OncoImmunology 1:9, 1–3; December 2012; © 2012 Landes Bioscience

# The acidity of the tumor microenvironment is a mechanism of immune escape that can be overcome by proton pump inhibitors

Matteo Bellone,[1,*] Arianna Calcinotto,[1] Paola Filipazzi,[2] Angelo De Milito,[3,4] Stefano Fais,[3] and Licia Rivoltini[2,*]

[1]Cellular Immunology Unit; San Raffaele Scientific Institute; Milan, Italy; [2]Unit of Immunotherapy of Human Tumors; Fondazione IRCCS Istituto Nazionale Tumori; Milan, Italy; [3]Department of Therapeutic Research and Medicines Evaluation; Unit of Antitumor Drugs; Istituto Superiore di Sanità; Rome, Italy; [4]Oncology-Pathology; Cancer Center Karolinska; Karolinska Institute; Stockholm, Sweden

**Keywords:** anergy, T cell, melanoma, prostate cancer, adoptive immunotherapy, active immunotherapy

We have recently reported that lowering the pH to values that are frequently detected in tumors causes reversible anergy in both human and mouse CD8⁺ T lymphocytes in vitro. The same occurs in vivo, in the tumor microenvironment and the administration of proton pump inhibitors, which buffer tumor acidity, can revert T-cell anergy and increase the efficacy of immunotherapy.



The tumor microenvironment is dynamic, as it evolves and adjusts its functions to cope with the compelling need of tumor cells to survive and grow. Thus, modifications of the cellular metabolism imposed by the altered microenvironment (often induced by oncogenic driver mutations), contribute to the shaping of the tumor milieu.[1] As an example, cancer cells craving for energy take up much more glucose than normal cells and mainly process it through aerobic glycolysis, the so-called "Warburg effect."[1] Such an altered metabolic pattern associates with an elevated production of lactate, proton accumulation and a reversed intra-extracellular pH gradient, causing a drop in extracellular pH.[2] While low pH values have been shown to select for more aggressive acid-resistant clones and to favor tumor invasion,[1,2] little is known about how an acidic tumor microenvironment affects T-cell immunity. We have recently shown that lowering the pH to values most frequently detected within the tumor mass (pH 6–6.5) causes the establishment of a state of anergy in both human and mouse tumor-specific CD8⁺ T lymphocytes in vitro.[3] This condition is characterized by a significant impairment in cytolytic activity and cytokine secretion, coupled to a reduced expression of both the α-chain of the interleukin-2 receptor (IL-2Rα) and the T-cell receptor (TCR), as well as to a diminished activation of STAT5 and ERK in response to TCR signaling. We have also found that tumor-infiltrating lymphocytes (TILs) obtained from B16 melanomas, whose extracellular pH is approximately 6.5 (as specifically measured by in vivo magnetic resonance spectroscopy), display a similar anergic phenotype.[3] Thus, tumor acidity negatively regulates tumor-specific effector T cells in both human and murine experimental settings (**Fig. 1A**), and might indeed contribute to the dysfunction of anti-tumor immunity.[4] While it has been previously reported that hypoxia and/or the metabolic alterations of cancer cells may contribute to immune suppression,[5-7] our results show that acidity per se represents a mechanism of immune escape. Further studies will help unraveling the mechanisms whereby acidity reduces T-cell fitness. Since perforin degranulation, cytokine release and proliferation are significantly impaired at pH 6.5, our hypothesis is that acidity alters the biochemical equilibrium that regulates physiological activities, including exocytosis, secretion and proliferation.

Interestingly, the anergic state observed in lymphocytes cultured at pH 6.5 is reversible upon pH buffering, although prolonged exposure to such an acidic environment or lower pH values cause permanent damage and T-cell apoptosis.[3] Thus, we investigated whether tumor pH buffering could result in improved CD8⁺ cell function in vivo. Proton pumps, such as vacuolar H⁺-ATPases (V-ATPases), are upregulated in cancer cells, protect them from intracellular acidity and apoptosis, and participate in the establishment of an acidic tumor microenvironment.[2] Upon treatment of melanoma-bearing mice with a high dose of esomeprazole (12.5 mg/Kg), a proton pump inhibitor (PPI) employed to reduce gastric acid secretion,[8] we registered a rapid increase in the intratumoral pH, reaching values aroung 7.0 within 60 min and maintaining them for hours. In vivo pH buffering with PPIs was associated with improved TIL effector functions in melanoma lesions (**Fig. 1B**). Additionally, we detected a more efficient production of interferon γ (IFNγ) on a per cell basis.[3] TILs collected from PPI-treated mice showed enhanced expression of pERK,[3] confirming our in vitro data and demonstrating

