TNF-α is involved in activating DNA fragmentation in skeletal muscle

N Carbó¹, S Busquets¹, M van Royen¹, B Alvarez¹, FJ López-Soriano¹ and JM Argilés*¹
¹Cancer Research Group, Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028-Barcelona, Spain

Intraperitoneal administration of 100 μg kg⁻¹ (body weight) of tumour necrosis factor-α to rats for 8 consecutive days resulted in a significant decrease in protein content, which was concomitant with a reduction in DNA content. Interestingly, the protein/DNA ratio was unchanged in the skeletal muscle of the tumour necrosis factor-α-treated animals as compared with the non-treated controls. Analysis of muscle DNA fragmentation clearly showed enhanced laddering in the skeletal muscle of tumour necrosis factor-α-treated animals, suggesting an apoptotic phenomenon. In a different set of experiments, mice bearing a cachexia-inducing tumour (the Lewis lung carcinoma) showed an increase in muscle DNA fragmentation (9.8-fold) as compared with their non-tumour-bearing control counterparts as previously described. When gene-deficient mice for tumour necrosis factor-α receptor protein I were inoculated with Lewis lung carcinoma, they were also affected by DNA fragmentation; however the increase was only 2.1-fold. These results suggest that tumour necrosis factor-α partly mediates DNA fragmentation during experimental cancer-associated cachexia.

British Journal of Cancer (2002) 86, 1012–1016. DOI: 10.1038/sj/bjc/6600167 www.bjcancer.com

Keywords: DNA fragmentation; skeletal muscle; TNF-α; cancer cachexia; apoptosis

Tumour necrosis factor-α (TNF) is a cytokine synthesised and released by blood monocytes and tissue macrophages in response to invasive stimuli, which exerts diverse metabolic effects (see Evans et al, 1989; Argilés et al, 1997 for a review). Although a large body of evidence suggests that this cytokine participates in the protein wasting and loss of nitrogen associated with cachectic situations (Argilés et al, 1997; Argilés and López-Soriano, 1999), the mechanisms underlying such actions still remain obscure. Both in man and mouse, TNF binds as homotrimer to two kinds of receptors, TNFR1 (p55) and TNFR2 (p75) (Hohman et al, 1989); in addition, lymphotoxin-α (LTα or TNF-β) also binds to the same receptors. The pleiotropic functions of TNF can be partially explained by the presence of its receptors in almost all types of nucleated cells. The expression of the genes encoding the two receptors is differentially regulated in different cells. TNFR1 is expressed constitutively and plays a central role in many biological processes, whereas less is known about TNFR2 expression (see Vandenaeele et al, 1995 for a review).

Cancer cachexia is perhaps the most common manifestation of advanced malignant disease. Cachexia occurs in the majority of cancer patients before death, and is responsible for the death of 22% of these patients (Warren, 1932), although lower percentages have been considered in more recent publications (Dworzak et al, 1998). The abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss and atrophy, anaemia and alterations in metabolism (see Argilés et al, 1997, for review). The degree of cachexia is inversely correlated with the survival time of the patient and it always implies a poor prognosis (Harvey et al, 1979; Nixon et al, 1980; De Wys, 1985). Perhaps asthenia is one of the most relevant characteristics of cachexia, reflecting the extensive muscle waste that takes place in the cachectic cancer patient (Argilés et al, 1992), and is also characterised by a general weakness as well as physical and mental fatigue (Adams and Victor, 1981). Actually, body protein depletion is one of the main trends of cachexia and it involves not only skeletal muscle but it also affects cardiac proteins, resulting in alterations in heart performance (Drott et al, 1986).

