The effect of 8-methoxypsoralen and longwave UV-radiation on growth rates of human epidermal cells in culture[1]

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Summary. Cell yields from human epidermal cell cultures 6 days after treatment with 0.1 µg/ml or 0.3 µg/ml 8-methoxypsoralen and 0.34 J/cm^2 longwave UV-radiation were significantly lower than nontreated controls ($p < 0.025$, $p < 0.005$ respectively) and significantly different from each other ($p < 0.025$).

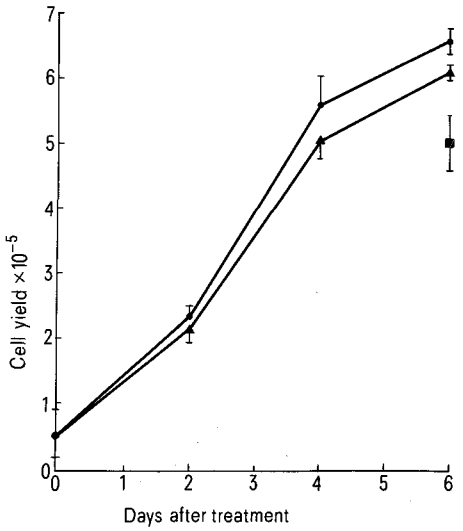
Treatment of psoriatic patients with 8-methoxypsoralen and longwave UV-radiation (PUVA) has resulted in a dramatic improvement in many cases[2,3] but the way in which the beneficial effects are mediated remains uncertain. Although PUVA both *inhibits* DNA synthesis[4,5] and causes considerable chromosomal damage in a variety of cells[6-9], other cellular effects including ultrastructural changes[10,11] and inhibition of certain immune functions[12-15] have been demonstrated. This has led to speculation that changes in immune functions in the skin may be important in promoting the clinical improvement in psoriatic patients on PUVA therapy[15]. The epidermis is the target organ during PUVA therapy. We have already shown that the sister chromatid exchange frequency is increased in epidermal cells exposed to PUVA[9] and in this study we report the direct effect of this treatment on epidermal cell proliferation in vitro.

Materials and methods. Second passage epidermal cells derived from a 75-year-old female were used for the experiments and were cultured with 3T3 mouse fibroblast feeder layers prepared as previously described[9].

Epidermal cell cultures were initiated by inoculating 1.7×10^4 cells in 2 ml of medium on to each feeder layer. The medium was F10 containing 2 mM glutamine, 100 µg/ml kanamycin, 100 IU/ml benzyl penicillin together with 20% foetal calf serum, 0.4 µg/ml hydrocortisone and 10^{-10} M cholera toxin. The cultures were incubated undisturbed for 5 days and then divided into 3 groups. The medium was replaced with F10 alone, or F10 containing 0.1 µg/ml 8-MOP or 0.3 µg/ml 8-MOP. Cultures were incubated in the dark for 0.75 h, then those with 8-MOP were irradiated with longwave UV-radiation (UVA) from 2 parallel Atlas 20 W fluorescent tubes giving 0.34 J/cm^2 of incident light on the cells. After irradiation feeder layers were selectively removed from all the cultures by washing with 0.02% EDTA in phosphate buffered saline (PBS)[16]. Fresh mitomycin-treated 3T3 cells suspended in medium + 10 ng/ml epidermal growth factor were added to all cultures except those harvested immediately. The cultures were incubated in the dark and the medium changed every 2nd day.

Before harvesting the cultures the feeder layers were removed. The epidermal cells were then dissociated by a 10-min incubation in 0.03% trypsin and 0.02% EDTA in PBS. These cells were suspended in medium and counted using a hemocytometer to determine cell yields.

Results. Control cultures (not exposed to 8-MOP or UVA) were harvested at day 0 and also with those treated with 0.1 µg/ml 8-MOP + UVA on days 2, 4 and 6 after treatment. Cultures treated with 0.3 µg/ml 8-MOP + UVA were harvested on day 6 only. 3 or 4 replica cultures were used for each culture type at each time. The results are shown in the figure. Cell yields from cultures treated with 0.1 µg/ml or 0.3 µg/ml 8-MOP + UVA on the 6th day after treatment were significantly lower than those from the controls ($p < 0.025$, $p < 0.005$ respectively) and different from each other ($p < 0.025$).



Cell yields from untreated control cultures and cultures treated with PUVA during the 6 day post-treatment period. Controls, ●——●; 0.1 µg/ml 8-MOP + 0.34 J/cm^2 UVA, ▲——▲; 0.3 µg/ml 8-MOP + 0.34 J/cm^2 UVA, ■. Bars represent SD.

