New Doctorial Cancer Research

Prostaglandin-Mediated Immune Modulation

Sheraz Yaqub

The Biotechnology Centre of Oslo, University of Oslo, Norway

Ph.D. Dissertation date: June 20, 2008
Supervisor: Professor Kjetil Taskén
ISBN: 978-82-8072-275-1

The immune system has several methods to regulate the immune response to avoid harming the host during its encounter with an invading microbe or foreign peptide. The recent discovery of regulatory T cells (Tregs) as a subpopulation of T cells that suppress immune responses and are essential for maintaining immunological tolerance have led to a considerable interest in evaluating the role of Tregs in health and disease and the mechanisms of suppression employed by these cells. Human Tregs constitute 5%–10% of the CD4+ T-cell population and are characterized by expression of CD25 and FOXP3. Naturally occurring Tregs develop in the thymus and suppress responder cells by a cell-contact-dependent mechanism, whereas adaptive Tregs are generated in the periphery from naive CD4+ T cells by various stimuli and suppress immune cells in both a cell-contact-dependent and independent manner. Several cytokines are known to suppress immune activation. Prostaglandin E2 (PGE₂) is one of the most potent immunosuppressive molecules and is synthesized by cyclooxygenase 2 (COX-2). PGE₂ plays a prominent role in inflammatory diseases such as arthritis and is also involved in tumorigenesis by promoting cell growth, angiogenesis, and tumor invasion. PGE₂ exerts its cellular effects by binding to EP1-EP4 receptors that belong to seven transmembrane G-protein coupled receptors.

We have shown that continuous antigen stimulation of human naive CD4+ T cells gives rise to adaptive Tregs that suppress autologous responder T cells. Furthermore, we reported a novel mechanism of Treg-mediated immune suppression where adaptive Tregs express COX-2 and suppress responder T cells in a PGE₂-dependent manner that can be reversed with COX-2 inhibitors and PGE₂-receptor antagonists. However, both the expression of FOXP3 and COX-2 and the suppressive phenotype of adaptive Tregs were temporally regulated and transiently present for less than a week. This may constitute a physiological mechanism of homeostasis in which Tregs are generated during an infection to suppress the immune response once the offending agent is cleared.
Tregs are present in various malignant diseases, both within the tumor as well as in peripheral blood and malignant fluids. We demonstrated that patients with colorectal cancer (CRC) have high levels of PGE$_2$ in plasma and that Tregs from peripheral blood of CRC patients inhibit antitumor immune responses that can be reversed with COX inhibitor and PKA antagonist (Rp-8-Br cAMP) to the same extent as depleting Tregs. These observations outline hope for a new strategy that targets Tregs in cancer patients both to increase the antitumor immune responses and to improve the effect of cancer vaccines.

Finally, we examined the immune suppression observed in severe sepsis. We mimicked the septic microenvironment by adding lipopolysaccharide (LPS) to human whole blood and measured an increased level of PGE$_2$ in the plasma as well as suppression of T-cell proliferation, which was reversible with Rp-8-Br cAMPS. When dissecting the mechanism at a cellular level, we found that monocytes were the main producers of PGE$_2$ on stimulation with LPS and that the suppression of T cells was mediated through the PGE$_2$-cAMP-PKA pathway, as it could be reversed by COX inhibitors, PGE$_2$ antibody, or PKA antagonist. Interestingly, we found that LPS-activated monocytes also induced FOXP3 expression in responder T cells, supporting the notion that PGE$_2$ has a versatile role in the immune system, both suppressing T cells and inducing suppressor cells to limit the immune response.

Comment by Steven M. Dubinett, M.D.

Professor of Medicine and Pathology, Chief, Division of Pulmonary and Critical Care Medicine; Director, UCLA Lung Cancer Research Program, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, 37-131 CHS, 10833 Le Conte Avenue, Los Angeles, CA 90095-1690; Tel.: (310)794-6566; Fax: (310)267-2829; sdubinett@mednet.ucla.edu

Prostaglandins (PGs) have been known to impact immune responses in a variety of settings. Important details of these relationships and the specific pathways whereby PGs mediate these effects have recently been elucidated. The work of Sheraz Yaqub makes an important contribution to this field. Dr. Yaqub and colleagues discovered that polyclonal in vitro stimulation of human peripheral blood mononuclear cells (PBMCs) leads to upregulation of COX-2 and production of PGE2 in a subset of CD4$^+$CD25$^-$ T cells. Concomitantly, induction of FOXP3 occurs in those cells, and after isolation they show suppression of autologous effector T cells.

The authors stimulated the cells either with the superantigen SEB or by adding anti-CD3/CD28-antibody-coated magnetic beads for up to four days, isolated the activated (CD25+) cell fraction, and further analyzed these cells phenotypically and functionally. Because the cells upregulated FOXP3 on activation and started to secrete the immunosuppressive cytokines IL-10 and TGF-β, the authors called the cells “adaptive” Tregs and showed that they suppress proliferation as well as cytokine production in cocultures with autologous effector T cells. Concomitantly
with FOXP3, these cells upregulated expression of COX-2 and secreted PGE2 both during differentiation and after isolation and restimulation. PGE2 secretion could be completely abrogated by the addition of COX inhibitors. PGE2 inhibited proliferation of T cells and led to a rapid rise of cAMP levels in treated cells. The suppressive effect of these adaptive Tregs could be completely reversed by the addition of COX inhibitors or antagonists of the PGE2 receptors EP2 and EP4, but not by adding blocking anti-IL-10 or anti-TGF-β antibodies. Finally, data are presented showing induction of FOXP3 in T cells on incubation with PGE2 and blocking of anti-CD3/CD28–mediated upregulation of FOXP3 in the presence of COX inhibitors. The paper finishes with a model illustrating and integrating the novel findings. Here the authors develop the hypothesis that adaptive Tregs—through secretion of PGE2—not only suppress activation of effector T cells by accumulation of cAMP in the targeted cells, but also support their own differentiation state via an autocrine process that sustains their own FOXP3 expression.1 These are important findings that elucidate the pathways for PGE2-dependent induction of the T regulatory phenotype.

Yaqub and colleagues went on to investigate these relationships in more detail including studies of murine cells to demonstrate in vivo induction of adaptive Tregs.2 In addition, this group adds to this literature by specifically addressing the activity of Tregs within the context of colorectal cancer and relating this with COX-2/PGE2.3 Novel relationships of PGE2 production and Treg activation in the context of sepsis-induced immunosuppression were also described.4,5

Mounting evidence from a number of studies suggest that the overproduction of PGE2 and its decreased catabolism can lead to malignant progression. One pathway for this to occur is via the suppression of cell-mediated immune responses. Yaqub and colleagues have contributed substantially to this field by highlighting the important relationships between PGE2-dependent signaling and induction of the Treg phenotype in cancer and the immune suppression associate with sepsis.

REFERENCES