IGF-I inhibits apoptosis induced by serum withdrawal, but potentiates TNF-α-induced apoptosis, in 3T3-L1 preadipocytes

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Abstract

We have previously shown that human preadipocytes in primary culture undergo apoptosis in response to serum deprivation and addition of tumour necrosis factor alpha (TNF-α), and have proposed that regulation of preadipocyte apoptosis *in vivo* may contribute to the overall control of adipose mass. In the present study we have investigated both pro- and anti-apoptotic factors, and the signalling pathways by which they act, in murine 3T3-L1 preadipocytes. Apoptotic indices (fraction of cells undergoing apoptosis) were determined by microscopic examination of acridine orange-stained cells, fluorescence-activated cell sorting of propidium iodide-stained cells, or phase-contrast video microscopy. Murine 3T3-L1 cells were more susceptible to apoptosis than human preadipocytes. In medium containing 10% newborn calf serum, the basal apoptotic index was very low (<2%), but the number of apoptotic cells increased significantly following serum withdrawal (10% after 24 h). Addition of TNF-α (6 nM) stimulated apoptosis in both serum-containing and serum-free media (apoptotic indices of 12% and 20% respectively after 24 h). IGF-I inhibited by approximately 50% the apoptosis induced by serum withdrawal, but increased by 25% the apoptosis induced by TNF-α in serum-free medium. It was shown by using specific inhibitors of lipid and protein kinases (LY294002, rapamycin, PD98059, SB203580) that both phosphoinositide 3-kinase and MAP kinase pathways contribute to the anti-apoptotic action of IGF-I on serum-starved cells, while phosphoinositide 3-kinase but not MAP kinase activity is required for the paradoxical pro-apoptotic action of IGF-I in the presence of TNF-α. We conclude that, in addition to its previously described anti-apoptotic action, IGF-I can also be pro-apoptotic in 3T3-L1 cells in the presence of TNF-α, and that both the anti- and pro-apoptotic effects of IGF-I require the activation of phosphoinositide 3-kinase.

Journal of Endocrinology (2000) **167**, 165–174

Introduction

The importance of apoptosis in many aspects of animal development is well established (Jacobson *et al.* 1997) although susceptibility to apoptosis and the biochemical mechanisms which modulate the process are variable between cell types. Insulin-like growth factor-I (IGF-I) is one of the major survival factors in serum, and has been shown in various cell types to protect from apoptosis induced in numerous ways, including withdrawal of growth factors, addition of etoposide or tumour necrosis factor alpha (TNF-α), hyperosmotic stress, irradiation, and overexpression of oncogenes or interleukin-1β-converting enzyme-related proteases (Harrington *et al.* 1994, Sell *et al.* 1995, Jung *et al.* 1996, Wu *et al.* 1996, Kulik *et al.* 1997, Parrizas *et al.* 1997, Porras *et al.* 1997). Furthermore, decreased expression of IGF-I receptors increases apoptosis of tumour cells (Baserga *et al.* 1997).

The precise mechanisms by which IGF-I receptors signal to apoptotic pathways are still unclear. The IGF-I receptor is a member of the tyrosine kinase superfamily, and following the binding of IGF-I the autophosphorylated receptor exerts its action by recruiting and phosphorylating substrates such as the insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and Shc (Butler *et al.* 1998). These substrates serve as docking sites for various downstream signalling molecules which bind via their SH2 domains. Among these, the best characterised are phosphoinositide 3-kinase (PI 3-kinase), which binds to IRS-1 and -2, and Grb2/Sos, which binds mainly to Shc. Recruitment and/or activation of these proteins leads in turn to the activation of the PDK/Akt/GSK-3 and

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Ras/Raf/ERK cascades respectively (Butler *et al.* 1998). The serine kinase PKB/Akt has been strongly implicated in mediating the anti-apoptotic actions of growth factors such as IGF-I (Dudek *et al.* 1997, Kennedy *et al.* 1997), probably via phosphorylation of BAD, one of the members of the Bcl-2 family of proteins (Datta *et al.* 1997). The role of the extracellular signal-regulated kinase (ERK) cascade is less clear, some studies suggesting an anti-apoptotic action and others not (Xia *et al.* 1995, Dudek *et al.* 1997, Kulik *et al.* 1997, Parrizas *et al.* 1997).

