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Intravenous administration of BCG in mice promotes natural killer and T-cell mediated antitumor immunity in the lung

Eduardo Moreo[1,2,3] , Santiago Uranga[1,2,3] , Claudia Guerrero[1,2,3] , Miguel Araujo[1,2,4] , Ana Belén Gómez[1,2,3] , Carlos Martín[1,2,3] , Nacho Aguiló[1,2,3]

1) UNIVERSIDAD DE ZARAGOZA, FACULTAD DE MEDICINA, GRUPO DE GENÉTICA DE MICOBACTERIAS 2) IIS ARAGÓN 3) CIBER ENFERMEDADES INFECCIOSAS, INSTITUTO DE SALUD CARLOS III 4) UNIVERSIDAD DE OVIEDO, DEPARTAMENTO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR, INSTITUTO UNIVERSITARIO DE ONCOLOGÍA

BACKGROUND

Intravesical administration of the tuberculosis vaccine Bacillus Calmette-Guérin (BCG) was one of the first immunotherapies approved by the FDA and is still nowadays the treatment of choice for a subset of non-muscle invasive bladder cancer patients. Intravenous (IV) administration of BCG has been recently reported to be highly effective at preventing tuberculosis infection in macaques by inducing strong T cell responses in the lung[1], as well as to alter bone marrow myelopoiesis, generating "trained" macrophages which confer protection against tuberculosis infection in the lung[2]. In this work we set out to characterize the therapeutic application of IV BCG in mouse models of lung cancer.

METHODS

We used mouse models of lung tumors based on IV inoculation of B16-F10 melanoma, LLC and TC-1 lung carcinoma tumor cells to study the therapeutic effect of IV BCG. We performed lung immune cell profiling by flow cytometry to study the mechanism of action, as well as functional assays with isolated immune cells. In vivo antibody-mediated depletion studies were carried to find immune cells responsible for the therapeutic effect.

RESULTS

First, we observed that therapeutic IV BCG administration extended mice survival in models of B16-F10 lung metastasis and orthotopic LLC and TC-1 lung tumors. Immune cell depletion studies revealed that both NK cells and $CD4^+$ and $CD8^+$ T cells were required for the therapeutic effect. IV BCG induced a tumor-specific $CD8^+$ T cell response in the lung in a process which relied on type 1 conventional dendritic cells (cDC1s). Mechanistically, BCG stimulated lung NK cells, which participated in the recruitment of cDC1s to the tumor bed and facilitated the development of adaptive immune responses by killing tumor cells in a perforin-dependent manner., which provided tumor-associated antigens to cDC1s. Remarkably, IV BCG also restrained tumor growth in B16-F10 and LLC lung tumors in which MHC-I expression was ablated, suggesting potent stimulation of NK-cell mediated cytotoxicity in the lung.

Further analysis revealed that IV BCG induced the generation of immunostimulatory macrophages, which were responsible for NK cell activation in lung tumors in a process completely dependent on T-cell derived interferon gamma. Intriguinly, transfer of bone marrow cells from mice treated with IV BCG to mice depleted of their bone marrow protected against tumor growth, suggesting that training of bone marrow progenitors contributes to the observed antitumoral effect.

Finally, we observed that IV BCG therapy upregulated PD-L1 in multiple immune cells in the tumor microenvironment (TME), suggesting the generation of an inflamed TME. Combination of IV BCG and antiPD-L1 antibodies further increased mouse survival in mouse models of lung tumors, which were otherwise resistant to checkpoint blockade as a monotherapy. Furthermore, combination of IV BCG with the chemotherapeutic drug doxrrubicin improved immune-mediated control of lung tumors by rendering tumor cells more susceptible to targeting by CD8+ and NK cells.

CONCLUSIONS

Here we describe a therapy based on IV administration of BCG which enabled antitumor immunity in the lung by the coordination of multiple immune cell populations, including both T and NK cells as well as *Batf3*-dependent cDC1s and bone-marrow derived macrophages.

The results presented here have been recently accepted for publication at Nature Communications and will be published online the 4th of October.

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