Preliminary Studies on Mouse Macrophage Cultures Infected with Trachoma Agents

Cells from the hematopoietic and reticulo-histiocytic system have been cultivated in vitro by many authors, and used for the study of the host-parasite relationship in bacterial[1-3] and viral infections[4-6].

Recently, macrophage cultures have been demonstrated to be susceptible to ornithosis agent[7] and leucocytes have been successfully infected with the meningopneumonitis agent[8], suggesting that circulating cells can play a role in the infection by Chlamydiae.

In the case of animal and human infections caused by viruses or by Chlamydiae, the importance of circulating cells has scarcely been studied.

Investigations carried out using mouse macrophage cultures infected in vitro with 2 different strains of trachoma agent are described in this paper.

Mouse peritoneal macrophage cultures were obtained according to ORFILA and LEPINAY[7], from pathogen-free adult white Swiss mice (Charles River, Paris) and cultivated in Leighton tubes, in HYLS medium (Hanks + lactalbumine hydrolisate 0.5% + calf serum 10% + yeast extract 0.1% + penicillin 100 U/ml + streptomycin 100 µg/ml). Usually, 10^6 cells/ml were used. 2–3 days after the inoculum, the cells, containing some vacuoli and granules, are attached to the glass and start to elongate. At the fifth to sixth day, the majority of the cells have a fibroblastic shape, and the intracytoplasmic granules and vacuoli disappear (Figure 1). At this point, the macrophage cultures were infected with trachoma agents, strain As_3 (TRIC/EAE/Asm-3 (1960) OT)[9] or strain T'ang (TRIC/China/Peking-2/OT)[10].

Both strains were cultivated in yolk sac of chicken embryonated eggs; yolk sac pools (YSP) were prepared by diluting the yolk sac up to 50% in HYLS medium.

Cell infection was carried out with 1 ml/tube of YSP diluted in HYLS. After adsorption at 37 °C for 3 h (except when otherwise stated), inoculum was removed, cell cultures were washed once and 2 ml/tube of medium were added.

After different times of incubation at 37 °C, cultures on coverslips were fixed and stained with MGG (May Grünwald-Giemsa) or with AO (Acridine Orange).

In mouse macrophage cultures inoculated with YSP from non-infected eggs, no CPE was observed.

After infection with trachoma agent suspensions, either strain T'ang or As_3, typical trachoma inclusion bodies can be observed. The morphological changes connected with the development of the trachoma inclusion bodies, were particularly studied on macrophage cultures infected with As_3 trachoma strain at different times after the infection.

At the 4th h after the infection, no inclusion bodies could be observed. At the 8th h, the AO stain revealed in a few cells the presence of a small orange-red intracytoplasmic area that can probably be identified with the first step toward the 'red ball' stage, already described in cell cultures infected with trachoma agents[11,12]. Cytopathic effect becomes more intense the 24th h. At this time, typical intracytoplasmic trachoma inclusions are seen, consisting of a vacuolum containing small dark bodies (MGG stain), which corresponded for their size to the 'initial bodies' (Figure 2).

The AO staining revealed that almost every cell cytoplasm contained red bodies (Figure 3).

At the 48th h, the total number of cells present in the culture was strongly decreased, because of the lysis caused by the trachoma infection, and the intracytoplasmic vacuoli contained inclusion bodies corresponding for their size to both 'initial' and 'elementary bodies'.

Also those cells which were devoid of inclusion bodies appeared to be deeply altered, being rounded and dark stained. These effects increase at 72 h after the infection.

