TNF autovaccination induces self anti-TNF antibodies and inhibits metastasis in a murine melanoma model

AM Waterston*,1, F Salway2, E Andreakos2, DM Butler2, M Feldmann2 and RC Coombes1

1Department of Cancer Medicine, Faculty of Medicine, Chelsea and Westminster Hospital, 369 Fulham Rd, Imperial college School of Medicine, London SW10 9NH, UK; 2Kennedy Institute of Rheumatology Division, Faculty of Medicine, Imperial college School of Medicine, London, UK

Keywords: TNF; melanoma; metastasis

TNF is a proinflammatory cytokine involved in the pathogenesis of chronic inflammatory diseases, but also in metastasis in certain types of cancer. In terms of therapy, TNF is targeted by anti-TNF neutralising monoclonal antibodies or soluble TNF receptors. Recently, a novel strategy based on the generation of self anti-TNF antibodies (TNF autovaccination) has been developed. We have previously shown that TNF autovaccination successfully generates high anti-TNF antibody titres, blocks TNF and ameliorates collagen-induced arthritis in DBA/1 mice. In this study, we examined the ability of TNF autovaccination to generate anti-TNF antibody titres and block metastasis in the murine B16F10 melanoma model. We found that immunisation of C57BL/6 mice with TNF autovaccine produces a 100-fold antibody response to TNF compared to immunisation with phosphate-buffered saline vehicle control and significantly reduces both the number (P < 0.01) and size of metastases (P < 0.01) of B16F10 melanoma cells. This effect is also observed when an anti-TNF neutralising monoclonal antibody is administered, confirming the essential role TNF plays in metastasis in this model. This study suggests that TNF autovaccination is a cheaper and highly efficient alternative that can block TNF and reduce metastasis in vivo and trials with TNF autovaccination are already underway in patients with metastatic cancer.

British Journal of Cancer (2004) 90, 1279–1284. doi:10.1038/sj.bjc.6601670 www.bjcancer.com

© 2004 Cancer Research UK

Keywords: TNF; melanoma; metastasis

TNF is an important proinflammatory cytokine involved in normal physiological immune and inflammatory processes. However, when inappropriately expressed, TNF also plays a role in the development of chronic inflammation and diseases associated with it (Andreakos et al, 2002). More recently, inappropriately expressed TNF was also shown to play a role in the development of cancer but in a more complex manner. Thus, in certain cancers TNF has been shown to induce haemorrhagic necrosis of tumours, whereas in others it has been shown to promote cancer (Carswell et al, 1975; Moore et al, 1999). What determines the TNF procarcinogenic or anticarcinogenic effects is not clear. It may be that this is dependent on the levels of TNF produced locally or the type of tumour and cancer involved (Balkwill, 2002).

Previous studies have suggested that one of the main mechanisms by which TNF promotes tumour growth is by upregulating metastasis. TNF activates key molecules involved in metastasis such as IL-8 (an angiogenic chemokine) and Groz/KC, as well as matrix metalloproteinases (MMP) and urokinase-plasminogen activator, molecules involved in ECM degradation and cellular migration (Wu et al, 1999; Shin et al, 2000). In addition, recombinant TNF injected into mice inoculated with a methylcholanthrene-induced fibrosarcoma increased the number of lung metastases (Orozs et al, 1993). Similarly, tumour cell lines infected with a retrovirus carrying the TNF gene augmented metastatic activity of the tumour (Quin et al, 1993). In contrast, blocking TNF using the human p55-IgG fusion protein in a murine B16-BL6 melanoma model reduced the number of metastatic lung tumours temporarily over 2 weeks but not after 3 weeks, possibly due to increased immune clearance of the fusion protein (Cubillos et al, 1997).

