γ Irradiated Mycobacteria Enhance Survival in Bladder Tumor Bearing Mice Although Less Efficaciously than Live Mycobacteria

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Abbreviations and Acronyms

BC = bladder cancer
BCG = Mycobacterium bovis bacillus Calmette-Guérin
CD = cluster of differentiation
IFN-γ = interferon-γ
IL = interleukin
NO = nitric oxide
PBMC = peripheral blood mononuclear cell
PBS = phosphate buffered saline
SEM = scanning electron microscopy
TEM = transmission electron microscopy
TNF = tumor necrosis factor
UAB = Universitat Autònoma de Barcelona
UV = ultraviolet

Purpose: γ Irradiated Mycobacterium bovis bacillus Calmette-Guérin has shown in vitro and ex vivo antitumor activity. However, to our knowledge the potential antitumor capacity has not been demonstrated in vivo. We studied the in vivo potential of γ irradiated bacillus Calmette-Guérin and γ irradiated M. brumae, a saprophytic mycobacterium that was recently described as an immunotherapeutic agent.

Materials and Methods: The antitumor capacity of γ irradiated M. brumae was first investigated by analyzing the in vitro inhibition of bladder tumor cell proliferation and the ex vivo cytotoxic effect of M. brumae activated peripheral blood cells. The effect of γ irradiated M. brumae or bacillus Calmette-Guérin intravesical treatment was then compared to treatment with live mycobacteria in the orthotopic murine model of bladder cancer.

Results: Nonviable M. brumae showed a capacity to inhibit in vitro bladder cancer cell lines similar to that of live mycobacteria. However, its capacity to induce cytokine production was decreased compared to that of live M. brumae. γ Irradiated M. brumae could activate immune cells to inhibit tumor cell growth, although to a lesser extent than live mycobacteria. Finally, intravesical treatment with γ irradiated M. brumae or bacillus Calmette-Guérin significantly increased survival with respect to that of nontreated tumor bearing mice. Both γ irradiated mycobacteria showed lower survival rates than those of live mycobacteria but the minor efficacy of γ irradiated vs live mycobacteria was only significant for bacillus Calmette-Guérin.

Conclusions: Our results show that although γ irradiated mycobacteria is less efficacious than live mycobacteria, it induces an antitumor effect in vivo, avoiding the possibility of further mycobacterial infections.

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MYCOBACTERIUM bovis bacillus Calmette-Guérin is still the gold standard treatment to avoid progression and recurrence in patients with nonmuscle invasive BC. BCG triggers a dual effect. 1) It is supposed that BCG inhibits the growth of the remaining tumor cells because in vitro experiments have demonstrated the capacity of BCG to inhibit the proliferation of bladder cancer cell lines. 2) BCG triggers an immunological cascade of events, including attracting different subsets of immune cells into the bladder, which helps eradicate tumor cells.[1–3] However, this efficacious treatment also has adverse events.[4] The most critical collateral effect is BCG infections due mainly to traumatic instillations.[5–7] BCG is instilled weekly into the bladder for 6 weeks but for optimal efficacy clinical trials suggest that BCG should be given on a maintenance schedule for at least 1 year.[8] This increases the risk of infection.

To avoid the possibility of BCG infection different strategies involving mycobacteria derived agents are currently under investigation. The first is based on a nanoemulsion of BCG cell wall extract,[9] which increases the attraction of the hydrophobic BCG cell wall to the bladder epithelium. This triggers a more efficient immune response than using the BCG cell wall without nanoemulsion. The other approximation is to use the cell wall plus nucleic acids from M. phlei in a formulation called MCNA (M. phlei cell wall-nucleic acid complex).[10] In a recent study Morales et al determined its safety and potential to treat BCG refractory cases.[10]

In this line of research 2 other therapeutic options were recently proposed at our laboratory. 1) Based on the fact that at least in the first instillations live mycobacteria are needed to induce a proper immune reaction,[1,11,12] we determined the antitumor capacity of M. brumae, a saprophytic mycobacterium. Like BCG M. brumae can activate the immune system and inhibit bladder cancer cells. In vitro, ex vivo and in vivo M. brumae has shown antitumor ability similar to that of BCG.[13] 2) We determined the antitumor activity of killed but metabolically active BCG in vitro and ex vivo.[14] In contrast to the proposal of using cell wall extracts, using killed but metabolically active mycobacteria implies using the whole bacterium, thus, avoiding missing any potential immunostimulatory compounds. Mycobacteria in that case are treated with γ irradiation, preserving a percent of active metabolism but preventing the mycobacteria from replicating. Thus, the fact that γ irradiated mycobacteria cannot cause infections warrants using them. However, to our knowledge the in vivo antitumor effect remains to be investigated.

To establish the true potential of γ irradiated mycobacteria as immunotherapeutic agents in the current series we first determined the antitumor capacity of γ irradiated M. brumae in vitro and ex vivo. We then determined for the first time to our knowledge the in vivo effect of intravesical instillations of irradiated mycobacteria compared with live mycobacteria in the orthotopic murine model of BC.

MATERIALS AND METHODS

Bacterial Strains and Treatments

M. bovis BCG Connaught (ATCC® 35745), M. phlei (ATCC 11758) and M. brumae (ATCC 51384^T) were grown on Middlebrook 7H10 medium (Difco Laboratories, Detroit, Michigan) supplemented with 10% OADC enrichment for 3, 2 or 1 week at 37C. In some experiments bacteria cells were subjected to different heat (60C and 121C) and irradiation (UV and γ) treatments as previously described.[14]

SEM and TEM Negative Staining

Mycobacterial structure was observed with electron microscopy. For conventional SEM experiments bacterial cell suspensions (10^5 cfu/ml) were filtered though a 0.2 μm nanopore Whatman® membrane and processed as previously described.[15] For TEM negative staining a suspension of isolated bacteria (10^6 cfu/ml) was applied to glow discharged, carbon coated copper grids and treated as described previously.[14]

Direct Growth Inhibition Experiments

In 2007 to 2011 we obtained human transitional carcinoma cell lines corresponding to high (T24 and J82) and low (RT112, 5637, SW780, MG-HU-3 and RT4) histopathological tumor grades (RTICCC-PRBB, Cancer Cell Line Repository). They were authenticated following short tandem repeat profiling. Cells were confirmed as negative for mycoplasma contamination by monthly screening using the MycoAlert™ assay. Tumor cells were maintained and infected with live or treated bacteria as described previously.[13–16] The formation of mycobacterial clumps was assessed by microscopy. Of the cells 90% were present as single cells or small clumps (data not shown). After 72 hours, or 120 hours for J82 cultures, cell culture supernatants were harvested, centrifuged and stored at −40C until use. Cell proliferation was measured by MTT colorimetric assay (Sigma®).

Mycobacteria Activated PBMC Macrophage Activation and Antitumor Capacity

Flow cytometry analysis of surface receptors in the murine macrophage-like J774 cell line was performed as described previously.[13,14] J774 macrophages were seeded