We have been interested in the role which apoptosis may play in the regulation of adipose mass. It is now accepted that not only the lipid content but also the number of adipocytes is variable in adult mammals. Adipocyte number may be increased by differentiation of preadipocytes, a process which has been widely studied (Prins & O'Rahilly 1997, Gregoire *et al.* 1998). The mechanisms by which adipocytes may be lost are less well understood, although apoptosis of mature adipocytes *in vivo* has been documented (Prins *et al.* 1994). To study the regulation of apoptosis of adipose-related cells we have used primary cultures of human preadipocytes, which may be maintained in culture while retaining their capacity for differentiation. We have shown that apoptosis is induced in these cells by serum deprivation and addition of TNF-α (Prins *et al.* 1997, Niesler *et al.* 1998), although the fraction of cells undergoing apoptosis was small (typically 5%) and variable between individuals. To extend studies of pro- and anti-apoptotic factors regulating apoptosis, and to examine the signalling pathways by which they act, we have turned to the murine 3T3-L1 preadipocyte cell line, which is more susceptible to apoptosis and more consistent in its responses. We report here that both serum deprivation and TNF-α induce apoptosis in 3T3-L1 cells, and that IGF-I can either inhibit or enhance the apoptotic effect depending on the inducing agent used.

Materials and Methods

Materials

TNF-α was purchased from Autogen Bioclear (Wiltshire, UK). LY294002 and rapamycin were from Alexis Biochemicals (Nottingham, UK), and PD98059 and SB203580 were from Calbiochem-Novobiochem (Nottingham, UK). Recombinant IGF-I was a generous gift from Dr John Wallace, University of Adelaide. Unless otherwise indicated, other reagents were from Sigma Chemical Co., Poole, Dorset, UK.

Cell culture

3T3-L1 preadipocytes were obtained from the American Tissue Culture Collection (VA, USA). Cells were cultured in DMEM containing 4·5 g/l glucose together with 10% newborn calf serum, 2 mM glutamine, 50 U/ml penicillin, 0·05 mg/ml streptomycin, and maintained at 37 °C in a 5% CO_2 gassed incubator. Experiments were conducted either in the same serum-containing medium (SCM) or in serum-free medium (SFM). The number of independent experiments performed for each analysis is indicated in the figure legends.

Electron microscopy

Detached preadipocytes were removed and the monolayer was washed twice with PBS and trypsinised. Cells in the supernatant and monolayer were pooled and washed once with PBS. Cells were then pelleted again and fixed in 3% glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·2) for 2 h, then processed and embedded in Epon/Araldite resin. Semi-thin resin sections (0·5–1 µm) stained with toluidine blue were examined by light microscopy at 1000 × magnification (oil immersion) to select areas for examination by electron microscopy. Ultra-thin resin sections (50–70 nm) stained with lead citrate were examined in a Philips CM10 electron microscope (Cambridge, UK).

Acridine orange assay for apoptosis

Apoptosis was routinely quantified by fluorescence microscopy of acridine orange-stained cells (McGahon *et al.* 1995). Acridine orange was added to cell culture medium to a final concentration of 10 µg/ml, and within 5 min cells were viewed with a fluorescence microscope with a filter combination suitable for reading fluorescein (rhodamine filter: excitation wavelength 485 nm; emission wavelength 530 nm). A minimum of 200 cells in at least four fields of view in each well were counted to derive an apoptotic index, defined as (number of apoptotic cells/total number of cells) × 100.

Phase-contrast video microscopy

Cells were subcultured into a 25 cm^2 flask 24 h prior to treatment. Immediately after treatment, the flask was placed on a heated platform (37 °C), monitored using an Olympus IX70 inverted phase-contrast microscope (London, UK) and filmed using a JVC Colour Video Camera and Monitor (Ibaraki-Ken, Japan), and a Panasonic Time Lapse Video Cassette Recorder (Osaka, Japan) (Harrington *et al.* 1994). The recorder was set to record at 1/480 the normal recording speed such that a 24 h incubation period would be viewed in a 3 min time frame. Apoptotic indices were calculated for fields of 50–100 cells by counting total and apoptotic cells at different times on the video record.

Analysis by fluorescence-activated cell sorting (FACS)

Fibroblasts were serum-starved with or without addition of IGF-I (13 nM) and TNF-α (6 nM) for 24 h. The cells in