Monoclonal antibodies and IgG fusion proteins are now an established approach to blocking TNF. We have previously used monoclonal antibodies against TNF to ameliorate disease in animal models of arthritis (Williams et al, 1992) as well as rheumatoid arthritis in the clinic (Elliot et al, 1994). Similarly, others have shown that administration of anti-TNF neutralising antibodies is successful in treating many other diseases such as Crohn’s disease, psoriasis and spondyloarthropathies (reviewed by Andreakos et al, 2002). However, there are major drawbacks to the long-term use of monoclonal antibodies, murine, chimeric or human, which include a variable degree of immunogenicity and major costs, $12–15 000 p.a. (£7–9000) (Dillman et al, 1994; LoRusso et al, 1995; Philpott et al, 1995). TNF autovaccination is an alternative, novel approach that circumvents the problem of immunogenicity and cost of nonautologous antibodies. It is based on the generation of autologous human autoantibodies by immunisation with a self-antigen to which a T-cell epitope has been added (Dalum et al, 1997). Autoantibodies against TNF are raised by immunisation with recombinant TNF protein containing inserted hen egg lysozyme (HEL) or ova albumin (OVA) epitopes. This leads to a T helper response specific for the foreign parts of the recombinant molecule and allows the generation of antibodies against TNF that neutralise and eliminate it (Dalum et al, 1999).
When we previously used TNF autovaccination in a murine collagen-induced arthritis model, we found that this resulted in the generation of high titres of anti-TNF antibodies that ameliorated the disease and decreased the severity of arthritis (Dalum et al., 1999).

In this study, we examined whether blockade of TNF could reduce metastasis in the murine B16F10 melanoma model (Poste et al., 1980) and compared the efficacy of anti-TNF monoclonal antibodies and the TNF autovaccination technology in reducing metastasis. Our results indicate that both the conventional anti-TNF monoclonal antibody treatment and TNF autovaccination are successful in reducing the number and size of lung metastases in this model and supports the use of these agents in clinical trials in patients with metastatic cancer.

MATERIALS AND METHODS

Immunisation with TNF autovaccination

The immunisation regime was first developed in 4- to 5-week-old male DBA/1 mice as described in our previous study in collagen-induced arthritis (Dalum et al., 1999). C57BL/6 mice (4-week old) (12/group) were immunised intradermally at the base of the tail with 100 μg of TNF autovaccine in phosphate-buffered saline (PBS) emulsified with complete Freund’s adjuvant (CFA), in a volume of 100 μl (Figure 1). Three subsequent boosts with TNF autovaccine in incomplete Freund’s adjuvant (IFA) were performed at two-weekly intervals. In the immunisation study, the control used was HEL in CFA and three boosters in IFA and the blood samples were taken prior to study commencement and at two-weekly intervals thereafter, for 12 weeks. For the B16F10 metastasis model, mice were immunised with PBS in CFA/IFA as a control. After 3 weeks, the mice were killed, lung tumours counted, and the diameter measured in two perpendicular planes. To reduce biased selection in the B16F10 melanoma metastasis model, the treatments were blinded coding them A and B prior to injection and the codes broken at the end of the experiment after the data were collected and analysed.

Blood samples were taken prior to immunisation and prior to tumour insertion. On killing the animals at the end of the experiments, a terminal bleed was also undertaken. The blood was allowed to clot and then centrifuged at 12 000 r.p.m. for 5 min and the top layer of sera was removed. The serum was stored at −20°C prior to use in immunological assays to determine antibody levels. The experiments were carried out according to Protocol 8 on License PPL 70 3031, issued by The Home Office Animal Procedures Section, London and adhered to the UKCCR guidelines (Workman et al., 1998).

Treatment with Anti-TNF monoclonal antibodies

C57BL/6 mice (6-week old) were injected intraperitoneally (i.p.) with 500 μg 100 μl−1 of anti-TNF monoclonal antibodies (CV14q) three times per week or PBS. Again to reduce biased selection, the treatments were blinded coding them A and B prior to insertion into the mice and the code was broken after the data were collected and analysed. The monoclonal antibodies were a rat mouse fusion protein (gift from Dr DB Scallon, Centocor, Malvern, USA). The day after the initial injection, 105 B16F10 murine melanoma cells in 200 μl of PBS were injected into the tail vein of each mouse. After 3 weeks, the mice were killed and the lung tumours counted and the diameter measured as described above.

