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REGULAR ARTICLE

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Fibrillar islet amyloid polypeptide (amylin) is internalised by macrophages but resists proteolytic degradation

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Abstract Pancreatic islet amyloid, formed from islet amyloid polypeptide, is found in 96% of Type II (non-insulin-dependent) diabetic patients. Islet amyloidosis is progressive and apparently irreversible. Fibrils immunoreactive for islet amyloid polypeptide are found in macrophages associated with amyloid, suggesting that deposits can be phagocytosed. To determine the mechanism for the recognition and internalisation of fibrils, mouse peritoneal macrophages were cultured with fibrillar synthetic human islet amyloid polypeptide. Fibrils did not exert a cytotoxic effect over 72 h of culture. The uptake and degradation of fibrils was analysed by quantitative light-and electron-microscopic immunocytochemistry and immunoreactivity was detectable in 86±3% cells within 6 h of culture. Neither polyinosinic acid (200 μg/ml) nor nocodazole (10 μg/ml) inhibited fibril uptake, suggesting that internalisation is not blocked by poly-ions and is independent of microtubule assembly. Inhibition of pseudopodia formation by cytochalasin B blocked fibriI uptake. Fibril aggregates became condensed in lysosomes to form protofilaments and were resistant to intracellular proteolysis. Fibrils can be phagocytosed by macrophages in vitro but amyloid-associated factors may block the recognition of fibrils in vivo preventing the removal of islet amyloid in diabetes.

Key words Islet amyloid polypeptide · Macrophages · Amyloid · Diabetes · Lysosomes · Macrosialin · Phagocytosis · Mouse (C34/HEH 101/H)

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Introduction

Amyloid deposits are present in pancreatic islets of Langerhans in over 96% of patients with Type II (non-insulin dependent) diabetes mellitus examined at post-mortem (Westermark and Grimelius 1973; Maloy et al. 1981). The extent of amyloidosis is variable; it can occupy up to 80% of the islet mass and is associated with the loss of insulin-secreting islet β-cells (Clark et al. 1988). Islet amyloidosis in Type II diabetes is unrelated to forms of systemic amyloidoses. Deposition of islet amyloid is progressive (de Koning et al. 1993) and is likely to play a major role in the deterioration of insulin secretion, which is a characteristic feature of Type II diabetes. The constituent peptide of islet amyloid is islet amyloid polypeptide (IAPP; also known as 'amylin'), which is a 37-amino-acid peptide synthesised and released by insulin-producing pancreatic β-cells (Clark et al. 1987). In Type II diabetes and in human insulinomas, extracellular IAPP is converted to a β-molecular conformation and oligomerises to form characteristic unbranching insoluble amyloid fibrils.

The role of resident macrophages in the pathobiology of islet and other forms of amyloidosis is uncertain. Amyloid deposition in all sites of the body is largely irreversible, although some amyloid accumulations can regress (Holmgren et al. 1993). In general, amyloid deposits are poorly immunogenic (Franklin and Pras 1969) and there is little evidence of an active immune response to amyloid deposition that could lead to regression of the deposits in vivo. The incidence of auto-antibodies to IAPP in diabetic man is extremely low (Clark et al. 1991; Gorus et al. 1992) and lymphocytotic infiltration of the islets is not associated with islet amyloid deposition. However, small numbers of macrophages can be found in pancreatic islets of normal mice and non-diabetic man and in the amyloid-containing islets of subjects with Type II diabetes (Westermark and Grimelius 1973; de Koning et al. 1994a). Resident macrophages containing IAPP-immunoreactive (IAPP-ir) fibrils are associated with amyloid in human insulinomas and monkey pancreatic islets (de

Koning et al. 1994b) suggesting phagocytosis of amyloid deposits. The mechanism by which IAPP is recognised by phagocytotic cells and internalised is unknown.

To examine the uptake of human IAPP (hIAPP) by macrophages and the fate of internalised material, murine peritoneal macrophages were elicited with polyacrylamide beads (Fauve et al. 1983) and cultured in the presence of fibrillar synthetic hIAPP for periods of up to 72 h. The uptake of fibrillar IAPP was determined by immunocytochemistry for light and electron microscopy. To establish the molecular mechanism of uptake of synthetic hIAPP, macrophages were also exposed to various pharmacological agents in vitro. The fate of internalised IAPP was determined by quantitative microscopy.

Materials and methods

Preparation of mouse peritoneal macrophages

Adult male C34/HEH 101/H mice (n=27) each received an intraperitoneal injection of 1 ml sterile phosphate-buffered saline (PBS) containing 1% w/v Bio-Gel P100 polyacrylamide beads (BioRad, Hemel Hempstead, UK) and returned to care in the University animal facility, which is managed in accordance with National and N.I.H. guidelines. After 5 days, the mice were killed by cervical dislocation and the peritoneal cavity irrigated with PBS at 37°C. Polyacrylamide beads, with adherent macrophages, were isolated from the peritoneal washings by centrifugation, washed in PBS and RPMI 1640 medium (Gibco, Paisley, UK) containing 2 mM L-glutamine and 10% fetal calf serum (Gibco, UK) and resuspended in RPMI medium prior to culture. A yield of 10×10^6 cells/animal was obtained. The cell population contained 92% of macrophages identified by fluorescent labelling of an antibody to F4/80 (Serotec, Kidlington, UK), a specific marker for the macrophage lineage. Cells were plated at 250 cells/mm^2 onto sterile 4-well microscope slides (Hendley, Loughton, UK), 24-well Nunclon dishes (Gibco, UK) or tissue-culture-treated polycarbonate filters (Costar, High Wycombe, UK). After incubation for 2 h at 37°C in 5% v/v CO_2, macrophages were released from the beads and were allowed to adhere to the culture matrix; non-adherent cells and polyacrylamide beads were washed away with warmed culture medium.

