Journal of the Arkansas Academy of Science

Volume 36 Article 30

1982

Dark Repair of Lethal Damage Induced in a Hybrid Mammalian Tissue Culture Cell Line by Ultraviolet Light

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Table 1. Physicochemical parameters for Springs I and II, Randolph County, Arkansas (26 April-13 June 1981).

Date	Spring	Air Temp. °C	Water Temp. °C	pH	nitrate ppm
26-IV	I	31	18	7.5	20
	II	30	15	7.5	22
10-V	I	23	15	8.0	20
	II	22	14	7.7	20
23-V	I	26	17	8.0	20
	II	26	15	7.2	20
13-VI	I	27	17	8.0	20
	II	27	16	7.5	20

Table 2. Aquatic macroinvertebrates, expressed as relative abundance, for Springs I and II, Randolph County, Arkansas (26 April-13 June 1981).

TAXA	Spring I	Spring II
GASTROPODA	0	7
AMPHIPODA		
Crangonyx forbesi (Hubricht Mackin)	150	41
Gammarus pseudolimnaeus Bousfield	18	0
Hyalella azteca (Saussure)	2	0
DECAPODA	0	1
HEMIPTERA		
Gerris remigis Say	8	23
Sigara grossolineata Hungurford	1	0
Microvelia americana (Uhler)	0	2
Microvelia austrina Buano	0	4
PLECOPTERA		
Perlesta placida (Hagan)	1	0
Isoperla	25	0
EPHEMEROPTERA		
Heptagenitidae	7	0
ODONATA		
Calopteryx maculata (Beauvois)	1	0
Argia plana Calvert	1	0
Plathemis lydia Drury	0	1
COLEOPTERA		
Laccophilus pictus Sharp	4	0
DIPTERA		
Culiseta inornata (Williston)	4	1
Tabanus atratus Fabricius	3	0
Total	225	80

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We thank Ron Smith for his assistance in identifying many of the insects. We also thank Larry Olson for his identification of the Culicidae.

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DARK REPAIR OF LETHAL DAMAGE INDUCED IN A HYBRID MAMMALIAN TISSUE CULTURE CELL LINE BY ULTRAVIOLET LIGHT*

Dark (non-photoreactivation) intracellular mechanisms that repair ultraviolet light (UV)-induced lesions in nuclear DNA are known to contriburte significantly to the resistance shown by many prokaryotic cells to such UV-induced effects as loss of colony forming ability, mutations, and division delay (Rupert and Harm, 1966; Smith, 1977). A number of studies indicate that at least two such mechanisms function in some mammalian cells (Cleaver, 1974). HeLa cancer tissue culture cells possess a mechanism that may be analogous to the "excision" repair mechanism found in many UV-resistant bacteria (Regan et al., 1968). Another mechanism has been detected in rodent cells (Cleaver, 1974) that is a post DNA replication repair process similar to "recombinational" (recom) repair found in some UV-resistant bacteria (Rupp and Howard-Flanders, 1968). A study by Meyn et al. (1974) suggests that both of these mechanisms function in many mammalian cells, but with varying efficiencies. For example, V79 hamster cells exhibit both excision and recom repair but the recom repair mechanism dominates, while HeLa cells appear to exhibit efficient exci-

sion repair, but little if any recom repair. Present understanding of the nature and interactions of excision and recom repair in mammalian cells is far from adequate and further investigation is certainly indicated. We report here our initial attempt in this direction by studying resistance to UV-induced cell killing in a hybrid cell line that may possess the genetic potential for efficient repair by both excision **and** recombination.

A hybrid cell line was developed by fusion of V79 hamster and HeLa tissue culture cells. The V79B hamster cell line described by Bender et. al. (1973) and a HeLa tissue culture line obtained from Dr. Joel Bedford of Colorado State University were fused by the polyethylene Glycol (P.E.G.) technique developed by Davidson and Gerald (1976). Single cells from this fused culture were then isolated into Falcon tissue-culture microtest plates, one cell per well. Approximately three percent of the isolated cells formed colonies that were the progenitors of cultures with significant plating efficiencies. The culture chosen for experimentation had a plating efficiency of 44 percent.

Routine techniques, such as cell culture maintenance, incubations, irradiations, single cell plating and survival (colony forming ability) assayes, described in detail elsewhere (Griggs and Bender, 1972), were applied. Vigorously growing log-phase cultures were selected for all experiments.

