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Selective Phagocytosis of Nucleated Erythrocytes by Cytotoxic Amebae in Cell Culture

Abstract. Strains of *Acanthamoeba,* which produce cellular damage resembling viral cytopathic effect, are known to occur in cultures of monkey kidney cells. Trophozoites of a similar strain were observed to engulf and denucleate chicken erythrocytes. Nonnucleated guinea pig erythrocytes were apparently left unchanged.

Recently two virus research groups (*1*) have reported on the occurrence of amebae of the genus *Acanthamoeba* as natural "contaminants" in monkey kidney cultures. These amebae were cytotoxic, producing cellular damage not unlike viral cytopathic effect. A similar strain of amebae was isolated in our virus laboratory. An accidental observation of the remarkable behavior of these organisms in relation to nucleated and nonnucleated erythrocytes should be recorded as information of possible importance to other investigators.

We isolated the amebae in 1957 from one of the tubes in a large group of rhesus monkey kidney cell cultures which were under observation for visible viral effects. Six days after the replicate monolayer tube cultures were prepared by the usual trypsinization method, this particular tube was inoculated with a routine throat swab specimen from a healthy child who was in contact with a febrile patient under surveillance. Nine days later (the 15th day after trypsinization) early cellular damage was noted near the borders of the sheet. This resembled the cytopathic effect produced by the enteroviruses, although progression of the cellular degeneration was noted to be unusually slow. Serial passages of the culture fluid were made; by the tenth passage the characteristic effect made its appearance on the second day with complete degeneration of the cellular sheet within a week. Although with passage the incubation period was thus markedly shortened, the titer of the culture fluid, using the cytopathic effect endpoint, was maintained at 10^8. Repeated attempts at reisolation from the original human specimen were unsuccessful.

It became apparent that this was not a known enterovirus, and we initiated additional studies. The monkey kidney passaged agent was found to grow well (as evidenced by the appearance of the specific effect) in monolayer cultures of human amnion, chorion, and HeLa cells; the shortest (overnight) incubation period was observed in dog kidney cell cultures. To elucidate further the nature of the presumably viral agent, we attempted the hemadsorption procedure, which at that time was being developed in our laboratory (*2*), on some of the tubes with visible cellular degeneration. Chicken and guinea pig erythrocytes were added to the monkey kidney culture tubes which were examined under low magnification (×150) for the characteristic hemadsorption patterns. We did not find hemadsorption with either type of erythrocyte, but clumping, suggestive of hemagglutination, occurred in the tubes to which chicken erythrocytes were added. The clumping apparently was caused by small round bodies which at first were assumed to be detached renal cells.

The possibility that the agent was a strain of cytotoxic amebae was considered, and we examined the tubes under higher magnification (×795) (*3*). The peripheral portions of the kidney cell sheet were found to contain intracellular and extracellular thick-walled cysts approximately 20 μ in diameter; some of the renal cells contained several cysts crowding the cell nucleus to the side; these bulging cells occasionally ruptured, releasing the cysts into the nutrient fluid which already contained numerous motile trophozoites.

Both types of erythrocytes were then added to hanging drop preparations of culture fluid to study the clumping of erythrocytes. Addition of nonnucleated guinea pig erythrocytes caused little change: the trophozoites showed no visible reaction or merely "palpated" nearby red blood cells with their pseudopodia; an occasional trophozoite engulfed a guinea pig cell but promptly rejected it seemingly unchanged. Addition of nucleated chicken red cells produced the characteristic clumping: the freely moving trophozoites attached to the erythrocytes and engulfed them. After a variable period of time the amebae ejected misshapen, apparently completely *denucleated,* erythrocytes.

Limited attempts at characterization of the pseudo-viral agent disclosed that it was removed completely from the cell culture fluid by filtration (Selas 03), by centrifugation at 2000 rev/min for 30 minutes (International No. 2) and by heating in a water bath at 56°C for 3 hours (although not after 1 or 2 hours). The amebae were preserved in the culture fluid at room temperature for at least 13 weeks; at 37°C for 2 weeks; at 4°C for 7 weeks and at −50°C for 8 weeks. The incubation period, as measured by the appearance of specific cytotoxic changes, was considerably prolonged with storage at lower temperatures.

The amebae were identified by Leon Jacobs as belonging to the genus *Acanthamoeba* (*4*). Our findings provide further evidence that the previous reports (*1*) of the isolation of the *Acanthamoebae* from monkey kidney cultures were more than spurious observations of air contamination of the culture material or of a unique presence of the organisms in certain monkey organs; on the contrary, this might be a common occurrence generally overlooked.

Awareness of these repeated experiences may prompt the virologist who finds unusual cytotoxic effects to consider cytotoxic amebae rather than a bizarre viral agent. The remarkably selective behavior of the trophozoites in denucleating chicken erythrocytes may provide another clue and a tool for investigation of metabolic requirements of artificially propagated amebae.

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