Fibril formation from synthetic hIAPP

Fibrillar $hIAPP_{1–37amide}$ (Peninsula Laboratories, San Diego, USA) was produced by adding 500 ml sterile dH_2O to 500 mg synthetic hIAPP and allowing the solution to stand at room temperature for 1 h. Aliquots of the fibrillar material were stored at –20°C until required. The fibrillar nature of stock solutions and tissue culture medium to which the hIAPP had been added was confirmed by preparing negatively stained samples for electron microscopy (Fraser et al. 1994).

Culture of elicited peritoneal macrophages with hIAPP

Cultured macrophages were exposed to a suspension of fibrillar hIAPP at a concentration of either 1.2 μM or 12 μM in RPMI for periods of 6–72 h prior to the preparation of cells for light or electron microscopy. To examine the time course of degradation of intracellular hIAPP, macrophages were exposed to 12 μM hIAPP for 6 h, followed by further culture periods of 24, 48 and 72 h in hIAPP-free media. To determine the mechanisms involved in IAPP uptake, macrophages were exposed to polyinosinic acid (poly-[I]; 10–200 μg/ml; Sigma, Poole, UK) during the uptake of hIAPP over a period of 6 h, or to nocodazole (NOC; 1–10 μg/ml; Sigma) or cytochalasin B (CB; 0.5–5 μg/ml; Sigma) for 20 min prior to and during exposure to 12 μM hIAPP. The effect of these agents on IAPP uptake was determined by quantitive cytometry of immunolabelled cells in relation to untreated control cells.

Immunocytochemistry for light microscopy

Microscope slides with adherent macrophages were washed with PBS and the cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. IAPP, macrophages and cellular proteins were identified with single or double label immunofluoresence techniques. Background was reduced with non-immune serum diluted 1:20 in PBS. IAPP was labelled with rabbit anti-rat IAPP (Peninsula, USA; diluted 1:1000), which cross-reacts with human IAPP as shown by histochemical techniques, followed by fluorescein-conjugated (FITC) swine anti-rabbit antiserum (DAKO, High Wycombe, UK; diluted 1:20). Double-label immunocytochemistry required rhodamine-conjugated (TRITC) goat anti-rabbit antiserum (DAKO; diluted 1:30) as the secondary antibody. Macrophages were identified with rat anti-macrophage F4/80 (diluted 1:50) and FITC-conjugated goat anti-rat antisera (ICN, Thame, UK; diluted 1:30). Phagolysosomes within macrophages were localised with rat anti-macrosialin monoclonal antibody FA/11 (a gift from Prof. S. Gordon, Oxford, UK; diluted 1:1) and FITC-conjugated goat anti-rat antiserum (ICN, UK; diluted 1:30). The specificity of the rabbit anti-IAPP antibody was confirmed by the loss of IAPP-ir following pre-absorption with 1 μg/ml of the antigen; the specificities of monoclonal antibodies F4/80 and FA11 have been described previously (Hume et al. 1984; Rabinowitz and Gordon 1991).

Quantitative cytometry

The numerical density of F4/80-ir macrophages either after exposure to fibrillar hIAPP or under control conditions was measured at various time points in the culture period, up to 72 h. Cells were viewed by Nomarski optics (magnification ×200) and quantitation was performed on 5 microscopic fields each of 0.5 mm^2, selected in a systematically random manner (Weibel 1969). Quantitative observations on cells exposed to hIAPP with or without other treatments were compared with those of cells in control wells on the same 4-well microscope slide to minimise the effects of any changes in plating density of cells or small variations in culture conditions.

Electron microscopy

Polycarbonate filters with adherent macrophages were washed with PBS and fixed in 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide and block-stained with 2% uranyl acetate. Specimens were dehydrated in increasing concentrations of ethanol followed by immersion in 100% propylene oxide, which curled the filters for easier ulltrathin sectioning. Specimens were embedded in Spurr's resin. Ultrathin sections were cut perpendicular to the long axis of the curled filter and dried onto nickel grids. Sections were immunogold-labelled for IAPP with rabbit anti-rat IAPP antibody (Peninsula, USA) and 15-nm protein A-gold (Biocell, Cardiff, UK) diluted 1:1000 and 1:20, respectively, in PBS containing 10 mg/ml ovalbumin (Grade V; Sigma). Contrast was enhanced with 2% uranyl acetate in 70% methanol and lead citrate. Image analysis was performed on electron micrographs of macrophages in trans-nuclear profile by using an IBAS image analyser (Kontron Electronik, Messergeräte, Germany). Measurements included sectional areas of cytoplasm, phagosomes and lysosomes of macrophages cultured under various conditions.