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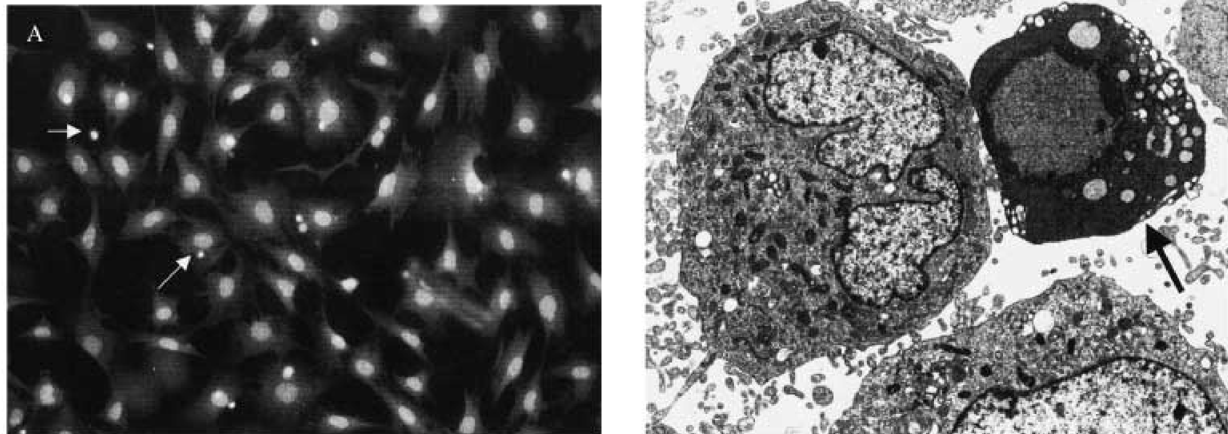

Figure 1 Evidence of apoptosis in 3T3-L1 preadipocytes. Cells were cultured in SFM with TNF-α (6 nM) for 24 h before preparation for light or electron microscopic examination. (A) Fluorescence microscopy of acridine orange-stained cells, with apoptotic cells indicated by arrows; (B) electron microscopy of normal and apoptotic cells.

the medium were collected and the attached cells trypsinised and washed, and both cell populations were incubated with propidium iodide in binding buffer on ice and in the dark, at the concentration suggested by the manufacturer (Nexis Research BV, Kattendijke, The Netherlands). Stained cells were analysed on a FACSort (Beckton Dickinson, Oxford, UK, single argon laser, excitation at 488 nm and green and red emission filters (515–545 nm and 600 nm)); 10 000 cells were counted per sample and the data were processed using the standard Lysis software (Becton Dickinson).

Inhibitor studies

Cells were treated for 24 h in SFM, with or without IGF-I (13 nM), TNF-α (6 nM) and the inhibitors LY294002, rapamycin, PD98059 or SB203580. Effective concentrations of inhibitors were taken from previous studies (Vlahos *et al.* 1994, Alessi *et al.* 1995, Shepherd *et al.* 1995, Jou *et al.* 1997, Parrizas *et al.* 1997). LY294002, PD98059 and SB203580 were dissolved in DMSO at a concentration of 10 mM, whereas rapamycin was dissolved in DMSO at a concentration of 10 μM. Inhibitors were added to the cells at a 1/1000 dilution. Cells were exposed to the inhibitors for 5 min prior to and during treatment and the apoptotic index assessed by acridine orange staining.

Results

Serum deprivation and TNF-α induce apoptosis in 3T3-L1 preadipocytes

Apoptosis of 3T3-L1 cells was demonstrated by acridine orange staining and electron microscopy, revealing nuclear condensation (Fig. 1), and by annexin V staining, revealing changes in membrane phospholipids (data not shown). The apoptotic index of cells in SCM was <2%, but removal of serum caused a time-dependent increase in the apoptotic index to $10{\cdot}3 \pm 0{\cdot}7\%$ after 24 h (Fig. 2). Incubation with TNF-α also increased apoptosis, in both the presence and absence of serum, and the effects of TNF-α and serum deprivation were roughly additive. After 24 h, apoptotic indices in the presence of TNF-α were $12{\cdot}0 \pm 1{\cdot}7\%$ in SCM and $20{\cdot}2 \pm 2{\cdot}5\%$ in SFM (Fig. 2).

IGF-I protects 3T3-L1 adipocytes from serum deprivation-induced apoptosis but increases TNF-α-induced apoptosis

IGF-I (13 nM) substantially inhibited the apoptosis induced by serum deprivation, although not so much as SCM (Fig. 3). This was found to be the maximally effective concentration of IGF-I as higher concentrations of IGF-I (up to 1000 nM) had no greater effect. Furthermore, although the effect of 1·3 nM IGF-I was similar to that of 13 nM, 0·13 nM IGF-I was clearly much less effective (data not shown). The effect of IGF-I was apparent when the apoptotic index was determined by acridine orange staining (55% reduction), phase-contrast microscopy (75% reduction) or FACS analysis (40% reduction). Incubation of serum-deprived cells with IGF-I resulted in a 15–20% increase in total cell number, but concomitantly decreased the number of apoptotic cells by 40% (Table 1). This indicates that in addition to its pro-proliferative nature, the effect of IGF-I on the apoptotic index was due to an absolute decrease in