Apoptosis, the programmed type of cell death, is an important physiological process in the development and homeostasis of multicellular organisms. Apoptotic cell death is characterised by a common pattern of morphological alterations such as chromatin condensation, membrane blebbing, DNA fragmentation and cell shrinkage (Huppertz et al, 1999). In cardiac muscle, apoptosis has been recognised as a component of many common pathologies including chronic heart failure, cardiac sudden death, viral myocarditis and ischaemia (Tanaka et al, 1994; Itoh et al, 1996; Kajstura et al, 1996). Moreover, during chronic heart failure, rat skeletal muscle atrophy has been related to apoptosis (Dalla Libera et al, 1999). Indeed, apoptosis has already been described associated with skeletal muscle atrophy (Allen et al, 1997; Tews and Goebel, 1997; Tews et al, 1997) and other diseases (see Sandri and Carrasso, 1999, for review).

During cancer cachexia, the activation of the ubiquitin-dependen
t proteolytic pathway seems to be responsible for the muscle protein mobilisation (Argilés and López-Soriano, 1996). Recently, a link between the apoptosome and the proteasome pathway has been described (Dimmeler et al, 1999). In addition, we have demonstrated that during experimental cancer cachexia, DNA fragmentation is increased in skeletal muscle (Van Royen et al, 2000). Therefore, it was the aim of the present investigation to examine if cytokines, TNF in particular, synthetised during the evolution of the tumour-induced cachectic process, were involved in the changes in skeletal muscle DNA content and integrity.
MATERIALS AND METHODS

Animals, tumour inoculation and TNF treatment

Male C57BL/6 mice (Criffà, Barcelona, Spain) weighing about 25 g were used. Mice were divided into two groups, namely controls and tumour hosts. The latter received an intramuscular (left thigh) inoculum of $5 \times 10^5$ Lewis lung carcinoma cells obtained from exponential tumours. The development of a nodule at the side of the injection, growing in size up to almost 20% of the animal weight in 2 weeks, was considered as an index of effectiveness of the inoculation. Nearly 100% of the injected animals developed hind-leg tumours, and all of them were affected by lung metastasis from day 7 onwards. On day 15 after tumour transplantation, animals were weighed and anaesthetised with ketamine/xylacine (Imalgene and Rompun respectively).

Concerning TNFRI-deficient mice, homozygous mice for a disrupted Tnfr I allele (Tnfr I/) were used. The gene targeting vector consisted of a genomic mouse DNA fragment (Rothe et al., 1993), in which exons 2 and 3 and part of exon 4 of the Tnfr gene were replaced by a neo cassette. This deletion disrupts the gene and removes the coding information for the cysteine-rich domains I and II of the receptor, which have been shown to be essential for ligand binding (Banner et al., 1993). Germ-line transmitters of the mutated Tnfr I allele were crossed with C57BL/6 mice and the resulting heterozygous mice interbred to yield homozygous mutant offspring. The F1 generation displayed the expected mendelian ratio 1:2:1 of wild-type (+/+), heterozygous (0/+ and 0/0) mutant mice, indicating that Tnfr I expression is not required for normal embryonic development.

In the experiments involving chronic TNF treatment, female Wistar rats (Interfauna Iberica) weighing 100–150 g were used. TNF was given intraperitoneally for 8 days at a dose of 100 μg kg$^{-1}$ per day (two administrations at 08:00 and 20:00 h). Control animals received 0.5 ml of vehicle (physiological saline). Human recombinant-derived TNF-α (specific activity $8.1 \times 10^7$ U mg$^{-1}$ protein, purity >99% containing less than 0.137 ng mg$^{-1}$ endotoxin) was generously given by BASEF/Knoll AG (Ludwigshafen, Germany).

All animals were maintained on a regular light-dark cycle (light on from 08:00 to 20:00 h) and had free access to food and water. The diet (BK Universal GJ/SL, Sant Vicenc del Horts, Barcelona, Spain) consisted of 45.5–48.5% carbohydrate (3.5% absorbible glucose, 43–5% starch), 18.5% protein and 3.1% fat (the residue being non-digestible material).

All animal experiments have been carried out with ethical committee approval. The ethical guidelines that were followed meet the standards required by the UKCCCR guidelines (Workman et al., 1998).