Results of the initial survival study (Figure 1) were somewhat surprising because the kinetics for the three cell lines did not differ significantly. It was anticipated that the hybrid line would exhibit a greater resistance to UV-induced cell killing, for the genetic potential for efficient repair by excision **and** recombination was contributed by respective cells hybridized. Eighty-six percent of the hybrid cells contained 98 chromosomes (22 V79 and 76 HeLa), in good agreement with the combined complements of the parent lines (22 for 98 percent of the V79 and 76 for 96 percent of the HeLa). The hybrid complement did not vary significantly during the experimentation.

Among the possible interpretations of the data of Figure 1 coupled with the chromosomal data is the possibility that at least one of the repair mechanisms functions with reduced efficiency in the hybrid cells. Enzymatic radiation repair systems are usually temperature sensitive (Rupert and Harm, 1966). If the recom and excision mechanisms exhibited significantly different temperature sensitivities, then comparison of the effects of variable post irradiation incubation temperatures on the survival kinetics of the hybrid and parent lines might indicate which mechanism functions more efficiently in the hybrid line. However, our data (Table) indicate no marked difference in response of irradiated cells to temperature variations. Since recom repair in the parent V79 line is inhibited by caffeine while excision repair in the parent HeLa line is not (Griggs, unpublished data), a pilot study (Figure 2) of the effects of caffeine on the repair of UV-induced lethal damage in the hybrid line was carried out in another attempt to identify the repair mechanism responsible for survival in the hybrid line. Caffeine greatly enhances the degree of cell killing induced by UV doses in the range 0-150 ergs/mm² in both the V79 parent line and the hybrid line, but has little, if any, effect on the HeLa parent line. Comparison of the similarities in kinetics for the V79 and hybrid lines, in the presence and absence of caffeine (Figures 1,2) suggest that the recom repair mechanism is responsible for practically all the associated repair. The excision mechanism is virtually shut down or suppressed.

*Research supported in part by NCI Grant CA 18809-06.

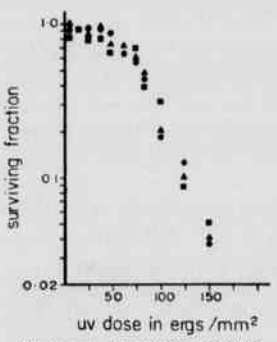

Figure 1. Survival of V79 (circles), HeLa (triangles), and V79-HeLa (squares) cells following UV irradiations. Entire experiment conducted at 37 °C. Plating efficiencies were V79 cells—84%, HeLa Cells—82%, Hybrid cells—44%. Surviving fraction = number of colonies assayed at each dose point/number of colonies assayed for control (0 ergs/mm² point).

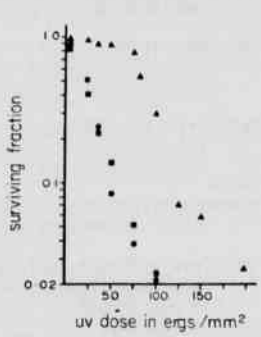

Figure 2. Survival of V79 (circles), HeLa (triangles), and V79-HeLa (squares) cells exposed to the indicated doses of UV and then incubated at 37 °C in medium containing 0.0008 moles per liter caffeine. Surviving fraction = number of colonies assayed at each dose point/number of colonies assayed for control (0 ergs/mm² point).

Table. Survival of V79, HeLa and V79-HeLa hybrid cells which were exposed to 100 ergs/mm² UV and then incubated at the temperatures indicated. 1000 cells were plated at each temperature for each cell line.

Temperature °C	Surviving Fraction* V79	HeLa	V79-HeLa
32	0.01	0.01	0.02
33	0.01	0.02	0.01
34	0.04	0.05	0.05
35	0.10	0.13	0.09
36	0.13	0.14	0.12
37	0.15	0.19	0.14
38	0.12	0.13	0.11
39	0.08	0.10	0.09
40	0.06	0.08	0.06

*Surviving Fraction = number of colonies assayed/number of cells plated.

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PROTECTIVE IMMUNE RESPONSES OF RABBITS TO *ASCARIS SUUM* LARVAL ANTIGENS

Parasitic nematode larvae frequently release physiologically active substances during and after hatching and moulting processes. These substances include enzymes which are often antigenic. Soulsby (1958) stated that most antigenic activity of *Ascaris suum* was associated with moulting. Sarles (1932) observed that *Trichostrongylus* larvae placed in immune homologous serum developed precipitates around various orifices such as the mouth, excretory pore and annus. Similar findings were reported for *Ascaris* and *Trichinella* (Mauss, 1941).