*Correspondence to: Matteo Bellone and Licia Rivoltini; Email: bellone.matteo@hsr.it and licia.rivoltini@istitutotumori.mi.it
Submitted: 08/28/12; Accepted: 09/01/12
http://dx.doi.org/10.4161/onco.22058

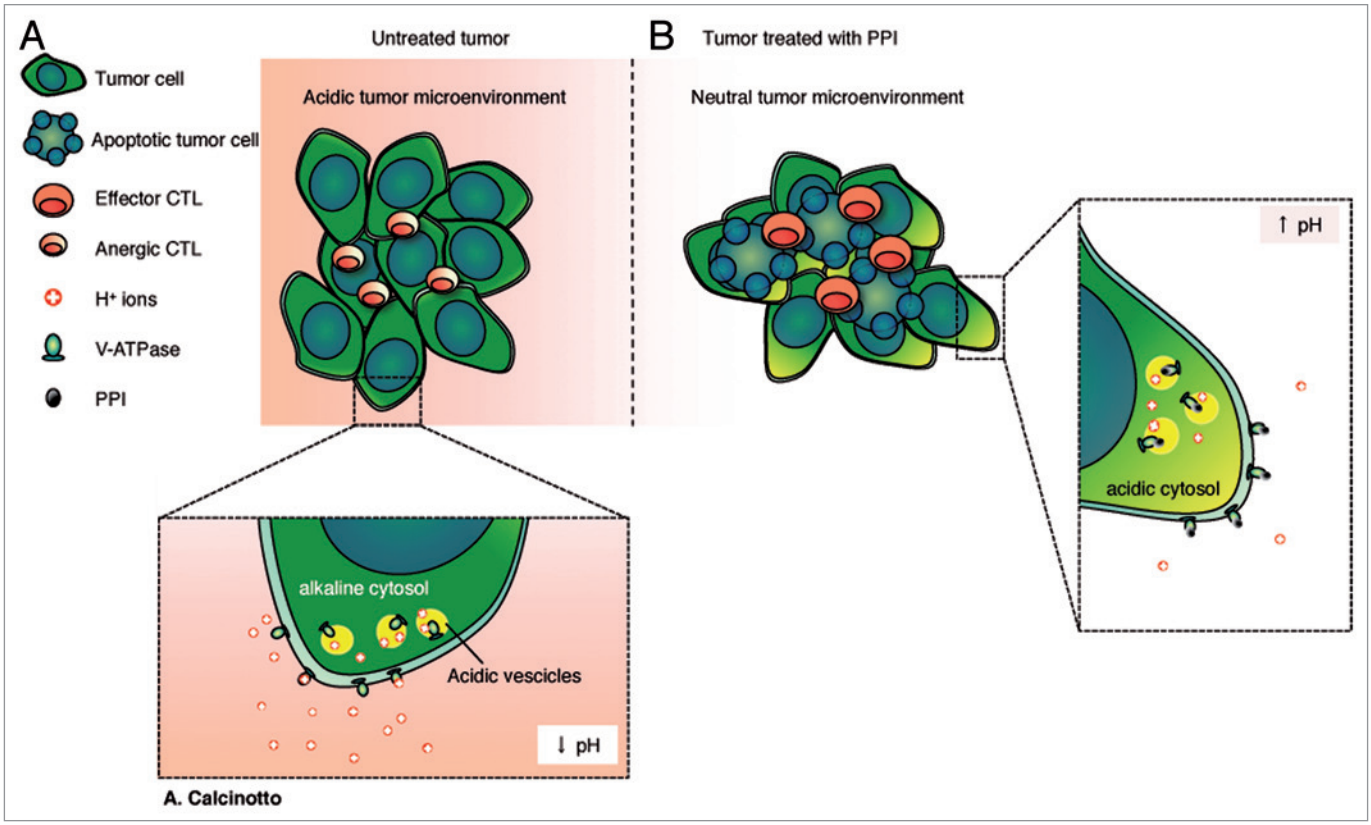


**Figure 1.** Effects of proton pump inhibitors on tumor cells and tumor-infiltrating lymphocytes. (A) Alkaline intracellular pH in tumor cells is maintained by specific regulatory mechanisms, including those mediated by vacuolar $H^+$-ATPases (V-ATPases). These pumps are devoted to extrude $H^+$ ions into the extracellular space, decreasing pH values in the tumor microenvironment. According to our data, local acidity favors the onset of T-cell anergy in infiltrating $CD8^+$ effector cells. (B) Proton pump inhibitors (PPIs) are lipophilic and weak base prodrugs that easily penetrate cell membranes and concentrate in acidic compartments, where they are very unstable and are transformed to biologically active inhibitors. Thus, in the presence of PPIs, tumor cells are no longer able to control intracellular pH and undergo apoptosis. PPIs also increase the extracellular pH, allowing $CD8^+$ T cells to overcome anergy and recover a tumoricidal activity. Thus, PPI treatment increases the therapeutic efficacy of T-cell based immunotherapies.

that anergy as induced by low pH can be overcome by PPI treatment.

PPIs are lipophilic and weak base prodrugs that easily penetrate cell membranes and concentrate in acidic compartments, where they are very unstable and are converted into sulfonamide forms, representing the active inhibitors.[8] Likely due to the high rate of aerobic glycolysis and/or to the overexpression of V-ATPase by tumor cells,[2] PPIs are rather tumor-selective immunomodulators, as they did not affect (at least in our hands) the T cells of organs lacking the acidic conditions required for prodrug activation, such as spleen, lungs and kidney.[3] The selective effects of PPIs on tissues characterized by a low pH might also explain why these drugs can be administered at very high doses without significant toxicity, as it occurs in the treatment of patients affected by the Zollinger-Ellison syndrome.[9]

