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Tuberculosis and lung cancer. An interesting case study

F. Mariani*, M. Bocchino#, G. Cappelli¶, T. Persechini+, V. Colizzi¶, E. Bonanno§, A. Ponticiello#, A. Sanduzzi#

ABSTRACT: *Tuberculosis and lung cancer. An interesting case study. F. Mariani, M. Bocchino, G. Cappelli, T. Persechini, V. Colizzi, E. Bonanno, A. Ponticiello, A. Sanduzzi.*

This report describes the case of a patient with lung cancer who completely recovered when he was suffering from tuberculosis. Since bacillus Calmette-Guerin (BCG) has beneficial effects in certain types of cancer, it was hypothesized that infection with *Mycobacterium tuberculosis* induced an effective response against the tumour. *M. tuberculosis*-infected blood T-lymphocytes of the patient were cultured with two lung tumour cell lines. T-lymphocytes *in vitro* remained attached to tumour cells that appeared reduced in number. Moreover, *M. tuberculosis* isolated from the patient was a strong inducer, in infected macrophages, of the expression of the inducible form of the nitric oxide synthase, that may regulate cytotoxic activity of human macrophages.

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**Experimental Medicine Institute-CNR, Rome, Italy. #Institute of Thoracic Disease, University of Naples "Federico II", Naples, Italy. ¶Dept of Biology, University of Rome "Tor Vergata", Rome, Italy. +Dept of Biology, University of Rome "ROMA TRE", Rome, Italy. §Dept of Pathology, University of Rome "Tor Vergata", Rome, Italy.*

Correspondence: A. Sanduzzi, Via A. Falcone 56, 80127 Napoli, Italy.

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Host defence against both *Mycobacterium tuberculosis* and cancer depends on activation and effector functions of a given subset of CD4-T helper lymphocytes (Th-1 cells), which mainly support cell-mediated immunity through the secretion of interleukin (IL)-2 and interferon (IFN)-γ. Furthermore, bacillus Calmette Guerin (BCG) has been demonstrated to have strong activity against several cancers (*e.g.* bladder cancer), probably by activating antitumour activity of macrophages. On the basis of these findings, this study investigated whether *M. tuberculosis* infection had any influence on the immune response against cancer, in a patient with lung cancer who completely recovered whilst suffering from pulmonary tuberculosis.

Case report

In October 1996, a 70-yr-old Caucasian male (height 169 cm, weight 71 kg), who had already suffered (in 1993) from lung cancer and had been treated with right upper lobectomy, presented to our clinic with astenia (lack of energy), cough and haemoptysis. Fibrebroncoscopy was performed and revealed the presence of a second neoplasm occluding the right intermediate bronchus. Two independent pathologists confirmed the occurrence of a poorly differentiated squamous cell carcinoma. The patient could not be submitted for the resection of the tumour because of compromised physical conditions and started on antiblastic therapy (vinblastine 10 mg, cisplatinum 100 mg and mytomicin 22 mg). Four weeks later, owing to the persistence of fever and sputum, antiblastic therapy was stopped and sputum smear examination was performed revealing the presence of acid fast bacilli (AFB), later confirmed as *M. tuberculosis* by culture. The patient was immediately treated with isoniazid 500 mg, rifampicin 600 mg and ethambutol 1500 mg. Sputum examination was repeated weekly. Although symptoms quickly improved and no drug-resistance was found, direct sputum smear examination and culture became negative for *M. tuberculosis* only 3 months after the beginning of therapy. At 9 months, a second fibrebroncoscopy showed no macroscopic features of the previous cancer and both bronchial cytology and histology were negative for malignancy. Finally, the radiographic examination of the thorax only revealed the presence of fibrotic sequelae in the residual right lung. Antituberculous therapy was then suspended and the patient has been followed every 6 months until now. He presents a good performance status without any clinical evidence of tuberculosis or cancer relapse.