Statistical analysis

Results of quantitative cytometry were expressed as the mean±SEM of data obtained from 4 experiments. Quantitative electron micros-

copy was performed on at least 8 cells per experiment and results were expressed as the mean±SEM for at least 3 experiments. A normal distribution was assumed and statistical comparison was performed with Student's *t*-test. Significant differences were assumed at values of $P<0.05$.

Results

Uptake of fibrillar hIAPP by cultured macrophages

Fibrillar hIAPP with typical amyloid fibril characteristics was detectable both in stock solutions and when diluted in culture media (Fig. 1). Cells were identified as macrophages by immunofluorescent labelling for the cell-surface macrophage antigen F4/80. No IAPP-ir could be detected in macrophages cultured in the absence of hIAPP. Cells were adherent to the polycarbonate filters and untreated microscope glass slides and had the typical appearance of macrophages (Fig. 2A). Following exposure to 12 μM fibrillar hIAPP for 6 h, the number of cells with elongated processes (Fig. 2B, C) increased; intracellular IAPP, identified by immunocytochemistry, was present in 92±3% of the macrophages.

Pseudopodia engulfing extracellular IAPP-ir material were visible by electron microscopy after 1 h in culture with hIAPP; after 6 h exposure to fibrillar hIAPP, 80% of trans-nuclear macrophage profiles contained IAPP-ir aggregates. After culture for 24 h with 1.2 μM or 12 μM hIAPP, IAPP immunoreactivity was detected in, respectively, 82±3% and 86±3% of the cells. A similar proportion of cells showed IAPP immunoreactivity after 72 h in culture. The amount of internalised IAPP-ir material (measured as areal density in electron micrographs; see Fig. 2A) did not increase with an increase of hIAPP in the culture medium; after macrophages had been cultured with 1.2 or 12 μM hIAPP for 24 h, IAPP immunoreactivity occupied, respectively 7±3% and 8±4%, of the cytoplasmic area.

The numbers of adherent macrophages decreased with the length of culture to 62±10% of the initial density after 48 h and to 22±7% (n=4) after 72 h (Table 1). However, the numbers of macrophages at each time point were unaffected by culturing with 12 μM IAPP. Ultrastructural analysis of macrophages showed that there were no abnormalities in the nucleus, cytoplasm or plasma membrane of the cells after 24 h or 72 h exposure to fibrillar hIAPP; the presence of abnormalities might have suggested fibril cytotoxicity.