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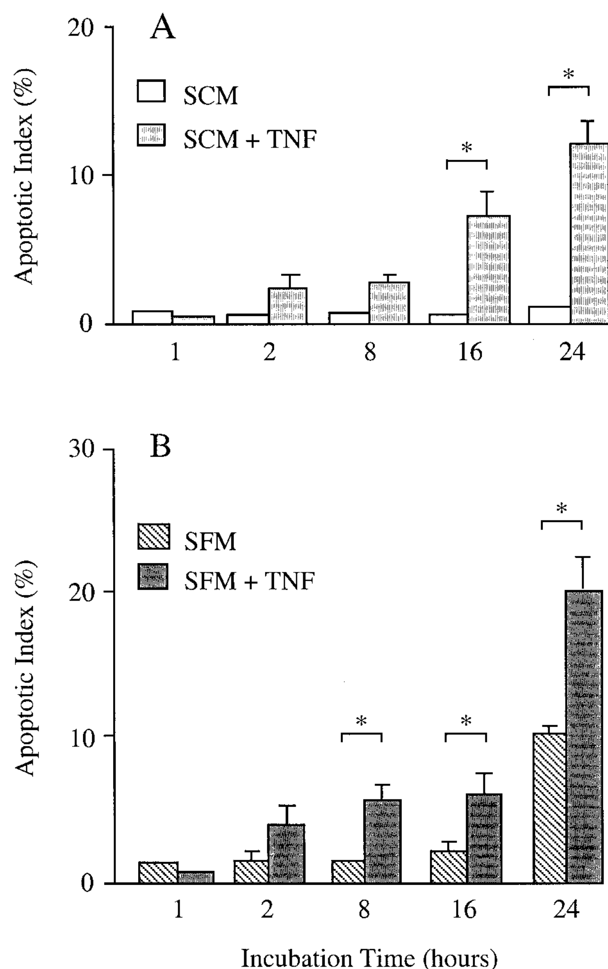

Figure 2 Time course of apoptosis induced by serum withdrawal or TNF-α. Cells were incubated with or without TNF-α (6 nM) either in SCM (A) or in SFM (B), and apoptotic indices were calculated following acridine orange staining. Values shown are means ± S.E.M., n=5 independent experiments. Statistically significant differences are indicated: *P<0·05, as determined by paired Student's t-test, otherwise P>0·05.

the number of apoptotic cells. IGF-I had no effect on TNF-α-induced apoptosis in SCM. However, in the absence of serum, IGF-I increased by approximately 25% the level of apoptosis induced by TNF-α, as assessed by acridine orange staining, phase-contrast microscopy or FACS (Fig. 3). Absolute values of the apoptotic index varied with the method used, and in particular appeared substantially higher when determined by phase-contrast microscopy. This may have been because video microscopy provided a cumulative measure of apoptosis rather than a snapshot at a given time, and/or because the various methods detected different aspects of the overall apoptotic process.

PI 3-kinase and MAP kinase mediate the anti-apoptotic action of IGF-I

To test the involvement of different signalling pathways in the anti-apoptotic action of IGF-I, 3T3-L1 preadipocytes were incubated for 24 h in SFM, with or without IGF-I, together with various inhibitors, before determination of apoptotic indices by acridine orange staining (Fig. 4). None of the inhibitors had a significant effect on apoptosis in the absence of IGF-I. The PI 3-kinase inhibitor LY294002 and the MAPK/ERK kinase (MEK) inhibitor PD98059 significantly decreased the anti-apoptotic effect of IGF-I, by 65% and 50% respectively, while the p70 S6 kinase inhibitor rapamycin and the p38 MAP kinase inhibitor SB203580 had no significant effect on apoptosis in the presence of IGF-I. It is concluded that both the PI 3-kinase and ERK MAP kinase signalling pathways participate in the anti-apoptotic action of IGF-I, and that p70 S6 and p38 MAP kinases are not involved in anti-apoptotic signalling under the conditions of these experiments.

PI 3-kinase but not MAP kinase mediates the pro-apoptotic effect of IGF-I

The effects of inhibitors on the potentiation of TNF-α-induced apoptosis by IGF-I were similarly examined (Fig. 5). The most dramatic observation was that TNF-α-induced apoptosis in the absence or presence of IGF-I was increased more than 2-fold by the p38 MAP kinase inhibitor SB203580. Against this background, it was not possible to say whether the relatively small increment in apoptosis induced by IGF-I was affected by p38 MAP kinase inhibition. Other inhibitors had no significant effect on TNF-α-induced apoptosis in the absence of IGF-I. However, the PI 3-kinase inhibitor LY294002 modestly but significantly diminished the potentiation of TNF-α-induced apoptosis by IGF-I. In some experiments it appeared that rapamycin also diminished the potentiation of TNF-α-induced apoptosis by IGF-I but overall this effect was not statistically significant. The MEK inhibitor PD98059 had no effect on apoptosis in the presence of IGF-I. It is concluded that the pro-apoptotic action of TNF-α is antagonised by tonic activity of p38 MAP kinase, and that PI 3-kinase, and possibly p70 S6 kinase, but not ERK MAP kinase, participates in the pro-apoptotic action of IGF-I in the presence of TNF-α.