Most importantly, we have shown that the treatment of tumor-bearing mice with PPIs increases the therapeutic efficacy not only of adoptive-cell immunotherapy but also of anticancer vaccines.[3] One limit of our work is that we did not investigate the effect in vivo of PPIs on the effector functions of other leukocytes such as regulatory T cells, B cells, natural killer (NK) cells, NK T cells, macrophages and myeloid-derived suppressor cells. While apparently the administration of PPIs did not favor the intratumoral accumulation of any of these cell populations,[3] others have reported that pantoprazole (an esomeprazole analog) given to tumor-bearing mice results in enhanced tumoricidal activity by macrophages.[10] We are presently investigating this issue.

Altogether, our findings show that the acidification of the tumor microenvironment is a novel mechanism of immune escape that can be overcome by drugs targeting pH-regulatory pathways, like PPIs, which can increase the clinical potential of T cell-based cancer immunotherapy.

### References


1. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011; 11:85-95; PMID:21258394; http://dx.doi.org/10.1038/nrc2981.
2. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4:891-9; PMID:15516961; http://dx.doi.org/10.1038/nrc1478.
3. Calcinotto A, Filipazzi P, Grioni M, Iero M, De Milito A, Ricupito A, et al. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. Cancer Res 2012; 72:2746-56; PMID:22593198; http://dx.doi.org/10.1158/0008-5472.CAN-11-1272.
4. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. Annu Rev Immunol 2007; 25:267-96; PMID:17134371; http://dx.doi.org/10.1146/annurev.immunol.25.022106.141609.

5. Henning T, Kraus M, Brischwein M, Otto AM, Wolf B. Relevance of tumor microenvironment for progression, therapy and drug development. Anticancer Drugs 2004; 15:7-14; PMID:15090737; http://dx.doi.org/10.1097/00001813-200401000-00002.
6. Lardner A. The effects of extracellular pH on immune function. J Leukoc Biol 2001; 69:522-30; PMID:11310837.
7. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 2007; 109:3812-9; PMID:17255361; http://dx.doi.org/10.1182/blood-2006-07-035972.
8. Olbe L, Carlsson E, Lindberg P. A proton-pump inhibitor expedition: the case histories of omeprazole and esomeprazole. Nat Rev Drug Discov 2003; 2:132-9; PMID:12563304; http://dx.doi.org/10.1038/nrd1010.
9. Mullin JM, Gabello M, Murray LJ, Farrell CP, Bellows J, Wolov KR, et al. Proton pump inhibitors: actions and reactions. Drug Discov Today 2009; 14:647-60; PMID:19443264; http://dx.doi.org/10.1016/j.drudis.2009.03.014.
10. Vishvakarma NK, Singh SM. Immunopotentiating effect of proton pump inhibitor pantoprazole in a lymphoma-bearing murine host: Implication in anti-tumor activation of tumor-associated macrophages. Immunol Lett 2010; 134:83-92; PMID:20837061; http://dx.doi.org/10.1016/j.imlet.2010.09.002.