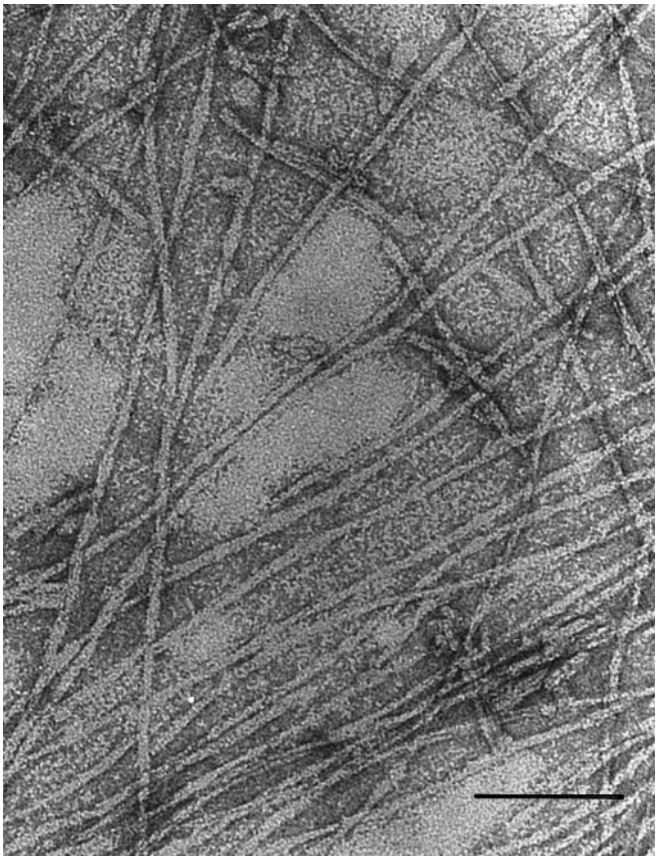


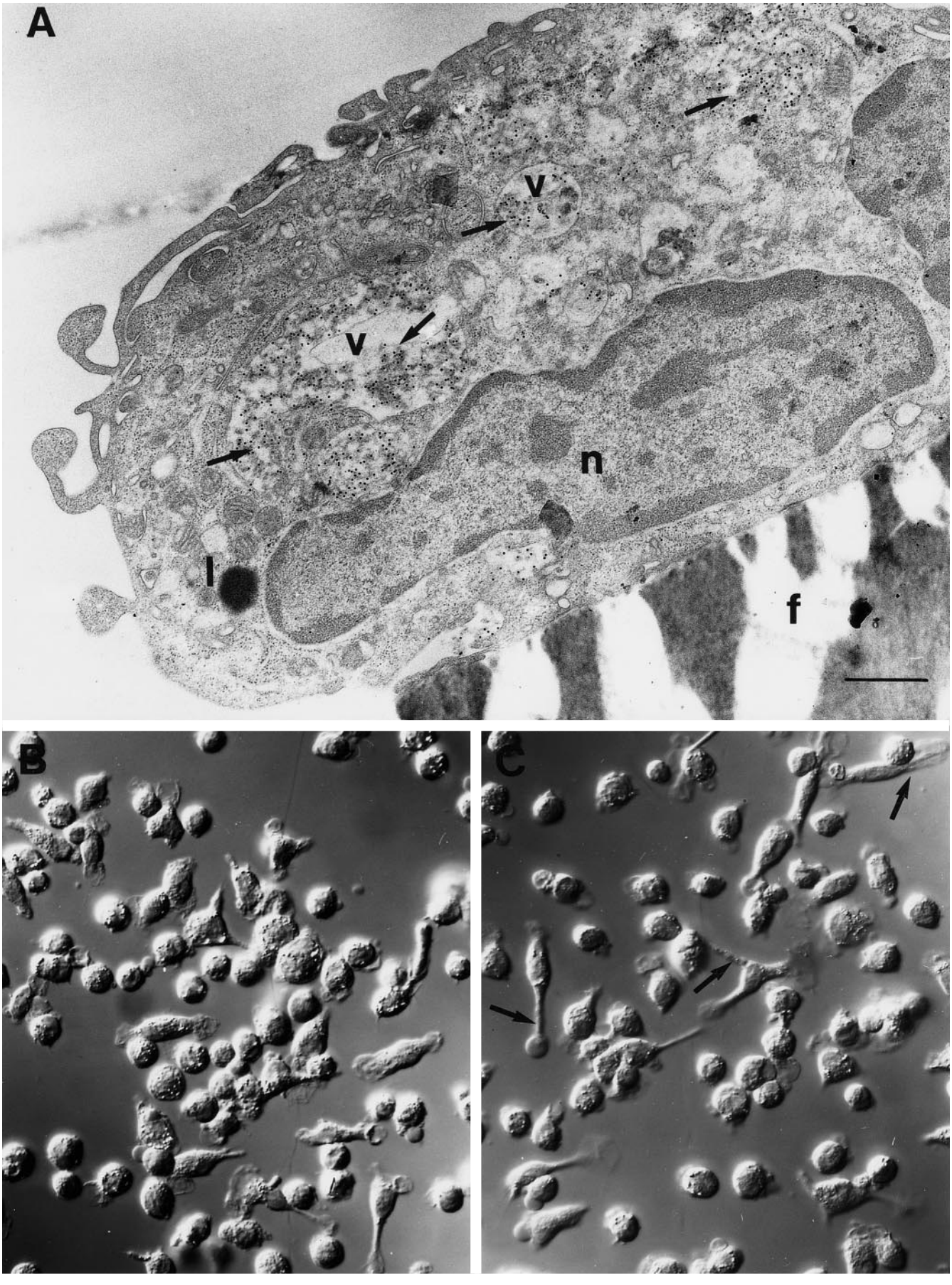
Fig. 1 Fibrils formed from synthetic human IAPP. Electron micrograph of fibrils negatively stained with uranyl acetate. Fibrils were visible in stock solutions and in culture media at concentrations of 1.2 or 12 μM. *Bar* 0.1 μm

Effects of inhibitors on uptake of fibrillar hIAPP by macrophages

To establish the mechanism by which hIAPP entered macrophages, cells were treated with (1) poly-(I), an agent that interferes with the cellular recognition of charged ligands, (2) NOC, which disrupts microtubule assembly required for endocytosis, and (3) CB, which disrupts cytoskeletal activity associated with pseudopodia formation. Compared with control cultures (Fig. 3A), exposure of macrophages to 10–200 mg/ml poly-(I) failed to prevent the uptake of hIAPP during 6 h of culture (Fig. 3B). Exposure of macrophages to NOC at concentrations up to 10 mg/ml also failed to block the uptake of

Table 1 Characteristics of macrophages (MΦ) in culture for various periods following a short exposure (6 h) to hIAPP. Although the number of cells diminished with time in culture, the proportion of macrophages and IAPP-ir cells was unchanged

Time after IAPP exposure (h)	Number of cells (cells/μm^2)		Proportion MΦ (%)	Cells containing IAPP-ir (%)
	Control	IAPP		
6	273±20	288±52	92±1	92±3
24	263±41	282±30	94±1	95±1
48	176±30	228±34	94±2	97±1
72	56±18	70±28	89±5	94±3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hIAPP over 6 h (Fig. 3C). However, treatment with 2 mg/ml CB for 1 h or 6 h completely inhibited IAPP uptake (Fig. 3D). CB-treated cells appeared more rounded, lost the ruffled appearance characteristic of macrophages and had fewer aggregates of fibrillar hIAPP adhering to their extracellular surfaces compared with cells under control conditions.

