J. Pharm. Pharmacol. 1998, 50 (Supplement): 39
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Mycobacterial cell wall–DNA complex induces apoptosis in cancer cells

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Live mycobacteria or mycobacterial preparations possess potent anti-cancer activity. This anti-cancer activity has been variously ascribed to cell wall components such as muramyl peptides, mycolic acid derivatives or peptidoglycan, or to immunostimulatory proteins. A mycobacterial cell wall complex (MCC) derived from the non-pathogenic organism *Mycobacterium phlei* (*M. phlei*) has been shown to have anti-tumour activity in animal tumour models (Chin et al, 1996) and in clinical studies in patients with carcinoma in situ of the bladder (Morales and Chin, 1997). We have demonstrated that MCC is a powerful inducer of a number of macrophage-derived cytokines such as IL-12 that have indirect anti-cancer activity, and that it can directly induce apoptosis in human bladder cancer cells (Filion et al, 1998).

In analysing the composition of MCC we found that it contains approximately 5% DNA in the form of short oligonucleotides (approximately 30-250 base-pairs in length). We have therefore evaluated whether MCC, DNA extracted from MCC or DNA extracted directly from *M. phlei* (*M. phlei* DNA) induces cytotoxicity or apoptosis in cancer cell lines. The induction of cytotoxicity was determined by the release of the cytoplasmic enzyme lactate dehydrogenase, and the induction of apoptosis was determined by a) inhibition of cancer cell proliferation, b) nucleosome-sized DNA fragmentation, c) the release of soluble nuclear mitotic apparatus protein (NuMA) and d) the presence of hypodiploid peaks in flow cytometric analysis of cellular DNA.

We have found that MCC, DNA extracted from MCC or *M. phlei* DNA are not directly cytotoxic towards a wide range of human and murine cancer cells. However, MCC, DNA extracted from MCC or *M. phlei* DNA directly induced apoptosis (incubation time 48-72 hours) in all cell lines in the concentration range 0.1-100 μg/ml in the absence of immune effector cells. The cancer cells tested were: human monocytic THP-1 leukemia, human pro-monocytic HL-60 leukemia, murine monocytic RAW 264.7 leukemia, human Jurkat T lymphoblast, murine B-16 melanoma, human colon SW260 adenocarcinoma, human oesophageal squamous OC2 carcinoma, human transitional bladder HT-1197 and HT-1376 carcinoma. The induction of apoptosis appeared to be independent of the cancer cell type and of the presence of a number of escape mechanisms associated with cancer progression, since all cell lines tested entered apoptosis after contact with MCC, DNA extracted from MCC or *M. phlei* DNA. The presence of multiple drug resistance phenotype, Fas-ligand resistance or p53/p21 mutations did not affect the ability of MCC or *M. phlei* DNA to induce apoptosis. Treatment of target cancer cells with the caspase inhibitor Z-VAD-FMK blocked the ability of MCC or of *M. phlei* DNA to induce apoptosis, indicating that the mechanism of action is in part dependent on caspase activation. The induction of apoptosis by *DNA* prepared from *M. phlei* was observed with both low and high molecular weight oligonucleotides in the range 10 base pairs to greater than 2×10^4 base pairs. DNase treatment of MCC, DNA extracted from MCC or *M. phlei* DNA significantly inhibited the induction of apoptosis, demonstrating that the DNA plays a pivotal role in inducing apoptosis. MCC was significantly more active than DNA extracted from MCC or *M. phlei* DNA (relative potencies 1.0, 0.1 and 0.09 respectively) indicating that the cell wall functions as an effective carrier and potentiating system for the apoptosis-inducing DNA. The ability of DNA from bacterial sources to act as an immunomodulator (Sparwasser et al, 1997) has been previously reported to be due to the presence of unmethylated CpG motifs (Krieg et al, 1995). Using cytosine methylase and the restriction enzyme *Bst*U I, which recognises the sequence 5'-CGCG-3', we have demonstrated that the induction of apoptosis does not appear to be related per se to the methylation state of putative CpG motifs present in MCC or in *M. phlei* DNA.

The results of this study clearly demonstrate that the ability of a cell wall complex prepared from *M. phlei* to induce apoptosis in cancer cells is related to the presence of DNA. The induction of apoptosis by mycobacterial DNA may help to explain the therapeutic activity of mycobacterial cell wall preparations in the treatment of cancer.

Filion, M.C., Lépicier, P., Morales, A. and Phillips, N.C. (1998) submitted for publication.

Chin, J.L. et al (1996) J. Urol. 156: 1189-1193.

Morales, A. and Chin, J.L. (1997) J Urol 157: A214.

Sparwasser, T., Miethke, T., Lipford, G., Erdmann, A., Häcker, H., Heeg, K. and Wagner. H. (1997) Eur. J. Immunol. 27:1671-1679.

22.Krieg, A., Yi, A-K., Matson, S., Waldschmidt, T.S., Bishop, G.A., R. Teasdale, R.,Koretzky, G.A. and Klinman, D.M. (1995) Nature 374:546-549.