Biochemicals

They were all reagent grade and obtained either from Roche (Barcelona, Spain) or from Sigma Chemical Co. (St Louis, MO, USA).

DNA and protein content

Samples of skeletal muscles were homogenised in an ammonium hydroxide/Triton X-100 extraction buffer (supplemented with protease inhibitors) and used for the determination of both protein (Bradford, 1976) and DNA (Downs and Wilfinger, 1983) content.

DNA fragmentation assay

Gastrocnemius muscles were homogenised and incubated at 48°C overnight in Kauffmann buffer (0.5 M TRIS, 2 mM EDTA, 10 mM NaCl, 1% SDS) in the presence of 200 μg ml$^{-1}$ of Proteinase K, and DNA was extracted with phenol/chloroform. After ethanol precipitation, the pellets were resuspended and the DNA integrity was checked in a 2% agarose gel electrophoresis and ethidium bromide staining. The percentage of DNA fragmentation was quantified by scanning densitometry. Liver from 8-h anti-Fas antibody-treated mice (Ogasawara et al., 1993) was used as a positive control of DNA fragmentation.

Plasma TNF levels

Circulating TNF was evaluated by a murine immunoassay (Genzyme, Cambridge, MA, USA).

Statistical analysis

Statistical analysis of the data was performed by means of the Student’s $t$-test.

RESULTS

As can be seen in Table 1, chronic administration of TNF to rats for 8 consecutive days results in a decrease (21%) in skeletal muscle protein content (soleus). The cytokine also induces a similar decrease in muscle DNA content although the results do not reach statistical significance. Interestingly, the protein/DNA ratio is unchanged as a result of TNF treatment. At this point, it seemed that DNA was probably being degraded at a faster rate as a consequence of TNF treatment. As can be seen in Figure 1, DNA fragmentation is clearly induced by the cytokine; indeed it caused an increase of 4.6-fold over the basal fragmentation observed in

| Table 1: Effect of chronic TNF treatment on protein and DNA content in rat soleus muscles |
|--------------------------------------|------------------|------------------|
| Experimental group                  | DNA (mg (100 g ibw)$^{-1}$) | Protein (mg (100 g ibw)$^{-1}$) | Protein/DNA |
| Control                             | 0.341 ± 0.029    | 108.0 ± 6.7      | 317 ± 20   |
| TNF treatment                       | 0.268 ± 0.047    | 85.0 ± 4.1*      | 317 ± 15   |

The results are mean values ± s.e.m. for five animals in each group. Significant difference of the differences (Student’s $t$-test). *$P<0.05$; ibw: initial body weight.

![Figure 1: DNA laddering in rats chronically treated with hTNF-α (100 μg kg$^{-1}$ body weight/day). Lane 1: DNA molecular weight marker; lanes 2–5: 40 μg of gastrocnemius muscle DNA from control (lanes 2 and 3) and TNF-treated (lanes 4 and 5) rats respectively; lane 6: 20 μg of liver DNA from anti-Fas-treated mice (positive control). The percentage of DNA fragmentation was quantified by scanning densitometry.](image-url)
the control animals. These data agree with our previous report (Van Royen et al, 2000) demonstrating that during experimental cancer cachexia, DNA fragmentation was an important event in skeletal muscle. For this reason, we decided to investigate if TNF was involved in this apoptotic event, since tumour-bearing animals generally show high circulating levels of the cytokine (Costelli et al, 1993). Bearing all this in mind, in the following experiments we used gene-deficient mice for TNFRI protein (TNFRI KO). As can be seen in Figure 2, tumour growth induced an important decrease in gastrocnemius weight (26%) in wild-type animals. In the gene-deficient mice, however, tumour burden only caused a 13% decrease in gastrocnemius weight (Figure 2). Interestingly, control non-tumour-bearing gene-deficient mice have a significantly smaller muscle mass (21%) as compared with the control wild-type mice (Figure 2). Similarly, gastrocnemius total protein content was clearly decreased (29%) by tumour burden in wild-type mice as well as in the TNFRI KO mice (18%) (Table 2). The DNA content was also decreased as a result of tumour growth in the wild-type (39%) and in the TNFRI KO mice (30%) (Table 2). Interestingly, the protein/DNA ratio was unchanged in the wild-type mice as a result of tumour burden whereas in the gene-deficient mice tumour growth resulted in a significant increase in this ratio (Table 2).