Characterisation of the intracellular compartment containing IAPP

Aggregates of IAPP were localised by immunofluorescence at intracellular sites within 1 h of culture with IAPP; at this time point, the distribution of IAPP immunoreactivity was largely extracellular and distinct from that of the phagolysosomal antigen macrosialin (Fig. 3E). However, after 6 h of exposure to hIAPP, immunoreactivity to IAPP was co-localised with that to macrosialin in intracellular compartments (Fig. 3F).

Ultrastructural analysis showed that IAPP immunoreactivity was localised within two types of membrane-bound organelles (Fig. 4). After 6 h of culture with hIAPP, membrane-bound electron-lucent phagosome-like organelles contained IAPP-ir material that was loosely aggregated and resembled a loose network of fibrils (Fig. 4A). Electron-dense IAPP-ir immunogold-labelled organelles resembling lysosomes were noted in 13% of macrophage profiles after 24 h and in 60% of the macrophages after 72 h of exposure to hIAPP; in some of these electron-dense bodies, short paired fibril-like structures 50 nm in length and 5 nm in diameter could be seen (Fig. 4B).

Intracellular degradation of IAPP by macrophages

To establish the time course of intracellular degradation of hIAPP, macrophages were loaded with hIAPP for 6 h and then examined at various times (24, 48 and 72 h) of subsequent culture in media without hIAPP. The proportion of cells containing IAPP immunoreactivity was unchanged after removal of IAPP followed by culture for up to 72 h, although the number of cells containing IAPP immunoreactivity decreased (Table 1). This reduction was attributable to a time-dependent decline in the total number of adherent cells. Electron microscopy revealed that, even after culture for 72 h in the absence of hIAPP, macrophages still contained substantial amounts of IAPP-ir material in intracellular compartments. Immunogold labelling for IAPP was present in 83±6%, 73±12% and 73±5% of macrophages after culture for 24, 48 and 72 h, respectively, in media without fibrillar hIAPP.

Fig. 2A–C Morphology of macrophages in tissue culture. **A** Macrophage cultures on a polycarbonate filter (*f*) in the presence of IAPP fibrils. Intracellular vacuoles (*v*) resembling lysosomes contained IAPP immunogold-labelled flocculent fibrils (*arrows*) after 24 h of culture with IAPP. *n* Nucleus, *l* lysosome, *Bar* 1.0 μm. **B, C** Light micrographs of macrophages cultured in the absence and presence of hIAPP fibrils, respectively. There was an increase in the number of cells with elongated processes (*arrows*) when macrophages were cultured with IAPP. ×520

Discussion

Macrophages containing fibrillar IAPP-ir material in lysosome-like bodies have been identified in amyloid-containing pancreatic islets (de Koning et al. 1994a). This observation has led to the hypothesis that IAPP fibrils in lysosomes are inadequately degraded and could, if extruded (Buktenica et al. 1987), contribute to amyloid deposition; extruded material could act as a nidus for polymerisation of additional monomers (Come et al. 1993; Jarrett and Lansbury 1993). Macrophages have been proposed to play an active role in amyloidosis by converting potentially amyloidogenic peptides (such as Alzheimer's Aβ-peptide) into fibril-forming components by intracellular proteolysis (Wisniewski and Terry 1973; Takahashi et al. 1989). Monocytic cells express Aβ (Banati et al. 1995) but there is no evidence for the production of IAPP by macrophages, a production that is limited to cells in the endocrine pancreas, the gut and some ganglia (Mulder et al. 1993, 1994). Alternatively, as scavengers of extracellular material, these phagocytotic cells may facilitate the removal of pre-formed extracellular accumulations of amyloid or fibrils. If macrophages are involved in the removal of islet amyloid, it is unclear how IAPP is recognised and internalised and whether intracellular proteolysis of fibrils is abnormal.

The uptake of hIAPP by macrophages could be effected by either receptor-mediated recognition of fibrillar IAPP or an unregulated mechanism. Opsonisation of the fibrils would be required for the recognition of fibrils by surface receptors binding the Fc region of IgG (Berken and Benacerraf 1966) and the complement components C3 and C1 (Lay and Nussenzweig 1968). Very low IgG concentrations (less than 10 mg/ml) are present in culture media and specific IAPP antibodies are unlikely. Direct interaction with receptors for mannosyl compounds (Ezekowitz and Stahl 1988) can also be excluded, because IAPP lacks a consensus amino-acid sequence for O-linked glycosylation (Betsholtz et al. 1989; Nishi et al. 1992) and synthetic human IAPP is not glycosylated. Binding to non-specific scavenger receptors (Krieger et al. 1993) causes internalisation either by classical endocytosis or by a specific phagocytotic zipper mechanism (Swanson and Baer 1995). Scavenger receptors have been characterised in murine macrophages (Freeman et al. 1990) as trimeric membrane receptors (Kodama et al. 1990) that have a high affinity for a number of classes of polyanionic ligands. These include polyribonucleosides, such as poly(I), which can act as a competitive inhibitor of this recognition system (Rohrer et al. 1990). However, since macrophages internalise hIAPP even in the presence of high concentrations of poly(I), uptake as a result of an ionic recognition signal seems unlikely. Scavenger receptors