Discussion

We used 3T3-L1 cells to study factors regulating apoptosis in cells of adipocyte lineage. Under appropriate conditions the preadipocytes displayed classical features of apoptosis as demonstrated qualitatively by electron microscopy and binding of annexin V, and as quantified by nuclear staining

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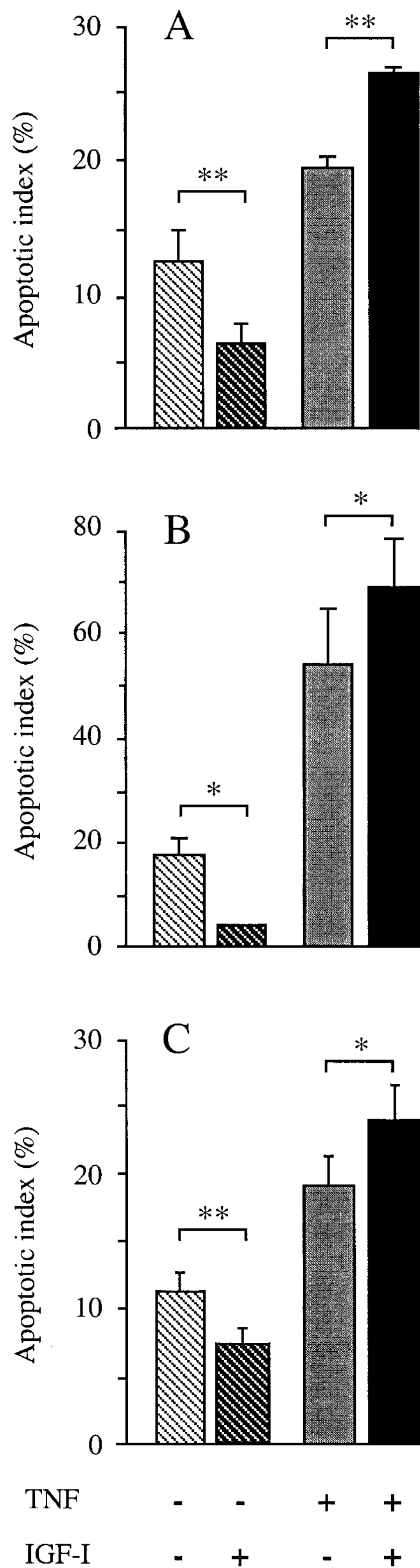

with acridine orange, video microscopy or FACS. We confirmed a previous report (Magun *et al.* 1998) that apoptosis is induced in these cells by serum deprivation, and showed that TNF-α increased apoptosis in both SFM and SCM. Apoptosis induced by both serum deprivation and TNF-α was time-dependent, with small increases at 2–8 h and more substantial effects at 16–24 h. The effects of serum deprivation and TNF-α were additive, with the combined apoptotic index reaching 20–25% after 24 h. These patterns of response are somewhat different from those seen with primary cultured human preadipocytes under similar conditions, where apoptosis did not increase after 2–4 h and remained at much lower levels (Prins *et al.* 1997). Moreover, in these human cells, TNF-α induced apoptosis only in the absence of serum.

The major novel finding of the present study is that IGF-I can act as either an anti-apoptotic or pro-apoptotic factor in 3T3-L1 cells depending on the conditions. IGF-I substantially reduced apoptosis induced by serum deprivation, although the inhibition was not so complete as when serum was present. This suggests that there must be additional factors in serum that are able to promote the survival of 3T3-L1 preadipocytes. One such may be platelet-derived growth factor, which has been shown to suppress apoptosis in differentiating fibroblasts in the presence of insulin (Staiger & Loffler 1998). As referenced elsewhere, an anti-apoptotic action of IGF-I has been previously demonstrated in numerous cell types and in response to a variety of stimuli, and the finding of a similar action in 3T3-L1 cells is not surprising.

However, the finding that addition of IGF-I increased apoptosis induced by TNF-α in SFM was unexpected. This increase in apoptosis, of approximately 25%, was demonstrated by three different methods which reflect different aspects of the apoptotic process. Aside from its effects with multiple other apoptotic stimuli, IGF-I has previously been shown to prevent TNF-α-induced apoptosis in BALB/c 3T3 fibroblasts, foetal brown adipocytes and oligodendrocytes (Wu *et al.* 1996, Porras *et al.* 1997, Ye & D'Ercole 1999) while insulin inhibited TNF-α-induced apoptosis in endothelial cells (Hermann *et al.* 2000). On the other hand, IGF-I has been reported to increase Fas-induced apoptosis in human osteoblasts (Kawakami *et al.* 1998), both IGF-I and insulin have been reported to increase apoptosis in serum-starved glioma and hepatoma cells (Yang *et al.* 1996, Xu *et al.* 1997) and