Figure 3 shows the result of the DNA fragmentation analysis. The Lewis lung carcinoma induced a marked increase in DNA fragmentation in wild-type mice (9.8-fold). The tumour also induced an increase in DNA fragmentation in the gene-deficient mice but this was much more modest (2.1-fold). Interestingly, control gene knockout mice have a higher rate of DNA fragmentation (3.9-fold) than that observed in the control wild-type mice (Figure 3). The circulating concentrations of TNF are shown in Table 3. It can be seen that tumour growth results in an increase in circulating cytokine both in wild-type (91%) and gene-deficient (178%) animals.

**DISCUSSION**

DNA fragmentation is a common feature of apoptotic cell death and we have previously suggested that the muscle wasting that accompanies cancer cachexia could be linked to an apoptotic phenomenon by which muscle cells lose not only protein but also DNA (Van Royen et al, 2000). Apoptosis has already been described in human (Tews and Goebel, 1997; Tews et al, 1997) and rat (Dalla Libera et al, 1999) atrophic muscle as well as in insect muscle (Schwartz et al, 1993). In patients with malignant tumours, anorexia, weight loss, emaciation and progressive alterations of vital functions are common features associated with cancer cachexia (De Wys, 1985). Although in some cases anorexia, gastrointestinal obstruction or malabsorption are responsible for the weight loss of cachectic patients (Balducci and Hardy, 1985), it cannot be wholly attributed to these causes and therefore it has been postulated to be due to a decrease in the energetic efficiency of the cancer patient. Among the factors involved in decreasing the energetic efficiency, skeletal muscle protein turnover seems to have a very significant role as we have previously shown (see Argilés et al, 1997, for review). In addition, apoptosis also seems to be present in cachetic muscle in different experimental tumour models (Van Royen et al, 2000). The basic aim of the

The results are mean values ± s.e.m. for four animals in each group. Statistical significance of the differences (Student’s t-test): *P<0.05, **P<0.01, ***P<0.001 (vs wild-type); ###P<0.001 (vs TNFRI KO). LLC: Lewis lung carcinoma; bw: initial body weight.

| Experimental group | DNA (mg 100 g bw⁻¹) | Protein (mg 100 g bw⁻¹) | Protein/DNA |
|--------------------|---------------------|-------------------------|-------------|
| Wild-type          | 0.578 ± 0.052       | 13.99 ± 7.0             | 259 ± 10    |
| Wild-type + LLC    | 0.350 ± 0.011**     | 99.2 ± 1.3***           | 286 ± 12    |
| TNFRI KO           | 0.588 ± 0.031       | 112.0 ± 3.5*            | 187 ± 6**   |
| TNFRI KO + LLC     | 0.411 ± 0.017###    | 92.2 ± 3.3##            | 229 ± 8###  |

| Experimental group | TNF (pg ml⁻¹) |
|--------------------|---------------|
| Wild-type          | 22 ± 7        |
| Wild-type + LLC    | 42 ± 2*       |
| TNFRI KO           | 18 ± 3        |
| TNFRI KO + LLC     | 50 ± 7**      |

TNF was determined by ELISA. The results are mean values ± s.e.m. for five animals in each group. Statistical significance of the differences (Student’s t-test): *P<0.05, **P<0.01 (vs wild-type).
present investigation was to see if the changes that occur in DNA in skeletal muscle during experimental cancer cachexia are linked to TNF. To test this hypothesis we have used two different experimental approximations: chronic TNF administration to healthy rats and experimental cancer cachexia (induced by the Lewis lung carcinoma in mice) in gene-deficient mice for TNFRI.