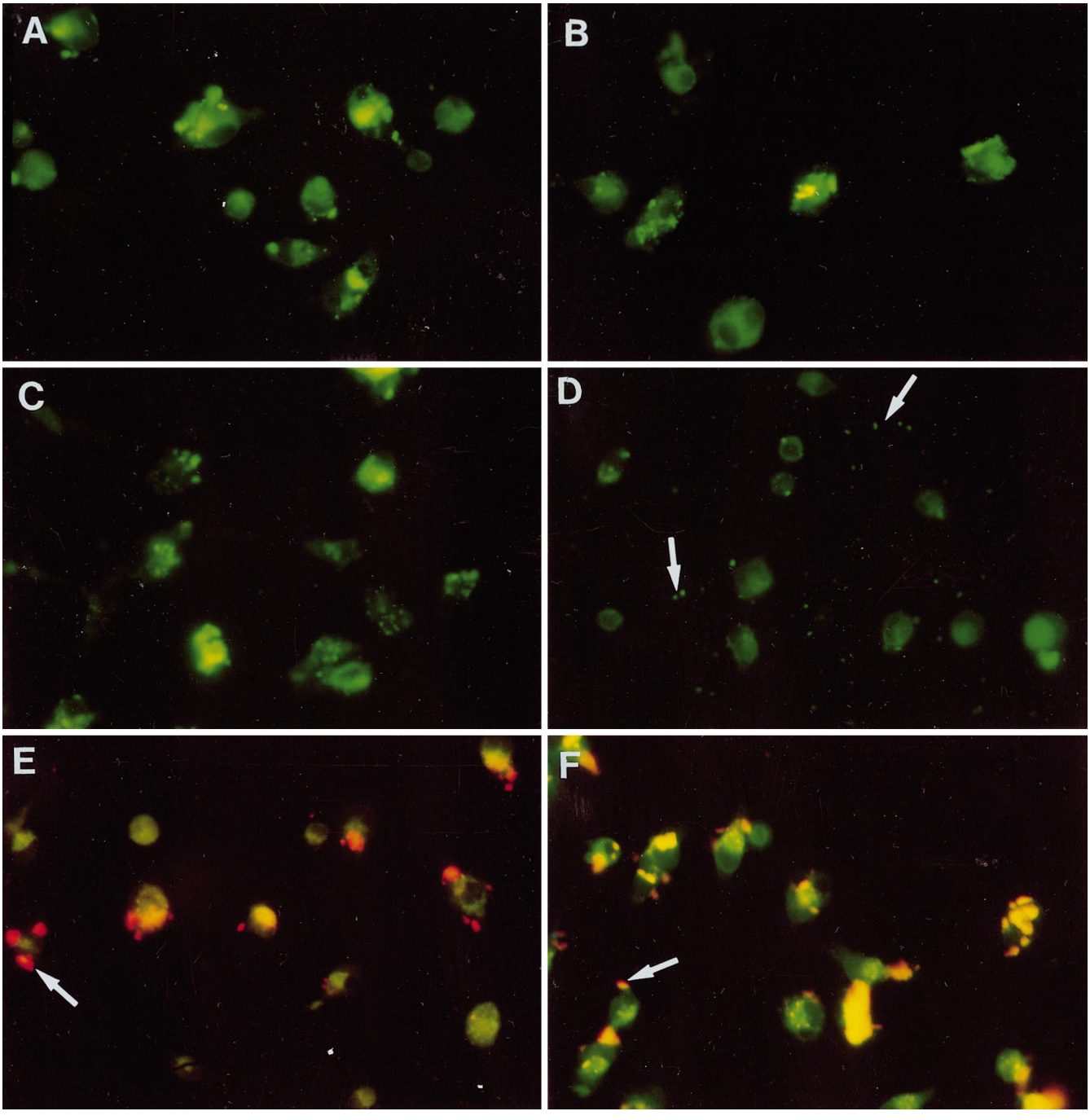

Fig. 3A–F Effects of inhibitors on the uptake of IAPP by macrophages and identification of the intracellular compartment containing IAPP. Cells were exposed to hIAPP (**A**) for 6 h without treatment, (**B**) with 200 μg/ml poly-(I), (**C**) with 5 mg/ml nocodazole or (**D**) with 5 mg/ml cytochalasin B. Exposure to neither poly-(I) nor nocodazole inhibited the uptake of IAPP and intracellular IAPP immunoreactivity (*green/yellow*) was not diminished compared with the control. Cytochalasin B reduced membrane ruffling and cells were more rounded. Cellular IAPP immunoreactivity was diminished, indicating the inhibition of hIAPP internalisation. Some fibrillar material was still adherent to the slide after washing (*arrows*). Fluorescence labelling of macrophages for IAPP (*red*) and the late-endosome marker, macrosialin (*green*), is shown after exposure to hIAPP for 1 h (**E**) or 6 h (**F**). Little IAPP is internalised after 1 h and the fibrils remain largely at extracellular sites (*red*, *arrow*); macrosialin immunoreactivity (*green*) labels intracellular lysosomes. However, after 6 h culture, internalised IAPP is co-localised with macrosialin (*red*+*green*=*yellow*) and only a little extracellular IAPP (*red*) remains (*arrow*). ×400