Figure 3 Effect of IGF-I on apoptosis induced by serum withdrawal or TNF-α. Cells were incubated for 24 h in SFM with or without TNF-α (6 nM) and IGF-I (13 nM) as indicated. Apoptotic indices were determined by acridine orange staining (A), phase-contrast video microscopy (B) or FACS analysis of propidium iodide-stained cells (C). Values shown are means ± S.E.M., $n=7$ independent experiments (A) or $n=3$ independent experiments (B, C). Statistically significant differences are indicated: $*P<0{\cdot}05$, $**P<0{\cdot}005$, as determined by paired Student's *t*-test, otherwise $P>0{\cdot}05$.

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Table 1 Changes in the cell number in response to 24 h culture with IGF-I. 3T3-L1 preadipocytes were cultured in SFM or SFM+TNF-α (6 nM) with or without the addition of IGF-I (13 nM) and the number of healthy and apoptotic cells (± S.E.M.) assessed by the acridine orange assay; n=8 independent experiments

	SFM	SFM+IGF-I	SFM+TNF-α	SFM+TNF-α+IGF-I
Healthy cells	133 ± 17	172 ± 23	178 ± 52	169 ± 46
Apoptotic cells	15 ± 5	9 ± 3	37 ± 12	49 ± 15

insulin has been reported to activate caspase-3 and induce apoptosis of serum-deprived myeloma cells by a PI 3-kinase-dependent pathway (Godbout *et al.* 1999). Moreover peptides derived from the IGF receptor C-terminus have been shown to induce apoptosis in tumour cells (Hongo *et al.* 1998, Liu *et al.* 1998, Reiss *et al.* 1999). Our findings show that, at least in 3T3-L1 preadipocytes, IGF-I can exert either an anti-apoptotic or pro-apoptotic effect depending on the absence or presence of serum and TNF-α. It remains to be determined whether 3T3-L1 adipocytes, let alone human adipocytes, show comparable responses. Apoptosis induced by TNF-α was greater in SFM than in SCM, suggesting that serum factors other than IGF-I inhibit TNF-α-induced apoptosis, and override the pro-apoptotic action of any IGF-I which is also present in the serum.

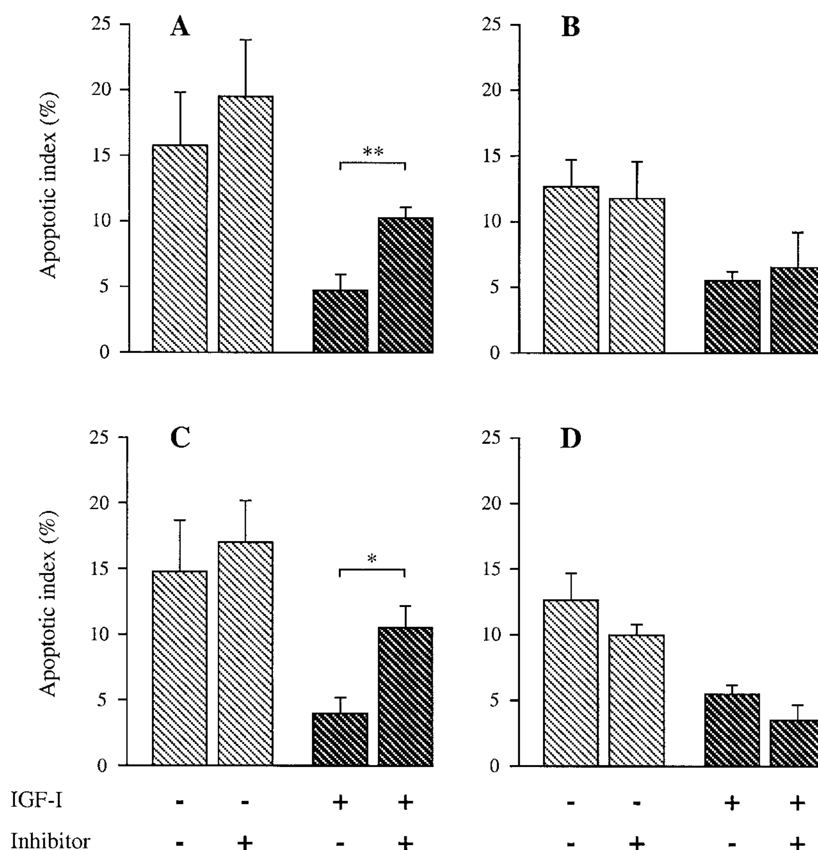

Figure 4 Effect of inhibitors on apoptosis in SFM. Cells were incubated for 24 h in SFM with or without IGF-I (13 nM) and inhibitors as indicated. (A) LY294002 (10 µM); (B) rapamycin (10 nM); (C) PD98059 (10 µM); (D) SB203580 (10 µM). Apoptotic indices were calculated following acridine orange staining. Values shown are means ± S.E.M., n=5 individual experiments (A, C) or n=3 independent experiments (B, D). Statistically significant effects of inhibitors are indicated: *P<0·05, **P<0·005, as determined by paired Student's t-test, otherwise P>0·05.