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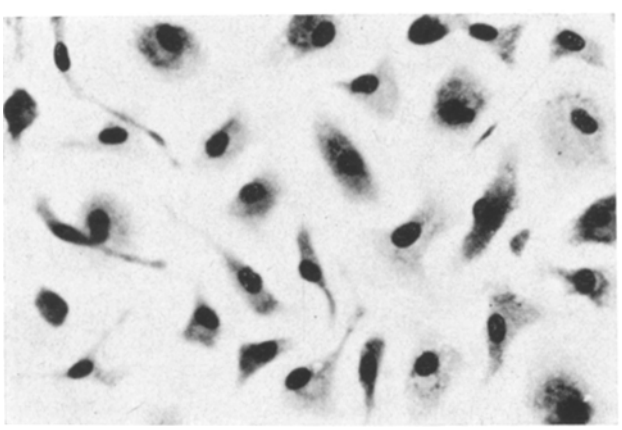

Fig. 1. Five days old non-infected macrophages culture. M.G.G. × 450.

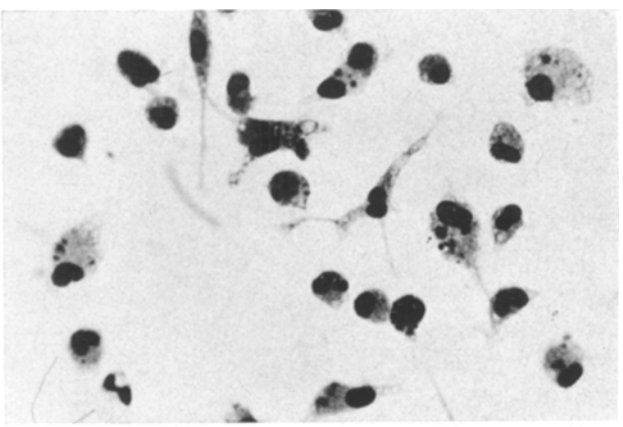

Fig. 2. Infected macrophages culture 24 h after infection. Vacuoli containing large and initial bodies. M.G.G. × 450.

The large intracytoplasmic vacuoli in some cases appeared to disrupt and liberate elementary body particles; besides, in some cells, vacuoli containing 'initial bodies' could be observed, suggesting the possibility of a second growth cycle (Figure 4).

After AO staining, both green and yellowish mature 'elementary bodies' and 'red balls' could be observed, in different cells (Figure 5).

The intensity of CPE, calculated as percentage of inclusion containing cells (ICC) depends on the inoculum dose and on the adsorption time at 37 °C.

A direct log-log linear relationship exists between the inoculum size and the percentage of ICC, either with As_3 or T'ang strain (Figure 6). The percentage of ICC increases along with the increase of the adsorption time, from 30 min up to 3 h at 37 °C. The cellular lysis caused by trachoma infection can be evaluated by counting the cell number/microscopic field in uninfected and infected cultures: 96 h after the infection, in the infected cultures the cell number is reduced of about 32%. On the contrary, the per cent of ICC increases during the time after the infection.

The data given in this paper demonstrate that trachoma agent can develop on in vitro cultured mouse macrophages. The morphological picture and the sequence of development of trachoma inclusion bodies in macrophages are similar to those observed in cultures of other kinds of cells[11–15].

The final stage of trachoma agent development is constituted by disruption of the vacuolum containing the elementary bodies, followed by cellular lysis. This event has been observed also by other authors using different cell cultures[15,16]. A second growth cycle is demonstrated by the presence of intracytoplasmic 'initial' and 'large' bodies at 72 h after the infection, and by the increase of the ICC percentage at the same and at following times.

In our hands, the susceptibility of macrophage cultures to trachoma infection seems to be 5 times lower than that of HeLa cell cultures.

The possible role of macrophages in the human trachomatous diseases has not been investigated, mainly because the most usual method of observation is the con-

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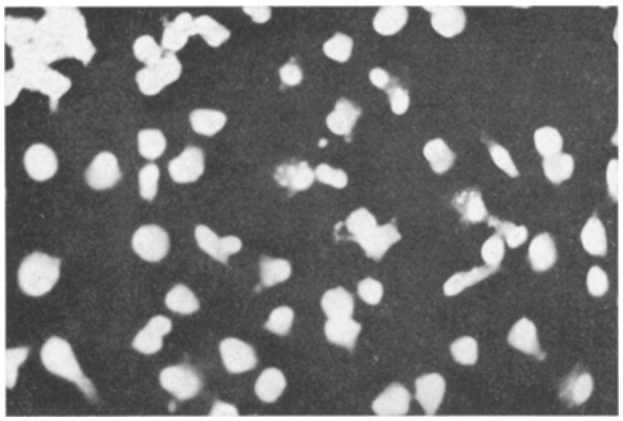

Fig. 3. Infected macrophages culture 24 h after infection. Vacuoli containing red balls. A.O. × 450.