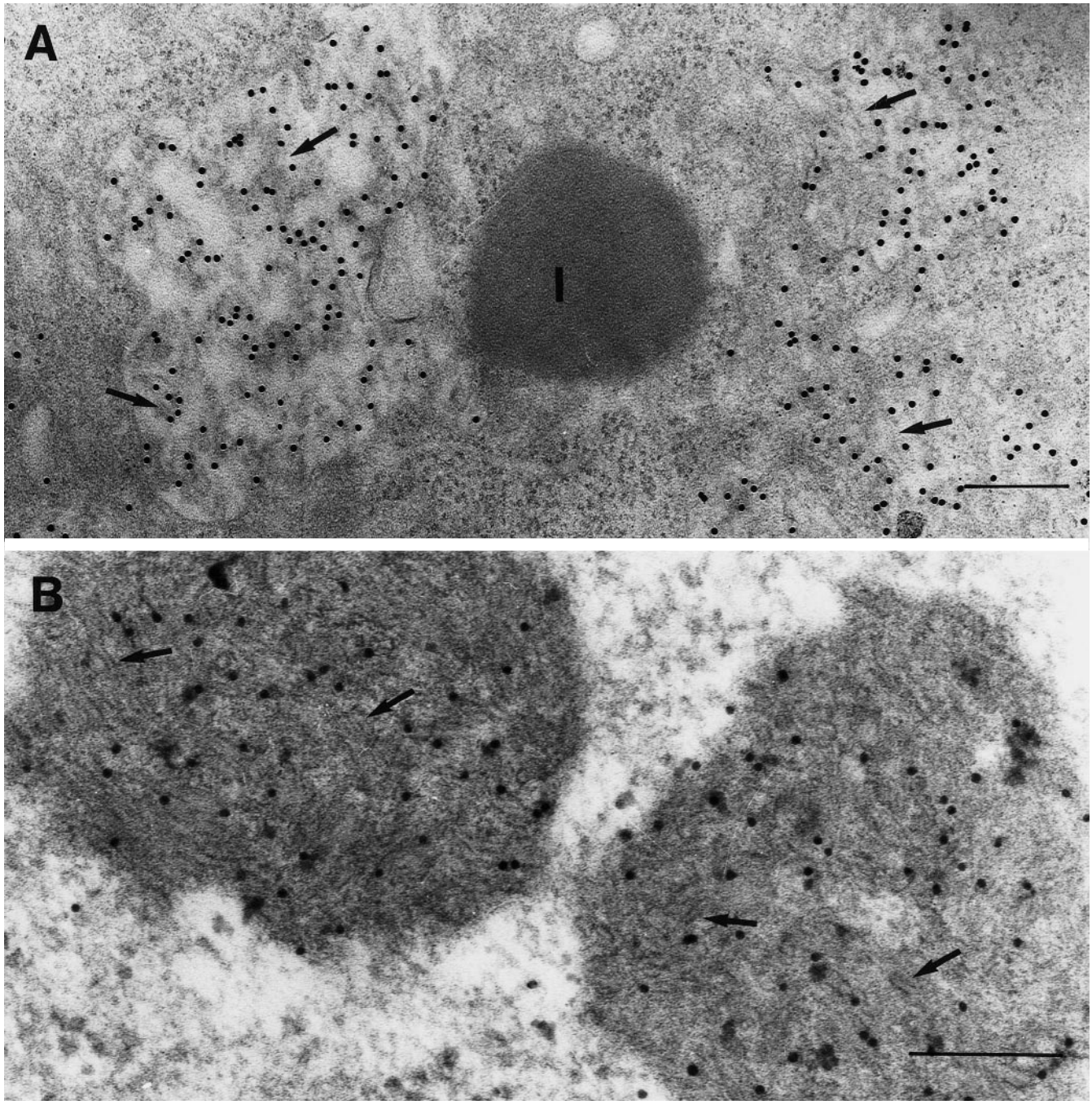

Fig. 4 Intracellular IAPP (immunogold-labelled for IAPP) in macrophages exposed to hIAPP for (**A**) 24 h and (**B**) 72 h. Flocculent aggregates of IAPP-ir material were contained in phagosome-like organelles after 24 h (*arrows*). *l* Dense secondary lysosome. After 72 h, IAPP-ir material is present as short fibrils with the dimensions of amyloid protofilaments (5–8 nm in diameter; *arrows*). *Bars* 0.2 μm

have been implicated in the binding of microglia to Aβ amyloid fibrils and the release of cytokines (Khoury et al. 1996) but phagocytosis of the Aβ fibrils by microglia has not been investigated. A receptor for advanced glycation end products has been implicated in the binding and neurotoxic effects of Aβ fibrils on microglia (Yan et al. 1996). However, synthetic IAPP shows no cytotoxic effects on peritoneal macrophages.

Fibrils could enter macrophages by endocytosis or phagocytosis. The fibrillar aggregates of IAPP visualised by electron microscopy are too large to enter the cell by fluid-phase pinocytosis. Internalisation of small particles (0.1–0.2 μm diameter) can occur by endocytosis via clathrin-coated invaginations of the plasma membrane (Steinman et al. 1976; Goldstein et al. 1979). NOC inhibits endocytosis by reversible binding to tubulin, thus preventing the assembly of microtubules (Robinson and Vandre 1995), which play an essential role in the internalisation of endocytotic vesicles (de Brabander et al.