Mills and Kent (1965) reported that the antigens found in incubation media were excreted and/or secreted by *Trichinalla spiralis* larvae and were involved in the formation of immune precipitates. They demonstrated that these materials, when injected into mice, confer a certain degree of immunity.

Thorson (1956) studied the effect of serum from a dog resistant to infection with *Ancylostoma caninum* and found that it inhibited proteinase activity in esophageal saline extracts of adult hookworms. He demonstrated (1956a) that injection of the extracts into dogs inhibited the growth and maturation of the worms in a challenge infection.

Rogers (1958) described the hatching mechanism of *Ascaris lumbricoides* and revealed the presence of chitinase and esterase in the hatching fluid (H.F.) released by the larvae during hatching. Hinck and Ivey (1976) detected proteinase activity in *Ascaris suum* hatching fluid and in the excretions and secretions (E.S.) produced by the second stage larvae after hatching. Finch (1977) also found proteolytic activity in E.S. and somatic extract of *Ascaris suum* third stage larvae. Hinck (1971) demonstrated that both antihatching fluid and infection antisera inhibited hatching fluid proteinase. The present study was conducted to determine if immunization with *Ascaris suum* larval antigens provided any protection from infection.

Mature, female *Ascaris suum* were collected at a local abattoir. The methods for collecting, processing and embryonating eggs and the techniques employed for in vitro hatching and processing of hatching fluid have been previously described (Hinck and Ivey, 1976).

Third stage larvae were collected by infecting approximately twelve week old rabbits *per os* with 1,000,000 infective eggs. Viability was determined by microscopically examining the eggs for the presence of motile, second stage larvae. The per cent of infective eggs was determined and appropriate dilutions were made using deionized water. Five days post-infection the rabbits were sacrificed and the lungs removed aseptically. The lungs were placed in a Virtis homogenizer along with 0.85 per cent NaCl and homogenized at high speed for approximately 20 seconds. Larvae were recovered by using a modified Baermann apparatus. After two hours, larvae were collected and washed with sterile deionized water to remove erythrocytes and hemoglobin. Washed larvae were centrifuged and either disrupted by sonic oscillation or incubated to produce excretions and secretions.

Larval somatic extract was prepared by subjecting larvae, suspended in 0.85 per cent NaCl, to sonic vibration at 80,000 cycles per second with a sonic oscillator (Kewaunee Scientific Equipment) for thirty minutes. The sonicate was incubated at 4 C for 2 hours, centrifuged for 1 hour at 15,000 X G at 4 C, dialyzed against four changes of deionized water, lyophillized and stored at 4C.

Excretions and secretions of larvae were collected by incubating larvae for 24 hours at 38 C in sterile Eagle's Medium containing 10 mcg/ml Garamycin (Schering), added to inhibit bacterial growth. After incubation the E.S. was processed as previously described for the somatic extract.

Two month old rabbits were injected subcutaneously with 3.0 mg of a particular larval antigen reconstituted in 0.85 per cent NaCl mixed with an equal volume of Freund's complete adjuvant. After three weeks, those rabbits which produced a positive Arthus reaction were given a challenge dose of 100,000 infective eggs. Five days later the rabbits were sacrificed and the larvae recovered by the modified Baermann technique. The larvae were quantitated by making serial dilutions followed by a microscopic count of all larvae in 0.02 ml. This count was multiplied by the dilution factor to give a total recovery count. Controls of nonimmunized rabbits were infected for comparison.

Immunization of rabbits with various larval antigen preparations resulted in lowered numbers of third stage larvae recovered as compared to nonimmunized controls (Table). Immunization with hatching fluid produced a 19 per cent decrease in larval recovery while immunization with the second stage E.S. resulted in a 17 per cent decrease. Enzyme studies with hatching fluid and second stage E.S. revealed similar levels of proteinase activity in the two preparations (Hinck, 1971). Measured proteinase activity in third stage E.S., however, was nearly half that of H.F. and second stage E.S. while other enzymes found in H.F. and/or second stage E.S. were not detected at all in third stage E.S. (Finch, 1977). It seems possible that enzymes produced during hatching and for some time afterward by the hatched second stage larvae might be important for the early intrahost migrations of the larvae. After reaching the lungs and moulting to the third stage it is likely that invasive enzymes would be of less value. An immune response to the enzymes produced during the early phases of the intrahost migration would conceivably have a more protective effect than a response directed to the third stage E.S. This idea is compatible with the data since only a 7 per cent decrease of larval recovery was noted in rabbits injected with third stage E.S.