

Poly-L-arginine 'opsonizes' nuclei for phagocytosis by mouse fibroblasts

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Cationic substances may play important roles in many biological systems. Arginine-rich polycations from neutrophils and eosinophils, and histones possess distinct bactericidal properties against a variety of microorganisms (1-4). Synthetic polyamino acids have been shown to agglutinate bacteria and mammalian cells (5-7), to hemolyze erythrocytes (6), to modulate blood coagulation and fibrinolysis (6, 8), to block Fc receptors for IgG on certain group A streptococci (9), and to enhance the adherence of mammalian cells to surfaces (10). A variety of polycations 'opsonize' various particles for phagocytosis by neutrophils and macrophages in culture (7, 11-15). Cationic polyelectrolytes have also been found to activate autolytic wall enzymes in *Staphylococcus aureus* (16) and to function as potent ligands for the activation of the respiratory burst in neutrophils and macrophages (17-19). One of us has additionally demonstrated that *Candida albicans* which had been 'opsonized' either with nuclear histone or with poly-L-arginine (PARG) was avidly phagocytosed *in vitro* by mouse fibroblasts as well as by human epithelial cells and HeLa cells (14).

The phenomenon of 'opsonization' by polycationic agents mimics the effects of immunoglobulins and may represent an archaic type of particle uptake by animal species which do not form real antibodies. The employment of polycations to enhance the uptake of microorganisms can be further expanded to include their organized structures. Here we describe the uptake of PARG-coated nuclei derived from human U 266 myeloma cells by mouse L 929 fibroblasts in culture. The possible implications of this phenomenon will be briefly discussed.

Materials and methods: Mouse L 929 fibroblasts were used as recipient cells of nuclei prepared from human U 266 myeloma cells. The culture medium was DMEM supplemented with 10% FCS, 2 mM L-glutamine, 4 mM Hepes, 50 U/ml penicillin, and 50 µg/ml streptomycin. Cell cultivation and incubation for coating of PARG was done at 37°C and 7.5% CO₂ in fully humidified air.

Enucleation was achieved by incubation of myeloma cells with 10 µg/ml cytochalasin B (dissolved in DMSO) for 1 h and subsequent Ficoll gradient centrifugation; except for the additional use of a 50% Ficoll cushion the procedure of Wigler and Weinstein (20) was followed. Upon centrifugation, the pellet consisted of > 90% cell nuclei as was determined by acridine orange staining and fluorescence microscopy. Nuclei were washed twice and were then incubated for 30 min either with 5 × 10⁻⁶ M PARG (MW 40 000; Sigma) dissolved in phosphate buffered saline containing Ca and Mg or with buffer only. Thereafter, the nuclei were washed again and 10⁺⁶ nuclei were pipetted onto half-confluent L 929 cell sheets grown in 35 mm Petri dishes. Such cultures were followed microscopically for up to 5 days. Some cultures were fixed and stained with Giemsa whilst others were prepared for transmission electron microscopy using standard techniques.

Results and discussion: Soon after the addition of PARG-coated nuclei to the fibroblast layer these nuclei adhered strongly to the surface of the target cells and could not be washed away. Internalization of the coated nuclei was already evident within the first 2 h of incubation. An uptake of 50-95% of the nuclei was observed after overnight incubation, whilst only a very few unopsonized nuclei were taken up by the fibroblasts. Nuclei which were not internalized within this period could still be seen attached to the fibroblast surface most of which were showing a strong tendency to autolyze; these extracellular nuclei disappeared upon further incubation. The Table demonstrates the frequencies of donor nuclei inside fibroblasts containing 1-3 nuclei (polykaryons). These frequencies show that multinucleate fibroblasts do not take up unusually high numbers of myeloma nuclei. This is unlike the case of *Candida albicans* (14), which was avidly taken up by polykaryons to a much larger extent than those fibroblasts that possessed a single nucleus.