1977; Newman et al. 1991; Young et al. 1992). However, NOC does not affect the uptake of IAPP. Furthermore, clathrin-coated membranes and microtubule bundles (Reaven and Axline 1973) are not visibly associated with areas of mouse macrophages that are actively engaged in the internalisation of IAPP. This suggests that classical endocytosis is not involved. Macropinocytosis, a process involving the ruffling of membranes, by which macrophages internalise extracellular material up to 15 μm in diameter (Cannon and Swanson 1992) in relatively large vesicles (Racoosin and Swanson 1992), is also unlikely to be involved in IAPP internalisation, because NOC inhibits the ruffling of stimulated human macrophages (Racoosin and Swanson 1992). However, the microscopical appearance of macrophages taking up IAPP in our cultures suggests that pseudopodia and therefore phagocytosis is the mechanism. Phagocytosis is a receptor-mediated event involving the extension of actin microfilaments to create pseudopodia that engulf extracellular material (Swanson and Baer 1995). CB inhibits the polymerisation of actin in the assembly of microfilaments. Therefore the inhibition of hIAPP uptake by CB confirms that IAPP is internalised by phagocytosis.

In many forms of amyloid, including IAPP amyloid in transgenic mouse islets (de Koning et al. 1994a, b) and in human insulinomas (Shirahama and Cohen 1971; Wisniewski et al. 1991), fibrils are engulfed by pseudopodia and are visible in intracellular lysosome-like structures. In the early stages of culture, IAPP and macrosialin, a macrophage-specific member of the lysosome-associated membrane protein family (Holness et al. 1993; Rabinowitz and Gordon 1991) have been identified in distinct compartments. However, after 6 h in culture, IAPP is co-localised with macrosialin immunoreactivity in intracellular vesicles, suggesting that IAPP accumulates in the lysosomal compartment (Rabinowitz et al. 1992). Later, the IAPP becomes further concentrated in electron-dense organelles that could be secondary lysosomes (Desjardins et al. 1994); fibrillar structures are visible in these organelles as short fibrils, less than 8 nm in diameter, and are arranged in pairs. This suggests that IAPP aggregates become condensed to form aligned protofilaments. If extruded, these protofilaments could form a nidus for fibril formation.

Macrophages have efficient proteolytic systems and cultured macrophages have been shown to degrade bovine serum albumin, bovine liver catalase and rabbit muscle enolase (Buktenica et al. 1987). However, IAPP immunoreactivity was present in lysosomes up to 72 h after exposure to hIAPP, even after removal of extracellular IAPP aggregates, indicating that human IAPP is degraded very slowly by lysosomal enzymes in macrophages. Accumulations of human IAPP have been identified in lysosomes of pancreatic β-cells of non-diabetic humans (Clark et al. 1988) and monkeys (Clark et al. 1989) and in hIAPP-transgenic mice but not in β-cells of normal rodents (de Koning et al. 1994b). Species-specific variations in the molecular structure of IAPP appear to confer not only amyloidogenic potential, but also extreme protease resistance. Amyloid fibrils formed from other peptides are also relatively protease-resistant (Franklin and Pras 1969). Furthermore, amyloid fibrils coated with serum amyloid P component (SAP) resist proteolysis in vitro (Tennent et al. 1995). It is unlikely that this is the protective factor for synthetic IAPP fibrils in culture media, which probably contain very low levels of SAP.

Fibrils formed from synthetic hIAPP have been reported to be toxic to isolated endocrine and certain other cell types in culture (Lorenzo et al. 1994). Other amyloidogenic peptides such as the Alzheimer Aβ peptide share this cytotoxic property (Behl et al. 1994) and it has been suggested that cell death occurs either by apoptosis (Lorenzo et al. 1994) or by necrosis (Behl et al. 1994). However, macrophage numbers are not influenced by culture with IAPP fibrils, suggesting that this type of cell is protected from fibril-induced cytotoxicity (Pryce et al. 1995).

Deposition of islet amyloid in Type II diabetes is linked with reduced islet function and a reduction of insulin-producing cells (de Koning et al. 1993) and, like most other forms of amyloid, this process appears to be irreversible. Our data suggest that macrophages are able to internalise fibrillar IAPP, even in the absence of a clearly defined receptor-mediated mechanism. Since islet amyloid deposition in Type II diabetes is progressive, it appears that fibril formation is more rapid than the sequestration and any degradation of fibrils by resident islet macrophages (de Koning et al. 1994b). Ineffective clearance of all types of amyloid deposits by macrophages may be attributable to protection by amyloid-associated factors. Many compounds are ubiquitous in islet amyloid deposits (Wisniewski and Frangione 1992) including amyloid P (Pepys et al. 1979), apolipoprotein E (Chargé et al. 1996) and heparan sulphate proteoglycans (Young et al. 1992). These factors could either enhance recognition of amyloid for internalisation into macrophages or block potential opsonisation of amyloid by antibodies in vivo. The modulation of macrophage activity by, for example, improvements to macrophage recognition systems could potentially promote the removal of islet amyloid and prevent the deterioration of islet function in Type II diabetes.

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