Different mediators have been suggested to account for cancer-induced cachexia, but basically the presence of both tumoural and humoral (mainly cytokines, TNF in particular) compounds is associated with depletion of fat stores as well as of muscular tissues (Argilés and López-Soriano, 1999). In fact, the balance between pro-inflammatory cytokines, their soluble receptors and the anti-inflammatory cytokines plays a key role in the development of the cachectic syndrome (Argilés and López-Soriano, 1998). Our research group has demonstrated that TNF is involved in the activation of the ubiquitin-dependent proteolysis that takes place during tumour growth (García-Martínez et al., 1994; Llovera et al., 1996, 1998). We clearly show here that TNF is also involved in triggering DNA fragmentation in muscle during cancer cachexia, mainly through the TNFRI. Indeed, chronic administration of recombinant human TNF, which can only bind rat TNFRI, clearly induces DNA fragmentation, and the use of a tumour model (where the levels of circulating TNF are highly increased) confirm this fact.

Indeed, the Lewis lung carcinoma is a cachectic tumour that induces an important decrease in body weight without significant changes in food intake, at least in the first two weeks of tumour growth (Llovera et al., 1998). Because TNFRI is absent from the cells of these animals, the data obtained here suggest that TNF can be involved in the muscle apoptotic mechanisms triggered by tumour growth through its binding with the TNFRI. However, TNFRI is not the sole receptor responsible for transduction of the death signal, even though it is the most important one. Under certain circumstances, TNFRII also either enhances the TNFRI death signal or, indeed, mediates death independently (Declercq et al., 1998; Haridas et al., 1998; Weiss et al., 1998). The mechanism of the TNFRII death signal has not been characterised. The results clearly show that in the gene-deficient mice apoptosis is not induced by tumour growth to the same extent as in the wild-type animals. In fact, TNF has been shown to trigger apoptosis in many cell types (Obeid et al., 1993; Ohta et al., 1994; Sidioti-de-Fraise et al., 1998) including cardiac muscle (Krown et al., 1996). In addition, a possible link between TNF and apoptosis has already been reported in ‘fast’ skeletal muscles in chronic heart failure (Dalla Libera et al., 1999). Interestingly high circulating levels of TNF are detectable in both rat (Costelli et al., 1993) and mouse (Llovera et al., 1998) tumour models used in this study.

Furthermore, TNF-treatment induces Bcl-2 dephosphorylation, targeting this anti-apoptotic protein for degradation by the ubiquitin proteolytic system (Dimmeler et al., 1999). Thus, TNF-induced apoptosis could be mediated by different cellular responses (Liu et al., 1996), which include the activation of TNFRII death domain and, as a consequence, the caspase cascade and amplification of this apoptotic pathway by means of ubiquitin-dependent Bcl-2 degradation. Nevertheless, TNF binding to its receptors also induces cell proliferation and survival signals mediated by Bcl-2 activation of NF-κB (Liu et al., 1996; Wang et al., 1996). Therefore, cell survival depends on a delicate balance between the different TNF signalling pathways. For this reason, future investigations in our laboratory will concentrate on ascertaining the role of this and other cytokines in the activation of the apoptotic process associated with cancer cachexia in skeletal muscle.

REFERENCES

Adams R, Victor M (1981) Asthenia. In Principles of Neurology Adams R, Victor M, (eds) pp 341 – 345 New York: McGraw-Hill
Allen DL, Linderman JK, Roy RR, Bigbee AJ, Grindeland RE, Mukku V, et al (2002) Cancer Research UK British Journal of Cancer (2002)
Adams R, Victor M (1981) Asthenia. In Principles of Neurology Adams R, Victor M, (eds) pp 341 – 345 New York: McGraw-Hill
Allen DL, Linderman JK, Roy RR, Bigbee AJ, Grindeland RE, Mukku V, et al (2002) Cancer Research UK British Journal of Cancer (2002)

ACKNOWLEDGEMENTS

Work supported by grants from the Fondo de Investigaciones Sanitarias de la Seguridad Social (00/1116) of the Spanish Health Ministry, and the Dirección General de Investigación Científica y Técnica (PM98-0199) from the Spanish Ministry of Education and Science.