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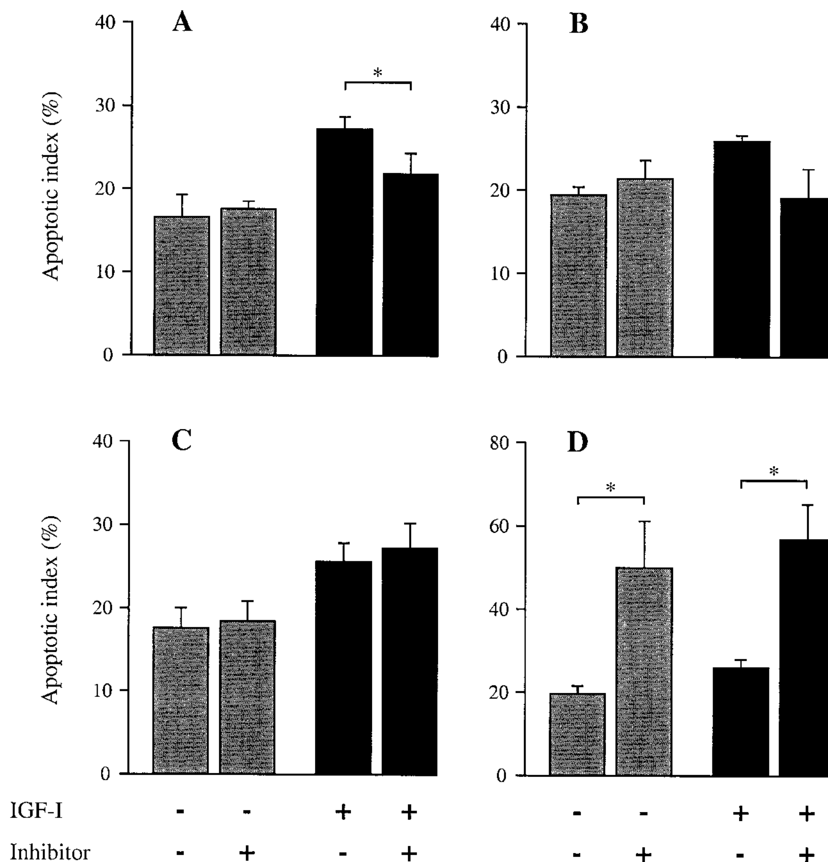

Figure 5 Effect of inhibitors on apoptosis induced by TNF-α. Cells were incubated for 24 h in SFM containing TNF-α (6 nM) with or without IGF-I (13 nM) and inhibitors as indicated. (A) LY294002 (10 μM); (B) rapamycin (10 nM); (C) PD98059 (10 μM); (D) SB203580 (10 μM). Apoptotic indices were calculated following acridine orange staining. Values shown are means ± S.E.M., $n=6$ individual experiments (A, C) or $n=3$ independent experiments (B, D). Statistically significant effects of inhibitors are indicated: *$P<0{\cdot}05$ as determined by paired Student's *t*-test, otherwise $P>0{\cdot}05$.

Inhibitors of specific intracellular signalling pathways were used to gain insights into the signalling mechanisms which mediate the anti- and pro-apoptotic actions of IGF-I. It was found that inhibition of either PI 3-kinase or MEK (and thus ERK MAP kinase activation) abrogated, but did not completely reverse, the anti-apoptotic action of IGF-I in SFM, while inhibition of p70 S6 kinase or p38 MAP kinase had no effect. Similar results have been reported in other cell types, with respect to the involvement of both PI 3-kinase and ERK pathways (Kulik *et al.* 1997, Parrizas *et al.* 1997, Navarro *et al.* 1998, Peruzzi *et al.* 1999) and lack of involvement of p70 S6 kinase and p38 MAP kinase (Kulik *et al.* 1997, Gunn-Moore *et al.* 1997, Parrizas *et al.* 1997, Gunn-Moore & Tavaré 1998, Minshall *et al.* 1999) in IGF-I-induced anti-apoptotic signalling. In the basal condition (SCM) the inhibitors of PI 3-kinase or MEK did not increase apoptotic indices even at the higher concentration of 50 μM (data not shown). This suggests that there are elements in serum which either stimulate other survival pathways not inhibitable by LY294002 or PD98059, or are able to sequester apoptosis-inducing factors secreted by the cells in an autocrine/paracrine manner.

