## 8 Tumour necrosis factor induced autophagy and mitochondrial morphological abnormalities are mediated by TNFR-I and/or TNFR-II and do not invariably lead to cell death.

John Prins[‡§], Elizabeth Ledgerwood[‡§], Paul Ameloot*, Peter Vandenabeele*, Paulo Faraco[‡], Nicholas Bright[§], Stephen O'Rahilly[‡§] and John Bradley[‡]

Departments of [‡]Medicine and [§]Clinical Biochemistry, University of Cambridge, Cambridge, CB2 2QR, U.K. and *Department of Molecular Biology, Flanders' Interuniversity Institute for Biotechnology, University of Gent, Gent, Belgium.

TNF affects many cellular metabolic processes, regulates gene expression, and may induce apoptosis or necrosis dependent on cell type and conditions of exposure. TNF also induces specific alterations in mitochondrial morphology and biochemistry. Ultrastructurally, these alterations include a swollen appearance characterised by increased matrix space and reduced number of cristae [1]. Biochemical abnormalities include inhibition of electron transport [2], generation of superoxide [1,3] and reduction in mitochondrial transmembrane potential [4]. Mitochondria have a central role in the apoptotic and necrotic processes, including in TNF-induced cytotoxicity [5], and TNF-induced functional mitochondrial damage precedes cytotoxicity. It has also recently been reported that TNF induces autophagy in an acute T-lymphoblastic leukemic cell line [6]. Autophagy is a major mechanism of intralysosomal proteolysis occurring in most cell types in both physiological and pathological states [7]. Autophagic vacuoles are formed when portions of the cytoplasm, sometimes including organelles, are surrounded by a sequestering membrane principally derived from the endoplasmic reticulum. These double membrane-bound structures then fuse with lysosomes to form autolysosomes and acquire protein degradative capacity. Autophagy is an energy-requiring process that is responsible for an increase in proteolysis thus enabling an acute cellular response to change in energy availability or expenditure. Autophagy is also a predominant feature of programmed (active) cell death in highly synthetic or secretory cells (type II cell death) [8]. Type II cell death is morphologically distinct from apoptosis (type I cell death) which classically occurs in highly mitotically active cells and is characterised by predominantly nuclear morphological changes with little autophagy [8].

TNF effects on cells are mediated via two distinct cell-surface receptors of 55/60 kD (TNFR-I) and 75/80 kD (TNFR-II), which are ubiquitously expressed [9]. The receptors utilise both independent and shared intracellular signalling pathways to mediate a variety of effects on cells. Cytotoxic effects are, in the main, mediated by TNFR-I whilst TNFR-II has proliferative effects, at least in lymphoid cells. In this study we sought to a) investigate ultrastructural responses to TNF, b) determine which TNF receptor(s) mediate these changes, and c) determine if these changes invariably lead to cytotoxicity.

Murine 3T3-L1 and human preadipocytes (which undergo TNF-induced apoptosis), murine WEHI 164 cells (which undergo TNF-induced necrosis) and human umbilical vein endothelial cells (which are insensitive to TNF) were cultured +/- TNF or receptor-specific TNF muteins. Ultrastructural analysis was undertaken by electron microscopy, and apoptosis and necrosis assayed using acridine orange and/or annexin V.

In all of the cell types studied TNF induced autophagy (Fig 1) in addition to classical mitochondrial morphological changes (Fig 2).

Abbreviations used: TNF , tumour necrosis factor-α; TNFR-, tumour necrosis factor–α-receptor-.

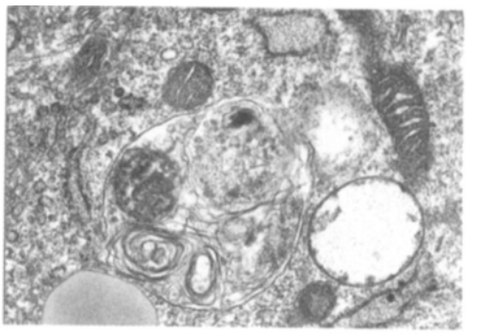

Figure 1. Autophagic vacuole in a TNF-treated preadipocyte

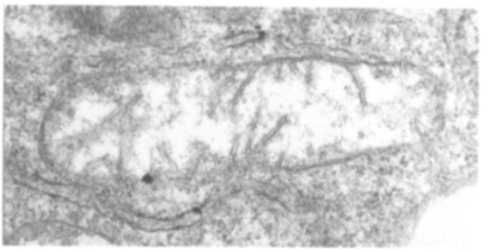

Figure 2. TNF-induced mitochondrial changes in a preadipocyte

Under control culture conditions (serum-containing medium) such changes were not seen. Culture in serum-free medium induced autophagy but not mitochondrial morphological abnormalities indicating that the two changes can result from independent signalling pathways. Specific ligation of TNFR-I and TNFR-II alone and in combination induced identical patterns of autophagy and alteration in mitochondrial morphology. TNF-induced mitochondrial morphological changes and autophagy were both observed as an isolated occurrence as well as in association with programmed cell death and necrosis.

Our results indicate that in addition to mitochondrial changes, autophagy forms part of the spectrum of cellular responses to TNF. Autophagy occurs in both cells that do and cells that do not undergo TNF-induced cytotoxicity and, along with mitochondrial morphological changes, can be mediated by either TNF receptor. Indeed whether a cell progresses to apoptosis or necrosis in response to TNF may depend on either the extent of mitochondrial damage and/or on the autophagic capacity of the cell.

1. Schulze-Osthoff, K, Bakker, AC, Vanhaesebroeck, B, Beyaert, R, Jacob WA and Fiers, W. (1992) J Biol Chem **267**: 5317-5323.
2. Lancaster, JRJ, Laster, SM and Gooding LR. (1989) FEBS Lett **248**: 169-174.
3. Goossens, V, Grooten, J, De Vos, K and Fiers, W. (1995) Proc Nat Acad Sci USA **92**: 8115-8119.
4. Marchetti, P, Castedo, M, Susin, SA, Zamzami, N, Hirsch, T, Macho, A, Haeffner, A, Hirsch, F, Geuskens, M and Kroemer G. (1996) J Exp Med **184**: 115501160.
5. Schulze-Osthoff, K, Beyaert, R, Vandevoorde, V, Haegeman, G and Fiers W. (1993) EMBO J **12**: 3095-3104.
6. Jia, L, Dourmashkin, RR, Allen, PD, Gray, AB, Newland, AC and Kelsey, SM. (1997) Br J Haematol **98**: 673-685.
7. Seglen, PO and Bohley, P. (1992) Experientia **48**: 158-172.
8. Zakeri, Z, Bursch, W, Tenniswood, M and Lockshin RA. (1995) Cell Death Differ **2**: 87-96.
9. Vandenabeele, P, Declercq, W, Beyaert, R and Fiers, W. (1995) Trends Cell Biol **5**: 392-399.