J | b | 27 | 1.3 |
| LP/J | b | 147 | 2.4 |
| BALB/cJ | d | 694 | 1.2 |
| DBA/2J | d | 358 | 2.4 |
| AKR/J | k | 774 | 1.2 |
| CBA/J | k | 661 | 1.0 |
| C3H/HeJ | k | 180 | 1.0 |
| C3H/StJ | k | 138 | 1.2 |
| 129/J | k | 443 | 0.5 |
| RF/J | k | 339 | 1.2 |
| DBA/1J | q | 953 | 1.4 |
| PUB/J | q | 124 | 0.8 |
| SWR/J | q | 385 | 1.7 |
| A.SW/J | s | 430 | 0.6 |
| SJL/J | s | 684 | 0.6 |
| SM/J | v | 92 | 1.6 |

* $^3$H-estradiol bound, expressed in fmoles/mg cytosol protein (pool of 10–15 uteri). ** Equilibrium dissociation constant (moles/l × $10^{-10}$).