Antibody detection assays

Microtitre plates were coated with 1 μg ml−1 of TNF in PBS at 100 μl well−1 and incubated overnight at 4°C. The plates were blocked with 2% bovine serum albumin (BSA) in PBS (200 μl well−1) for 1 h at 25°C and washed with 0.5% Tween in PBS after this and all subsequent steps. A measure of 100 μl of one in three serial dilutions of serum samples and the positive and negative control sera were incubated for 1 h at 25°C. The detection antibody (100 μl well−1) was diluted in 0.5% BSA in PBS and incubated for 1 h at 25°C. A sheep anti-mouse polyclonal detection antibody conjugated to horseradish peroxidase was used. These samples were developed with a peroxidase substrate system TMB, the reaction was stopped with 1 M H2SO4 and absorbance read at 450 nm. Since standard known amounts of antibodies to these antigens were unavailable, eight serial dilutions of sample sera were made and their titres taken as the dilution that gave an OD corresponding to that of the negative control sera. The negative control sera were from unimmunised mice and the positive control sera were pooled sera from previously immunised mice that had been tested by ELISA and found to have antibodies that bound TNF.

Cell culture and detection of KC

B16F10 murine melanoma cells were grown at 37°C in 5% CO2 in RPMI supplemented with 10% foetal bovine serum (BioWhittaker), penicillin (100 U ml−1) and streptomycin (100 μg ml−1). B16F10 cells were plated in 96-well plates at 2 × 104 cells ml−1 and 200 μl well−1. The cells were stimulated with LPS at 10 μg ml−1; some cells were further stimulated with murine TNF (mTNF) at 10 or 100 ng ml−1 and other cells with media as a control. The plates were incubated 37°C. Supernatants were removed at 24 and 72 h and stored at −20°C for immunological detection assays. KC was measured by ELISA as described previously (Sarawar et al., 2002). Detection range for the KC was 10 000–4.5 ng ml−1.
RESULTS

TNF autovaccination induces high levels of anti-TNF autoantibody production

We investigated the ability of the novel TNF autovaccination to induce high anti-TNF antibody titres. We immunised C57BL/6 mice with the TNF autovaccine in the presence of CFA/IFA and compared the antibody responses to mice immunised with HEL in CFA/IFA. We found that in the TNF autovaccination group, the anti-TNF antibody titres started rising at week 4 peaked at week 8 and reached plateau levels thereafter (Figure 2). This was compared to the HEL control group that had no anti-TNF antibody response. This was similar to the antibody response seen in BALB/c and CH3 mice (Dalum et al., 1999), but different from the biphasic antibody response seen at 6 and 10 weeks in DBA/1 mice after immunisation with TNF autovaccine (data not shown).

Anti-TNF monoclonal antibodies on lung metastases in the B16F10 melanoma model

First, to confirm the role of TNF in metastases in the B16F10 melanoma model, we used an anti-TNF neutralising antibody, CV1q. Mice were treated i.p. with either PBS or anti-TNF monoclonal antibodies (CV1q), and then B16F10 melanoma cells were injected into the tail vein. We found that mice treated with anti-TNF monoclonal antibodies had significantly less and smaller metastases compared to the mice treated with PBS (Figure 3A and B).

TNF blockade through immunisation using a novel TNF autovaccine on lung metastases in vivo in the B16F10 melanoma model

Having shown that blocking TNF can have an effect on metastasis, we examined the effect of immunisation with TNF autovaccine in the B16F10 melanoma model in three independent experiments. Mice were immunised with TNF autovaccine or PBS in CFA and boosted with IFA. The B16F10 melanoma cells were injected into the tail vein of each mouse 4 weeks after their first immunisation and the tumours were allowed to grow for 3 weeks.

We found that the group of mice immunised with the TNF autovaccine showed significant reduction in metastatic lesions compared with those treated with PBS (Figure 4A). The sizes of the metastases were also significantly reduced in the mice immunised with TNF autovaccine compared to the PBS control group (see Figure 4B). The reason for not using HEL with CFA as the control was that in previous experiments it had led to increased murine morbidity. The morbidity may have been due to the use of a strong antigen such as HEL with CFA inducing ulcerations on the surface of the animals; this was not seen with TNF autovaccination. Upon killing the animals, a terminal bleed was carried out. In these experiments, blood was also sampled from mice prior to immunisation and prior to tumour implantation. The anti-TNF antibodies were then measured in the sera using an ELISA-based detection system (Figure 5). We found that the anti-TNF titres in the TNF autovaccination group increased dramatically by the end of the experiment unlike the PBS control group.
TNF synergises with other stimulants to induce KC production in B16F10 cells