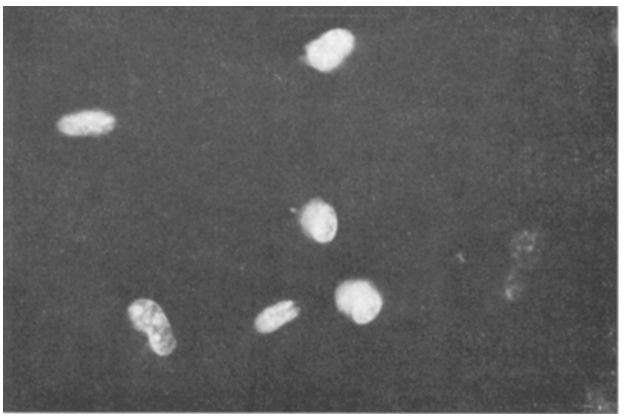

Fig. 5. Infected macrophages culture 72 h after infection. Vacuoli containing elementary or initial bodies. A.O. × 1000.

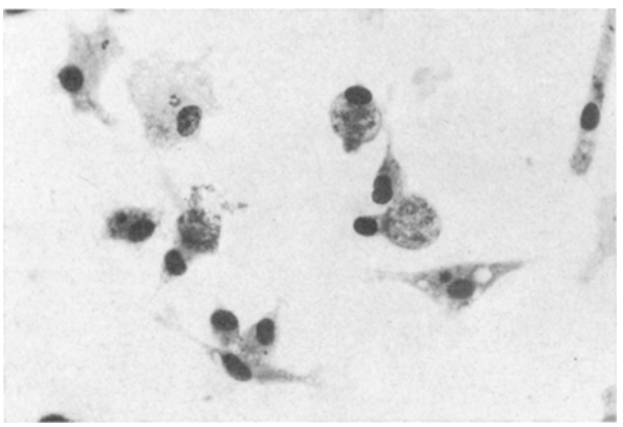

Fig. 4. Infected macrophages culture 72 h after infection. Several vacuoli filled with elementary bodies; 1 vacuolum is disrupted and spreading out elementary bodies. 3 cells contain initial bodies. M.G.G. × 450.

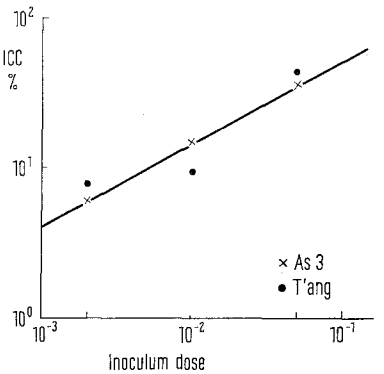

Fig. 6. Dose-effect relationship between the concentration of infecting inoculum and per cent ICC in macrophages culture. On the abscissa, inoculum dose; on the ordinate, inclusion containing cells (ICC) per cent.

junctival smears examination, in which a differentiation between epithelial and reticulo-endothelial cells becomes particularly difficult if based, as commonly performed, solely upon morphological criteria. It cannot be excluded, however, that the macrophages present in the sub-epithelial layers of the conjunctiva, as normal constituents or evoked by the phlogistic process, can play a role in the infectious process as well as in the mechanism of immunity.