Discussion. Exposure of epidermal cells in culture to the levels of 8-MOP and UVA used here caused slower growth giving lower cell yields compared with the controls. This finding might be expected from the chromosomal damage which this treatment causes[9] and is consistent with our observation that the proportion of dividing cells in similar cultures exposed to high concentrations of 8-MOP with UVA was considerably reduced[9]. Using fibroblast cultures Pohl and Christophers[5] also found a decreased growth rate after treatment with PUVA.

Since the 8-MOP concentrations used here are equivalent to those found in the serum of psoriatic patients on PUVA therapy, it seems probable that this treament will have a direct inhibitory effect on cell proliferation in the skin. Although differences between cultures after a single dose of PUVA and the controls are not great they are significant. Such an effect in vivo may be sufficient to explain the clinical improvement in psoriatic patients on PUVA therapy particularly as this usually involves repeated treatments during a therapeutic regime which extends over several weeks.

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2 Parrish, J.A., Fitzpatrick, T.B., Tannenbaum, L., and Pathak, M.A., New Engl. J. Med. *291* (1974) 1207.
3 Lakshmipathi, T., Gould, P.W., McKenzie, L.A., Johnson, B.E., and Frain-Bell, W., Br. J. Derm. *96* (1977) 587.
4 Baden, H.P., Parrington, J.M., Delhanty, J.D.A., and Pathak, M.A., Biochim. biophys. Acta *262* (1972) 247.
5 Pohl, J., and Christophers, E., J. Invest. Derm. *71* (1978) 316.
6 Carter, D.M., Wolff, K., and Schnedl, W., J. Invest. Derm. *67* (1976) 548.
7 Latt, S.A., and Loveday, K.S., Cytogenet. Cell Genet. *21* (1978) 184.

8 Mourelatos, D., Faed, M.J.W., and Johnson, B.E., Experientia *33* (1977) 1091.
9 West, M.R., Johansen, M., and Faed, M.J.W., J. Invest. Derm. *78* (1982) 67.
10 Wennersten, G., Acta derm.-vener., Stockh. *59* (1979) 21.
11 Ree, K., Johnsen, A.S., and Hovig, T., Acta path. microbiol. scand. A *89* (1981) 81.
12 Morison, W.L., Parrish, J.A., Woehler, M.E., Krugler, J.L., and Block, K.J., J. Invest. Derm. *76* (1981) 484.
13 Bridges, B.A., and Strauss, G., Nature *283* (1980) 523.
14 Briffa, D.V., Parker, D., Tosca, N., Turk, J.L., and Greaves, M.W., J. Invest. Derm. *77* (1981) 377.
15 Morhenn, V.B., Benike, C.J., and Engleman, E.G., J. Invest. Derm. *75* (1980) 249.
16 Rheinwald, J.G., and Green, H., Cell *6* (1975) 331.

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Physical characterization of the *E. coli dnaC* region carried by a plaque forming *λdnaC* transducing phage

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Summary. The physical map of the 11.5 kb DNA segment containing the *E. coli dnaC* gene carried by λp*dnaC* transducing phage was constructed and the *dnaC* gene within this segment was localized by subcloning it into plasmid pBR322. Based on the physical structure of λp*dnaC*, the formation of the λp*dnaC* by nonhomologous recombination is dicussed.

Among the proteins required for DNA replication in *E. coli*, the *dnaC* gene product is particularly interesting because it is needed for both initiation and elongation processes[2,3]. Previously, we had isolated 2 plaque forming λ phage derivatives transducing the *dnaC* gene, λp*dnaC*-17 and λp*dnaC*-37, through a) directed integrative suppression of a *dnaA* mutation by an F′ plasmid carrying *dnaC*, b) isolation of λd*dnaC* and c) conversion of λd*dnaC* into λp*dnaC* with the help of λ*imm*21*b*2*nin*5 prophage[4]. We describe here the physical map of λp*dnaC*, which may serve as a basis for functional studies.