In conjunction with the in vivo experiments, the ability of mouse recombinant TNF to induce the expression of cytokines/chemokines such as IL-6, IL-1, VEGF, GMCSF and KC, and also TNF was examined as this could account for some of the metastatic effects of TNF. However, we found no detectable IL-6, IL-1, VEGF, GMCSF or endogenous TNF when B16F10 cells were left unstimulated or stimulated with TNF and or LPS in vitro, in vivo this may differ. In contrast, the chemokine KC (the murine equivalent of human MGSA/Gro2), previously described to play a role in metastasis (Smith et al, 1998), could be detected by ELISA when B16F10 cells were cultured for 24 and 72 h in vitro with either TNF or anti-TNF neutralising monoclonal antibodies. TNF increased KC production above background levels after 72 h when 100 µg ml⁻¹ of TNF was added to B16F10 cultures. Anti-TNF neutralising antibodies added to B16F10 cells did not reduce KC (Figure 6). Interestingly, we found that under certain circumstances TNF can induce KC expression at 24 and 72 h. Thus, by adding 10 µg ml⁻¹ LPS at the onset of the culture, the production of KC was increased in B16F10 cells both at 24 and 72 h (Figure 6).
mice, TNF autovaccination generated autologous antibodies and therefore the antibodies would not have been recognised and cleared by the mouse immune response. Both the autoantibodies generated by immunisation with TNF autovaccine and the anti-TNF monoclonal antibodies reduced the levels of metastases measured after 3 weeks, which shows that their effect lasted longer than that seen with the human TNF receptor fusion protein (Cubillos et al, 1997). The optimal time for tumour insertion was based on the previous data by Dalum et al (1999) and on the immunisation of C57BL/6 mice (Figure 2). These data showed that the anti-TNF antibodies measured in mice treated with TNF autovaccination were present from 4 to 10 weeks. Others have shown a subsequent decline in anti-TNF antibody levels at 3–6 months (data not shown). Consequently, the time for tumour insertion was chosen as 4 weeks to correspond to the rise in antibody levels as seen in Figure 5 in which the anti-TNF antibodies were measured in mice with melanoma. Unfortunately, the examination of the TNF autovaccine in established tumours in the B16F10 melanoma model was not possible because of two reasons. First, the B16F10 melanoma model is an acute rapidly progressing model that develops metastases within 2–3 weeks. As TNF autovaccination requires 4–6 weeks to induce optimal TNF autoantibodies, the tumour burden by that time would have been too great, with many mice already dead or in great pain for the procedure to be ethically acceptable. Second, most of the TNF tumour-promoting effects that have been described concern processes that occur during metastasis. Therefore, our main purpose was to investigate if blocking TNF by TNF autovaccination had an effect on the establishment of metastasis from cells in circulation.

We then investigated potential mechanisms involved in the reduction of metastases seen with TNF autovaccination. Owing to the small amounts of serum obtained from each mouse, we were unable to perform a range of cytokine evaluations. The only inflammatory marker measured was serum amyloid P, which showed no difference in levels between the treated groups (data not shown). As we were unable to examine any cytokines in the mice sera, we examined the effects of blocking TNF in vitro. We found that at high doses of TNF or in the presence of LPS, TNF upregulates KC in B16F10 cells. Interestingly, studies of mice that had their primary tumours removed surgically and were then injected with LPS or saline had increased lung metastases in the LPS injected group, indicating that LPS may activate mechanisms involved in the metastatic processes (Pidgeon et al, 1999). Furthermore, in vivo and in vitro studies have shown that LPS can increase vascular permeability, tumour invasion as well as increase inducible nitric oxide synthase and MMP2 production, two factors known to play a role in metastasis (Harmey et al, 2002). It may be that in vivo LPS or LPS-like pathways also upregulate KC, although these groups did not measure this. Although LPS may not be physiologically relevant in this context, our study still demonstrates that under certain conditions that may be met in vivo, TNF can upregulate KC production. Certainly, in humans TNF alone is able to upregulate Gro (the human equivalent of KC) in keratinocytes (Li and Thornhill, 2000), fibroblasts and human melanoma cell lines (Shattuck et al, 1994). Interestingly, KC is a proinflammatory chemokine that has been found to play a key role in inducing neovascularisation of corneal epithelial cells (Strieter et al, 1995) and in promoting metastasis in squamous carcinoma cells (Smith et al, 1998). Thus, part of the effect of blocking TNF by TNF autovaccination may be due to a decrease in the production of KC. Decreased KC production, in particular, may result in the reduced ability of B16F10 cells to invade lung tissue and also reduce neovascularisation of tumours in the lung, consequently decreasing the size and numbers of metastatic lesions. In our cell system, however, we were unable to demonstrate any reduction in KC when anti-TNF was added to B16F10 cells, which maybe due to insufficient TNF production by B16F10 cells despite LPS stimula-