TNF-α and apoptosis in skeletal muscle
N Carbó et al

Dalla Libera L, Zennaro R, Sandri M, Ambrosio GB, Vescovo G (1999) Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. Am J Physiol 277: C982 – C986
De Wys WD (1985) Management of cancer cachexia. Semin Oncol 12: 452 – 460
Declercq W, Denecker G, Fiers W, Vandenaeyelee P (1998) Cooperation of both TNF receptors in inducing apoptosis: involvement of the TNF receptor-associated factor binding domain of the TNF receptor 75. J Immunol 161: 390 – 399
Dimmeler S, Breitschopf K, Haendeker J, Zeiher AM (1999) Dephosphorylation targets Bcl-2 for ubiquitin-dependent degradation: a link between the apoptosome and the proteasome pathway. J Exp Med 189: 1815 – 1822
Downs TR, Wilfinger WW (1983) Fluorometric quantification of DNA in cells and tissue. Anal Biochem 131: 538 – 547
Drott C, Ekman L, Holm S, Waldenstrom A, Lundholm K (1986) Effects of tumor-load and malnutrition on myocardial function in the isolated working rat heart. J Mol Cell Cardiol 18: 1165 – 1176
Dzworzak F, Ferrari P, Gavazzi C, Maiorana C, Bozzetti F (1998) Effects of cachexia due to cancer on whole body and skeletal muscle protein turnover. Cancer 82: 42 – 48
Evans RD, Argilés JM, Williamson DH (1989) Metabolic effects of tumour necrosis factor-α (cachectin) and interleukin-1. Clin Sci 77: 357 – 364
García-Martínez C, Llovera M, Argilés JM, López-Soriano FJ (1994) Ubiquitin gene expression in skeletal muscle is increased by tumour necrosis factor-α. Biochem Biophys Res Commun 201: 682 – 686
Haridas V, Darnay BG, Natarajan K, Heller R, Aggarwal BB (1998) Overexpression of the p80 TNF receptor leads to TNF-dependent apoptosis, nuclear factor-kappa B activation, and c-Jun kinase activation. J Immunol 160: 3152 – 3162

© 2002 Cancer Research UK
British Journal of Cancer (2002) 86(6), 1012 – 1016
Experimental Therapeutics
Harvey KB, Bothe A, Blackburn GL (1979) Nutritional assessment and patient outcome during oncological therapy. *Cancer 43*: 2065–2069

Hohman HP, Remy R, Brockhaus M, van Loon APMG (1989) Two different cell types have different major receptors for human tumor necrosis factor (TNFα). *J Biol Chem* **264**: 14927–14934

Huppertz B, Frank HG, Kaufmann P (1999) The apoptosis cascade: morphological and immunohistochemical methods for its visualization. *Anat Embryol* **200**: 1–18

Itoh G, Iie T, Tamura J, Suzuki M, Suzuki Y, Ikeda M, Nomura M (1996) Apoptosis and human ischemic myocardial damage, including conduction system. *Basic Appl Myol* **6**: 237–240

Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P (1996) Apoptotic and necrotic cell death are independent contributing variables of infarct size in rats. *Lab Invest* **74**: 86–107

Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, Glembotski CC, Quintana PJE, Sabbadin RA (1996) Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. *J Clin Invest* **98**: 2854–2865

Liu ZG, Hsu H, Goeddel DV, Karin M (1996) Dissection of TNF receptor-1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. *Cell* **87**: 565–576

Lloversa M, Carbó N, Garcia-Martínez C, Costell P, Tessitore L, Baccino FM, Agell N, Bagby GJ, López-Soriano FJ, Argilés JM (1996) Anti-TNF treatment reverts increased muscle ubiquitin gene expression in tumor-bearing rats. *Biochem Biophys Res Commun* **221**: 653–659