The structures of λp*dnaC*-17 and λp*dnaC*-37 are identical since they produced the same restriction cleavage patterns (data not shown). According to the procedure for the isolation and genetic characterization of λp*dnaC*[4], its genotype must be λ*imm*21*nin*5*int*$^+$*attP*$^+$*dnaC*$^+$. Indeed, electron microscopic heteroduplex studies (fig. 1,A) showed that it carries the *imm*21 substitution, the *nin*5 deletion and an 11.3±0.7 kb *dnaC* substitution (19 molecules analyzed). The *dnaC* substitution begins at λ map unit[5] 0.45 and terminates at λ map unit 0.57. This extension corresponds very closely to that of the *b*2 deletion[5], which covers 5.76 kb between λ map units 0.453 and 0.573. We have also analyzed λp*dnaC* DNA with restriction enzymes (fig. 1,C–F) and constructed a restriction cleavage map (fig. 2). The results confirmed that the *dnaC* segment replaced the *b*2 region of the λ genome. The *Bam*HI and *Hin*dIII sites[5] on the λ genome at λ map units 0.466 and 0.568, respectively, are lost due to the substitution, but the *Eco*RI site[5] at map unit 0.445 is still present. Three *Eco*RI sites[5] carried on wild type λ DNA at map units 0.653, 0.810 and 0.931 are absent because the *att*P-*int*-*imm*21-*nin*5-*R* region of λp*dnaC* originated from the λ*imm*21p*lac*5*nin*5 derivative λ616[4,6], on which these *Eco*RI sites are missing (see also fig. 3). From these analyses we conclude that the approximately 11.5 kb *dnaC* segment contains 2 *Eco*RI sites, 2 *Bam*HI sites, 1 *Bgl*II site and 2 *Hin*dIII sites. These are shown in figure 2.

To localize the *dnaC* gene further, λp*dnaC* DNA was cleaved with *Eco*RI, mixed with *Eco*RI cleaved pBR322[7], ligated and transformed to *E. coli* C LD332 *dnaC*ts[4] with selection for temperature resistant (tr), ampicillin resistant (Ap^r), and tetracycline resistant (Tc^r) transformants. Isolation and restriction cleavage analysis of plasmids[8] from these transformants revealed that they were pBR322 derivatives containing the 8.3 kb *Eco*RI-3 fragment (see figs 1 and 2). Similarly, pBR322 containing the 4.7 kb *Bam*HI-5 fragment inserted in its *Bam*HI site gave tr, Ap^r, Tc^s transformants of LD332. That production of tr transformants is not dependent on recombination of the *dnaC* segment into the host chromosome was shown by obtaining tr, Ap^r, Tc^s transformants of *E. coli* K12 NY60 *dnaC*ts *recA*[2] with appropriately (*Eco*K) modified pBR322:*Bam*HI-5 plasmid. Growth or tr transformants is thus rendered possible by complementation. Since the orientation of the *Bam*HI-5 fragment inserted into pBR322 did not affect

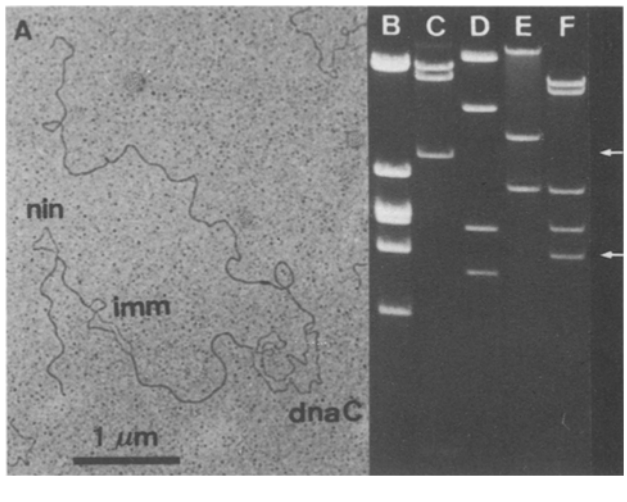

Figure 1. Heteroduplex molecules between λp*dnaC* and λ DNA, and restriction cleavage patterns of DNA of λ and λp*dnaC*. Preparation of λ phages, formation of heteroduplex molecules, extraction of phage DNA and restriction cleavage analysis were performed as described before[4,9]. *A* Heteroduplex between DNA of λp*dnaC* and λ*cI*857*S*7. Substitution loops for the immunity region (*imm*) and for the *dnaC* segment and the *nin*5 deletion loop are marked. *B* to *F* Restriction cleavage patterns of λ DNA and λp*dnaC*. Electrophoresis was carried out in a 0.8% Agarose gel. *B* λ*cI*857*S*7 DNA cleaved with *Eco*RI; *C* λp*dnaC* with *Eco*RI; *D* λp*dnaC* with *Hin*dIII; *E* λp*dnaC* with *Bgl*II; *F* λp*dnaC* with *Bam*HI. The arrows point to the *Eco*RI-3 and *Bam*HI-5 fragments, both of which contain the *dnaC* gene.