Figure 6 Effects of TNF and LPS on KC production in B16F10 cells. Cells were plated out in triplicate at 2 × 10⁶ ml⁻¹ in 200 μl well⁻¹. To each well was added 10 μg ml⁻¹ LPS, 10 or 100 ng ml⁻¹ mTNF, 10 μg ml⁻¹ murine anti-TNF monoclonal antibodies or media as a control as shown in the legend. Supernatants were removed at 24 and 72 h to measure KC levels by ELISA. Statistical significance was measured by the one-way ANOVA test. These are pooled results from two experiments.

in patients, the potential variability in antibody responses will be undesirable as it may result in variability in efficacy or make interpretation of data difficult. However, this problem should we hope be circumvented by using promiscuously binding T helper epitopes in the recombinant vaccines.

The induction of high levels of anti-TNF antibodies reduced the number of metastases reaching and establishing themselves in the lungs as well as their size in the B16F10 murine melanoma model when compared to mice immunised with PBS in CFA. Prior to this, we ensured that blocking TNF by a known mechanism, monoclonal antibodies to TNF, could also reduce tumour metastasis. For this, we examined the effect of treating mice with anti-TNF monoclonal antibodies upon tumour insertion and had a reduction in the lung metastases compared to the PBS-treated control group. Further support for this comes from findings of Cubillos et al (1997), who used a human TNF receptor fusion protein in a B16BL6 melanoma model. Although it was found that the human soluble TNF fusion protein significantly inhibited lung metastases, this effect was short lived possibly due to the immunogenicity of the human protein increasing its clearance. In our system, the TNF monoclonal antibody, CV19q, is a rat/mouse fusion protein, and as the two species share more similarity, the rat portion does not produce as strong an anti-rat immune response when injected into
REFERENCES

Andreakos ET, Foxwell BM, Brennan FM, Maini RN, Feldmann M (2002) Cytokines and anti-cytokine biologicals in autoimmunity: present and future. Cytokine Growth Factor Rev 13: 299 – 313

Balkwill F (2002) Tumour necrosis factor or tumour promoting factor? Cytokine Growth Factor Rev 13: 135 – 141

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumours. Proc Natl Acad Sci USA 72: 3666 – 3670

Cubillos S, Scallon B, Feldmann M, Taylor P (1997) Effect of blocking TNF on IL-6 levels and metastasis in a B16-BL6 melanoma mouse model. Anticancer Res 17: 2207 – 2212

Dalum I, Butler DM, Jensen MR, Hinderson P, Steinaa L, Waterston AM, Green SN, Feldmann M, Elsner HJ, Mouts´en S (1999) Therapeutic antibodies elicited by immunization against TNFz. Nat Biotechnol 17: 666 – 669

Dalum I, Jensen MR, Gregorius K, Thomasen CM, Elsner HJ, Mouts´en S (1997) Induction of cross-reactive antibodies against a self-protein by immunization with a modified self-protein containing a foreign T helper epitope. Mol Immunol 34: 1113 – 1120

Dillman RO, Shawler DL, McCullister TJ, Halpern SE (1994) Human anti-mouse antibody response in cancer patients following single low-dose injections of radiolabeled murine monoclonal antibodies. Cancer Biother 9: 17 – 28

Elliot MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Dalum I, Jensen MR, Gregorius K, Thomasen CM, Elsner HJ, Mouts´en S (1994) Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha and interleukin-1 in rheumatoid arthritis. Lancet 344: 1050 – 1110