It was hypothesized that infection with *M tuberculosis* had influenced the immune response against cancer. Therefore, the patient's peripheral blood mononuclear cells (PBMCs) were cultured *in vitro* in the presence of two lung tumour cell lines, A427 and Calu1, and then infected with both *M. tuberculosis* isolated from the patient's sputum and a laboratory strain, H37Rv. A decreased number of tumour

cells cultured with *M. tuberculosis*-infected PBMCs *versus* the lab strain was found. These cells differed morphologically from those cocultured with uninfected patient's cells or with healthy donors's PBMCs. In particular, the A427 lung tumour cells appeared smaller with a scanty cytoplasm, having the tendency to form clumps and detach from the plate. Moreover, T lymphocytes surrounded and remained attached to Calu1 cells (fig. 1). To ascertain whether a nitric oxide (NO)-mediated mechanism may play any role in the antitumour immune response, 5 day-old monocyte-derived macrophages of two healthy donors were infected with *M. tuberculosis* H37Rv or with the patient's strain. A detectable expression of the inducible form of nitric oxide synthase (iNOS), evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, was appreciated when the cells of both donors were infected with the patient's *M. tuberculosis*, but not when treated with the H37Rv strain (fig. 2).

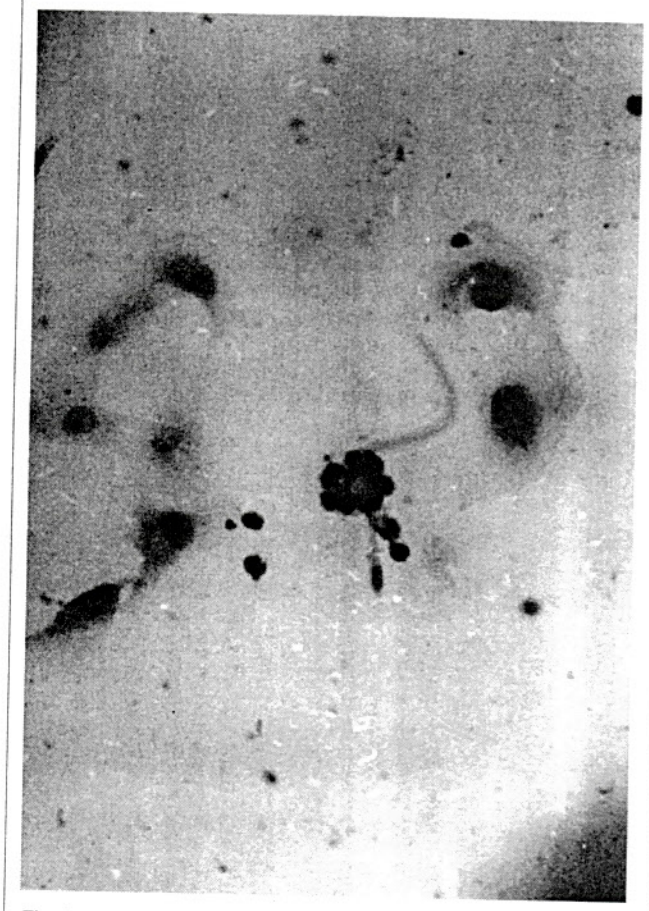

Fig. 1. – The lung tumour cell line Calu 1, infected with *M. tuberculosis* isolated from the patient, was cultured for 24 h in the presence of patient's peripheral blood mononuclear cells. Before fixation, cultures were gently washed and non adherent cells removed. Note that some lymphocytes are tightly attached to adherent epithelial cells (Hematoxylin-eosin stain 600×).