Lloversa M, Carbó N, Garcia-Martínez C, Costell P, Tessitore L, Baccino FM, Agell N, Bagby GJ, López-Soriano FJ, López-Soriano FJ, Argilés JM (1998) Role of TNF receptor 1 in protein turnover during cancer cachexia using gene knockout mice. *Mol Cell Endocrinol* **142**: 183–189

Nixon DW, Heymsfield SB, Kutner MM, Ansley J, Lawson DH (1980) Protein-caloric undernutrition in hospitalized cancer patients. *Am J Med* **68**: 491–497

Obeid LM, Linardic CM, Karolak LA, Hannun YA (1993) Programmed cell death induced by ceramide. *Science* **259**: 1769–1771

Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nakata S (1993) Lethal effect of the anti-Fas antibody in mice. *Nature* **364**: 806–809

Ohita H, Yalamy I, Sweeney EA, Hakomori SI, Igarashi Y (1994) A possible role of sphingosin in induction of apoptosis by tumour necrosis factor-α in human neutrophils. *FEBS Lett* **355**: 267–270

Rothe J, Lesslauer W, Lötscher H, Lang Y, Koebel P, Köntgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H (1993) Mice lacking the tumour necrosis factor receptor are resistant to TNF-mediated toxicity but susceptible to infection by Listeria monocytogenes. *Nature* **364**: 798–802

Sandri M, Carraro U (1999) Apoptosis of skeletal muscles during development and disease. *Int J Biochem Cell Biol* **31**: 1373–1390

Schwartz LM, Jones ME, Kosz L, Kух K (1993) Selective repression of actin and myosin heavy chain expression during the programmed cell death of insect skeletal muscle. *Dev Biol* **158**: 448–455

Sidoti-de-Fraisse C, Rincheval V, Risler Y, Mignotte B, Vayssière JL (1998) TNF-α activates at least two apoptotic signaling cascades. *Oncogene* **17**: 1639–1651

Tanaka M, Ito H, Adachi S, Akimoto H, Hishikawa T, Kasaijima T, Marumo F, Hiroe M (1994) Hypoxia induces apoptosis with enhanced expression of fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. *Circ Res* **73**: 426–433

Tews DS, Goebel HH (1997) Apoptosis-related proteins in skeletal muscle fibers of spinal muscular atrophy. *J Neuropathol Exp Neurol* **56**: 150–156

Tews DS, Goebel HH, Meinck HM (1997) DNA fragmentation and apoptosis-related proteins of muscle cells in motor neuron disorders. *Acta Neurol Scand* **96**: 380–386

Van Royen M, Carbó N, Busquets S, Alvarez B, Quinn LS, López-Soriano FJ, Argilés JM (2000) DNA fragmentation occurs in skeletal muscle during tumor growth: a link with cancer cachexia? *Biochem Biophys Res Commun* **276**: 533–537

Vandenabeele P, Declercq W, Beyaert R, Fiers W (1995) Two tumor necrosis factor receptors: structure and functions. *Trends Cell Biol* **5**: 392–399

Wang CI, Mayo MW, Baldwin Jr AS (1996) TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. *Science* **274**: 784–787

Warren S (1932) The immediate cause of death in cancer. *Am J Med Sci* **184**: 610–613

Weiss T, Grell M, Siemienski K, Muhlenbeck F, Durkop H, Pfizenmaier K, Scheurich P, Wajant H (1998) TNFR80-dependent enhancement of TNFR80-induced cell death is mediated by TNFR-associate factor 2 and is specific for TNFR80. *J Immunol* **161**: 3136–3142

Workman P, Balmain A, Hickman JA, McNally NJ, Rohas AM, Mitchison NA, Pierrepoint CG, Raymond R, Rowlatt C, Stephens TC, Wallace J (1998) UKCCCR guidelines for the welfare of animals in experimental neoplasia (second edition). *Br J Cancer* **77**: 1–10