Riassunto. L'infezione di culture in vitro di macrofagi di topo con due diversi ceppi di agente del tracoma, determina la comparsa di tipiche inclusioni intracitoplasmatiche, e, in seguito, di lisi cellulare. Lo sviluppo delle inclusioni è stato studiato a tempi diversi dall'infezione. Sulla base dell'osservazione morfologica, si suppone che l'agente del tracoma possa moltiplicarsi in tali culture cellulari, determinando un secondo ciclo di infezione.

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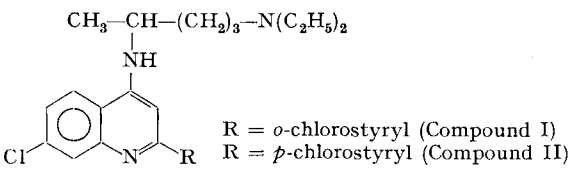
Antiviral Activity of some Derivatives of 2-Styrylquinoline

Various styrylquinolines have been shown to possess antimicrobial activity against nemathelminthes[1], protozoa[2,3], fungi[3], and bacteria[3]. In addition, inhibition of tumors has been reported from time to time[4-6]. These studies and the recent finding that chloroquine inhibited replication of a DNA bacteriophage[7] have encouraged us to investigate the antiviral activity of this broad group of compounds. Two 4-diethylaminoalkylamino derivatives of 7-chloro-2-styrylquinoline[3] (I, II) were studied in our standard virus-assay systems and found to possess significant activity against Semliki Forest Virus (SFV).



R = *o*-chlorostyryl (Compound I)
R = *p*-chlorostyryl (Compound II)

In vivo activity of 2 styrylquinolines against Semliki Forest Virus

Experiment No.	Compound	Drug dose (mg/mouse at stated times)	Treatment regimen[a]	Challenge dose of Virus (LD_{50}/mouse)[b]	Antiviral activity (% S/T)[c]
I	1	2.5	A	2.0	50
		0.25			0
		0.025			0
	–	–	–	–	–
	2	2.5	A	2.0	50
		0.25			0
		0.025			0
	control	–	–	2.0	0
II	1	1.0	B	2.5	100
	2	1.0	B		70
	control	–	–	2.5	10
III	1	1.0	C	3.5	30
	control	–	–	3.5	0
IV	1	1.0	D	16	54
	control	–	–	16	0

[a] Animals were treated by s.c. injection: (A) 2 h before, and then 24 and 48 h after infection; (B) 24 and 4 h before, and at 4, 24 and 72 h after infection; (C) at 30 min; 24, 48 and 72 h after infection; (D) same protocol as B except that last dose was given 48 h after infection. [b] Route of infection was i.p.; LD_{50} = lethal dose, 50% endpoint. [c] Per cent surviving animals 2 weeks after challenge.

The drugs were solubilized in distilled water and injected s.c. using multiple dosage schedules as summarized in the Table. At non-toxic concentrations, both compounds protected significant numbers of mice from the lethal effects of SFV infection especially when treatment regimens included prechallenge drug doses. When compound I was incubated in vitro with approximately 3.5 LD_{50} (mouse) of SFV prior to injection into mice by the i.p. route, more than 85% of the test animals survived. 2 weeks after challenge, survivors from all experiments appeared to be healthy and free from the signs of paralysis so characteristic of SFV infection in this species. The small skin lesion, which occurred at the site of injection, was grossly resolved at this time.

Since SFV is a neurotropic virus, but at the same time is an uncommon natural human or animal pathogen, it would be of interest to see whether these or other styrylquinolines would act against human neurotropic viruses. Preliminary data from this laboratory suggest that compound I and II may have marginal activity against influenza virus (type A, PR_8) in mice, but not in eggs. Neither compound appears to possess significant activity against vaccinia virus in eggs or in rabbits.

The mode of antiviral action of these 2 styrylquinolines remains to be elucidated.

Zusammenfassung. In Mäuseversuchen wurde gezeigt, dass gewisse Styrylquinoline in vivo eine Wirkung gegen Semliki-Forest-Virus haben.

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