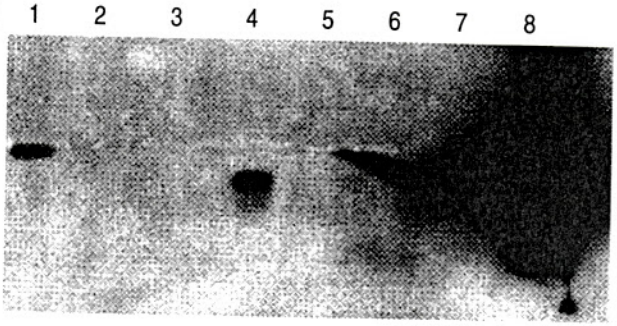

Fig. 2. – Induction of iNOS mRNA expression in monocyte-derived macrophages (MDM). Adherence-purified monocytes obtained from an healthy donor were cultured for 5 days, stimulated with two different concentrations (optimal and sub-optimal) of IFN-γ and LPS, and then infected with the *M. tuberculosis* H37Rv or with patient's strain. RT-PCR products were analysed for iNOS expression by Southern blotting. Lane 1: Unstimulated MDM; Lane 2: MDM stimulated with the optimal dose of IFN-γ and LPS; Lane 3: IFN-γ and LPS stimulated MDM infected with the *M. tuberculosis* laboratory strain, H37Rv; Lane 4: IFN-γ/LPS stimulated MDM infected with *M. tuberculosis* (clinical isolate); Lane 5: MDM stimulated with the sub-optimal dose of IFN-γ and LPS; Lane 6: IFN-γ/LPS stimulated MDM infected with the *M. tuberculosis* laboratory strain, H37Rv; Lane 7: IFN-γ/LPS stimulated MDM infected with *M. tuberculosis* (clinical isolate); Lane 8: positive control (plasmid containing the PCR target sequence).

Discussion

These findings suggest the hypothesis that *M. tuberculosis* infection has activated host T-lymphocytes to control cancer cell proliferation. This seems to be supported by the *in vitro* occurrence (in *M. tuberculosis*-infected cocultures of patient's PBMCs with two different lung cancer cell lines) of both morphological changes, quite suggestive of cell activation, and changes in tumour cell numbers. Moreover, the observation that patient's *M. tuberculosis*, but not the H37Rv laboratory strain, was able to increase the expression of the iNOS also suggests *in vivo* activation of cells like macrophages that are involved in the host defence. NO is a potent antimycobacterial agent which, regulated by the BCG gene, has been shown to regulate resistance or susceptibility to infection in murine/mice macrophages [1, 2]. The role of NO in human tuberculosis has not yet been clarified, but an increase in exhaled NO has been reported to occur, through iNOS activity, in infected patients as compared to control subjects [3–5]. In addition, the Nramp1 gene (the human homologue of BCG) has recently been identified as a protective locus against tuberculosis [6]. The role of NO in immunity against tumours is also unclear [7]. However, the cytostatic/cytotoxic activity of human macrophages has recently been attributed to the up-regulation of iNOS activity and NO production [8]. Although there is no direct evidence that the infection in this case has been responsible for the tumour regression, the results suggest that infection with an intracellular pathogen like *M. tuberculosis*, stimulating cell-mediated immunity that is also active against cancers, may have offered the host a protective mechanism against the tumour. NO produced by *M. tuberculosis*-infected macrophages may be a key mediator linking innate and adaptive immunity, thus leading to the control of both tuberculosis and cancer.

In conclusion, even though new research is being done [9,10], the mechanisms involved in the antitumour activity exerted by infection with mycobacteria still remain to be elucidated. It is strongly believed that further studies on a larger series are needed to understand the close interaction occurring between antimicrobial and antitumour immunity, and to consider the use of less virulent strains (*e.g.* bacillus Calmette-Guerin) as a therapeutic approach for lung cancer.

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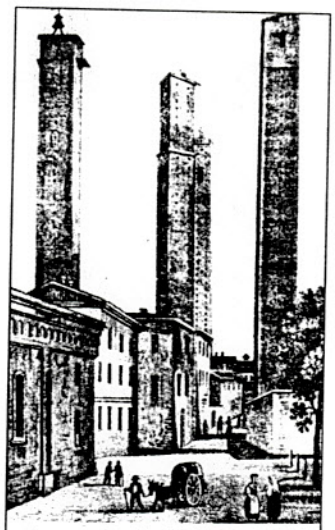

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