Although the primary focus of our work was on the actions of IGF-I, a striking finding was the potentiation of TNF-α-induced apoptosis by the p38 MAP kinase inhibitor SB203580, in both the absence and presence of IGF-I, although this inhibitor was without effect when apoptosis was induced by serum withdrawal. This suggests that, in 3T3-L1 cells, the p38 MAP kinase pathway exerts a tonic inhibitory effect on pro-apoptotic pathways which are activated by TNF-α but not serum deprivation. The p38 MAP kinase is activated by environmental stresses and pro-inflammatory cytokines and has previously been implicated in both anti- and pro-apoptotic effects depending on stimulus and cell type. Inhibition of p38 MAP

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kinase has been shown to increase TNF-α-induced, FasL-induced and constitutive apoptosis (Nemoto *et al.* 1998, Roulston *et al.* 1998, Kankaanranta *et al.* 1999). However, activation of p38 MAP kinase is apparently required for apoptosis induced under other conditions (Xia *et al.* 1995, Kummer *et al.* 1997, Frasch *et al.* 1998).

The pro-apoptotic effect of IGF-I in the presence of TNF-α was abrogated by inhibition of PI 3-kinase, and possibly p70 S6 kinase, but not the ERK MAP kinase pathway. This spectrum of inhibitor sensitivity suggests that different signalling pathways underlie the pro- and anti-apoptotic actions of IGF-I in 3T3-L1 cells. TNF-α and related ligands have themselves been reported to activate PI 3-kinase and downstream signalling pathways including Akt in some cell types, although under these circumstances inhibition of PI 3-kinase potentiates cytotoxic activity (Ozes *et al.* 1999, Pastorino *et al.* 1999, Wong *et al.* 1999, Reddy *et al.* 2000). However, contrary to its generally anti-apoptotic role when signalling via Akt, PI 3-kinase has been implicated in pro-apoptotic signalling in human Jurkat T cells induced to apoptose by exposure to FasL or ligation of MHC class I molecules, via stimulation of JNK and Ras respectively (Gulbins *et al.* 1996, Skov *et al.* 1997).

The mechanisms of cross talk between the TNF-α and IGF-I signalling systems are unclear. It has been shown that TNF-α inhibits signalling from the insulin receptor (Feinstein *et al.* 1993, Hotamisligil *et al.* 1994). This action is apparently mediated by phosphorylation of IRSs by unidentified serine/threonine-specific protein kinases (Kanety *et al.* 1995, Hotamisligil *et al.* 1996), including JNK (Aguirre *et al.* 2000). This in turn renders IRS proteins inhibitory to insulin receptor tyrosine kinase, decreasing tyrosine phosphorylation of IRSs and recruitment of proteins such as PI 3-kinase. The insulin and IGF receptor tyrosine kinases differ in the efficiency with which they phosphorylate IRS-1 and activate PI 3-kinase (Ursø *et al.* 1999), and it cannot be taken for granted that TNF-α-induced IRS phosphorylation will affect insulin and IGF signalling equally. However, it has been shown that TNF-α inhibits IGF-I-induced tyrosine phosphorylation of IRS-2 (Venters *et al.* 1999), and IGF-I-stimulation of protein synthesis and gene transcription (Urban *et al.* 1996, Frost *et al.* 1997). We hypothesise that TNF-α does not simply inhibit signalling by the IGF receptor, but selectively modulates signalling so that pathways involving PI 3-kinase, which normally act to inhibit apoptosis, instead take on a pro-apoptotic role. It is presently unclear at what level of signalling the anti- and pro-apoptotic actions of PI 3-kinase diverge, and how the activity of PI 3-kinase is channelled to effect distinct responses in different metabolic circumstances. In principle this could involve acute modulation of protein kinases, or change in gene transcription and protein synthesis, leading ultimately to alterations in the ratio of activities or concentrations of pro-apoptotic versus anti-apoptotic Bcl-2 members.

In summary, we have shown that IGF-I does not function only as a survival factor, and that in 3T3-L1 cells it can act to increase the level of apoptosis induced by TNF-α while still acting via PI 3-kinase. It remains to be determined whether a similar phenomenon is demonstrable with other growth factors and in other cell types.

Acknowledgements

We are grateful to the Cambridge Commonwealth Trust (C U N), the Wellcome Trust (J B P, International Research Fellow) and the British Diabetic Association (K S and B U, project grant RD95/0001102) for financial support. We also thank Dr Nick Bright for assistance with electron microscopy and Dr Martin Bennett for use of the video microscopy equipment.

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Received 15 June 2000
Accepted 26 June 2000

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