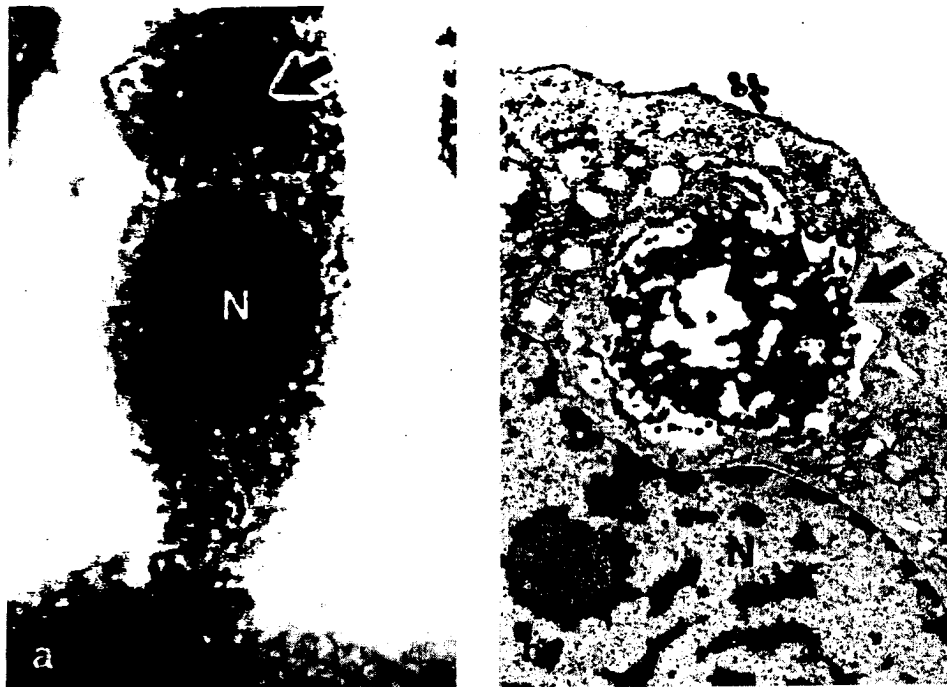
Uptake of U 266 nuclei into the cytoplasm of L 929 cells: Number of internalized nuclei (n = 59) versus number of fibroblast nuclei per cell

No. of myeloma nuclei	No. of fibroblast nuclei per cell		
	1	2	3
1	38	4	3
2	6	6	1
3	1	0	1

Determinations were made from Giemsa stained overnight incubations.

Figure (a) shows a Giemsa stained fibroblast containing an intracellular structure compatible with a donor nucleus; Figure (b) demonstrates by electron microscopy that the nucleus showing signs of degradation is surrounded by a cellular membrane. No evidence of fusion of this membrane with granules was found. Within 5 days of culture the donor nuclei turned progressively paler in the recipient cytoplasm, thus obviously being intracellularly degraded.

The data presented suggest that polycationic agents may function as 'opsonins' not only for microorganisms (15) but also for much larger structures such as whole mammalian nuclei. Although the mechanism of opsonization is not fully clear, it may be postulated that PARG, which binds to the nuclear surface, interacts via electrostatic forces with negative charges on the surface of the fibroblasts and triggers the transmembranal signal involving the cytoskeleton of the cell. A phagosome-like structure is formed similarly to such structures found in 'professional' phagocytic cells.



Internalized U 266 cell nucleus (arrows) in the cytoplasm of L 929 fibroblasts (N = fibroblast nucleus): (a) Giemsa stained cell (magnification $\times 4600$); (b) portion of an ultrathin section showing a membrane-surrounded donor nucleus in the process of degradation (magnification $\times 9600$)

It was hoped that the internalized nucleus might also fuse with the membrane of the nucleus of the recipient fibroblast and that such possible fusion might be employed to study uptake of genetic material. In view of the rapid degradation of the nuclei within the phagocytic vacuoles one should perhaps seek methods to prevent degradation and to facilitate the desired fusion. This might yield a useful tool for introducing genetic material into mammalian cells.

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