Harmey JH, Bucana CD, Lu W, Byrne AM, McDonnell S, Lynch C, Bouchier-Hayes D, Dong Z (2002) Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumour cell invasion. Int J Cancer 101: 415 – 422

Kiriakidis S, Andreakos E, Monaco C, Foxwell BM, Feldmann M, Paleolog E, Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M, Maini RN (1998) Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. Arthritis Rheum 41: 1258 – 1265

Philpott GW, Schwarz SW, Anderson CJ, Dehdashti F, Connett JM, Zinn KR, Meares CF, Cutler PD, Welch MJ, Siegel BA (1995) Radio-immunoPET: detection of colorectal carcinoma with positron-emitting 64Cu-labeled monoclonal antibody. J Nucl Med 36: 1818 – 1824

Pidgeon GP, Harmey JH, Kay E, Da Costa M, Redmond HP, Bouchier-Hayes DJ (1999) The role of endotoxin/lipopolysaccharide in surgically induced tumour growth in a murine model of metastatic disease. Br J Cancer 81: 1311 – 1317

Poste G, Doll J, Hart JR, Fijler IJ (1980) In vitro selection of murine B16 melanoma variants with enhanced tissue-invasive properties. Cancer Res 40: 1636 – 1644

Quin Z, Kruger-Krasagakes S, Kunzendorf U, Hock H, Diamantstein T, Blankenstein T (1993) Expression of tumour necrosis factor by different tumour cell lines results either in tumour suppression or augmented metastasis. J Exp Med 175: 355 – 360

Sarawar SR, Lee BJ, Anderson M, Teng YC, Zuberi R, Von Goegen S (2002) Chemokine induction and leukocyte trafficking to the lungs during murine gammaherpesvirus 68 (MHV-68) infection. Virology 293: 54 – 62

Shattuck RL, Wood LD, Jaffe GJ, Richmond A (1994) MGSA/GRO transcription is differentially regulated in normal retinal pigment epithelium and melanoma cells. Mol Cell Biol 14: 781 – 802

Shin KY, Moon HS, Park HY, Lee TY, Woo YM, Kim HJ, Lee SJ, Kong G (2000) Effects of tumour necrosis factor-alpha and interferon-gamma on expressions of matrix metalloproteinase-2 and -9 in human bladder cancer cells. Cancer Lett 159: 127 – 134

Smith CW, Chen Z, Dong G, Loukinova E, Pagram MY, Nicholas-Figueroa L, Van Waes C (1998) The host environment promotes the development of primary and metastatic squamous cell carcinomas that constitutively express pro-inflammatory cytokines IL-1alpha, IL-6, GM-CSF, and TNF. Clin Exp Metast 16: 655 – 664

Srieter RM, Polverini PJ, Kunel SL, Arenberg DA, Burdick MD, Kasper J, Dzuba J, Van Damme J, Walz A, Marriott D, Chan SY, Roczniak S, Shanafelt AB (1995) The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 270: 27348 – 27357

Williams RO, Feldmann M, Maini RN (1992) Anti-tumour necrosis factor antibodies ameliorates joint disease in murine collagen-induced arthritis. Proc Natl Acad Sci USA 89: 9784 – 9788

Workman P, Twentyman P, Balkwill F, Balmain A, Chaplin D, Double J, Embleton J, Newell D, Raymond R, Stables J, Stephens T, Wallace J (1998) United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia (Second Edition). Br J Cancer 77: 1 – 10

Wu W, Yamaura T, Murakami K, Ogasaewara M, Hayashi K, Murata J, Saiki I (1999) Involvement of TNF-alpha in enhancement of invasion and metastasis of colon 26-L5 carcinoma cells in mice by social isolation stress. Oncol Res 11: 461 – 469

ACKNOWLEDGEMENTS

We thank Ferring Pharmaceutical for the funding of this research, Pharmexa for providing valuable advice with regard to the TNF autovaccination, and thank Cancer Research (UK) and the Arthritis and Rheumatology Campaign.

TNF autovaccination in a murine melanoma model

AM Waterston et al

Experimental Therapeutics

British Journal of Cancer (2004) 90 (6), 1279 – 1284

© 2004 Cancer Research UK