REGULATORY B CELLS PRESENT IN LYMPH NODES DRAINING A MURINE TUMOR

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Abstract

In cancer, B cells have been classically associated with antibody secretion, antigen presentation and T cell activation. However, a possible role for B lymphocytes in impairing antitumor response and collaborating with tumor growth has been brought into focus. Recent reports have described the capacity of B cells to negatively affect immune responses in autoimmune diseases. The highly immunogenic mouse tumor MCC loses its immunogenicity and induces systemic immune suppression and tolerance as it grows. We have previously demonstrated that MCC growth induces a distinct and progressive increase in B cell number and proportion in the tumor draining lymph nodes (TDLN), as well as a less prominent increase in T regulatory cells. The aim of this research was to study B cell characteristics and function in the lymph node draining MCC tumor and to analyze whether these cells may be playing a role in suppressing antitumor response and favoring tumor progression. Results indicate that B cells from TDLN expressed increased CD86 and MHCII co-stimulatory molecules indicating activated phenotype, as well as intracellular IL-10, FASL and Granzyme B, molecules with regulatory immunosuppressive properties. Additionally, B cells showed high inhibitory upon T cell proliferation ex vivo, and a mild capacity to secrete antibodies. Our conclusion is that even when evidence of B cell-mediated activity of the immune response is present, B cells from TDLN exhibit regulatory phenotype and inhibitory activity, probably contributing to the state of immunological tolerance characteristic of the advanced tumor condition.

Key words: tumor immunity, B regulatory cells, tolerance

The involvement of the host immune system in the control of cancer progression has been assessed for several years; nowadays, it is vastly accepted that antitumor immunity occurs and that tumors have evolved an elaborate assembly of tricks to avoid immune-mediated rejection. The mechanisms responsible of immune cell dysfunction in patients with cancer involve a wide diversity of soluble immunosuppressive factors (such as TGFβ), IL-10 and the inhibitory ligands FasL and TRAIL) released by tumor cells or by various suppressor cells in the tumor microenvironment, including regulatory T cells (Tregs). In the last decades Tregs have been the major target...
of efforts to therapeutically modulate their inhibitory activity in order to achieve tumor remission or prevent recurrences\(^2\,^3\). More recent studies are currently focusing on the role of B cells in regulating the immune response. Although these cells have been classically associated with antibody secretion, antigen presentation and antitumor-T cell activation, a possible role for B lymphocytes in impairing antitumor response and collaborating with tumor growth has been brought into focus due to the recently described participation of a regulatory subset of B cells in autoimmune diseases\(^4\).

The mouse sarcoma MCC is a highly immunogenic tumor widely used to study the effect of tumor development on the immune system\(^5\,^6\). As MCC grows, its immunogenicity declines and a state of tolerance against the tumor arises\(^7\,^8\). We have recently demonstrated that MCC growth is accompanied by a marked and progressive increase in B cell number and proportion in the tumor draining lymph nodes (TDLN), along with a less prominent increase in Tregs\(^7\). While the participation of Tregs in the inhibition of the antitumor immune response has been broadly assessed\(^9\) the role of B cells is still a matter of debate. The aim of this paper was to study the characteristics of the B cell population present in the tumor draining lymph nodes, and to analyze whether these cells may play a role in favoring the establishment of tolerance and tumor progression.

As we previously showed\(^7\), MCC growth increased B cell number at the TDLN during the immunogenic phase, with a peak at day 15 after tumor inoculum (Fig. 1a). We therefore obtained cells from TDLN of tumor bearing mice (TBM) on day 15 and analyzed B cell phenotype and function. As control, non tumor bearing mice (non-TBM) B cells were obtained from inguinal and axillary LN. We found that B cells from TBM exhibited activated phenotype characterized by increased CD86 and MHCII expression.
Interestingly, these cells also expressed IL-10, FASL and Granzyme B (Fig. 1c), which were shown to induce immunosuppression in other systems. On the other hand, B cells isolated from TBM showed inhibitory effect upon allogenerically-induced T cell proliferation (Fig. 2a), while no effect was observed on MCC cultures (data not shown). Finally, a moderate titer of specific anti-MCC antibodies was detected in the serum of TBM, indicating that B cells retain their ability as antibody secreting-cells.

**Discussion**

Immune antitumor response emerges from the balance between regulatory and activated cells. It is now accepted that tumor presence leads to the appearance of regulatory immunosuppressive cell populations that not only affect endogenous antitumor response but also weaken the efficacy of immunotherapies. Regulatory T cells have been vastly assessed as one of the main inhibitory cell population. However, multiple regulatory mechanisms have been proposed in the last years for B cells in different abnormal immune system conditions. Some of them need cell to cell contact to be effective (FasL and PDL2) while others are mediated by B cell- secreted soluble molecules, TGFβ, Granzyme B and IL-10.

Together with signs of immune activation, such as increased expression of the surface markers CD86 and MHCII and secretion of antibodies against MCC, B cells from lymph nodes draining the tumor seemed to be predominantly immunosuppressive, as suggested by the expression of IL-10, FasL and Granzyme B. Importantly, these cells were able to inhibit in vitro- induced T cell proliferation, which could indicate that the negative effect of B cells on tumor immunity is mostly indirect through affecting T cells.

The presence of regulatory B cells was recently proposed to favor tumor progression in other models. Interleukin-10 secreted by B cells- was shown to reduce IFNγ secretion by cytotoxic CD8+ T cells11 and increase Treg cells presence12, thus impairing antitumor reaction. On the other hand, Granzyme B expressed by B cells infiltrating tumors had the capacity to negatively regulate T cell proliferation13.

Results presented herein indicate that along with some evidences of B cell- mediated activation of the immune response, the tumor also induces the emergence of a population of B cells with regulatory characteristics. We propose that these cells through different mechanisms such as IL-10, Granzyme B and FasL expression, could impede a proper antitumor response and collaborate with the immunological tolerance detected in advanced tumor bearing hosts.

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References