A new paradigm for an old story: the role of regulatory b cells in cancer

Health & Medicine

A common feature between cancer escape and autoimmune diseases is an inappropriate involvement of the regulatory immune system, albeit for opposing purposes. While autoimmune disease is a reflection of the failure to control responses to self, cancer is a result of an exaggerated use of these controls to abrogate antitumor effector responses. Although the importance of regulatory B cells [Bregs, the definition first used by Mizoguchi to describe B cells exerting protection from colitis in mice (Mizoguchi et al., 1997)] in protection from autoimmunity is now accepted, their involvement in cancer escape remains poorly understood. The conundrum of Bregs is that, if their numbers are low (in analogy with Tregs), their existence and importance may be concealed by the overwhelming response of effector B cells. For example, aberrant activation of B cells promotes autoimmune diseases, such as rheumatoid arthritis (RA), type 1 diabetes mellitus (T1D), multiple sclerosis (MS), and systemic lupus erythematosus (SLE). As such, the depletion of B cells with anti-CD20 antibody rituximab impairs antigen-specific $CD4^+$ T cell activation (Bouaziz et al., 2007) and ameliorates RA, MS, and T1D (Townsend et al., 2010). Yet, treatment with rituximab can also exacerbate the disease in some patients with ulcerative colitis, or even induce other diseases, such as psoriasis with psoriatic arthropathy and colitis in patients with Graves disease and non-Hodgkin lymphoma, respectively (Dass et al., 2007 ; Goetz et al., 2007 ; Mielke et al., 2008). The increased numbers of B cells in peripheral blood of transplant patients is positively associated with a rare but long-term drug-free clinical tolerance (Newell et al., 2010 ; Pallier et al., 2010 ; Sagoo et al., 2010). Although these clinical examples clearly indicate the importance of B cells, a current issue is how to

segregate the role of Bregs from suppressive activity of B cells that has been known for more than 30 years. As first proposed by Morris and Moller in late 1960s (Morris and Moller, 1968), B cell-produced immunoglobulin can elicit immune suppression by directly triggering ITIM-mediated suppressive signaling in target cells upon binding with inhibitory FcγRIIB (Ravetch and Bolland, 2001) or by indirectly modulating dendritic cells (DCs) via activating FcγR (Morris and Moller, 1968).

The first evidence of suppressive B cells (Bregs?) that functioned independently of their immunoglobulin was shown by Shimamura et al. (1982) about 30 years ago. Confirming this, the absence of B cells was linked with exacerbated autoimmune responses in mice deficient in B cells, such as mice that lack mature B cells (Wolf et al., 1996) and CD19 B cells (Yanaba et al., 2008). To date, the protection from autoimmune diseases in mice was linked with several unique subsets of IL-10-producing Bregs, such as CD1d ^{High} B1b cells (CD5 $^{-}$ B220 ^{Low} CD11b $^{+}$ IgM $^{+}$ CD1d ^{High} ; Mizoguchi et al., 2002), B10 regulatory cells (IL-10-producing CD1d ^{High} CD5 $^{+}$ B cells; Yanaba et al., 2008), and CD1d ^{High} Tim-1 $^{+}$ CD5 $^{+}$ Bregs (Ding et al., 2011). Although little is known about human Bregs, protection from SLE was recently linked with an impairment of regulatory activity of CD19 $^{+}$ CD24 ^{High} CD38 ^{High} B cells (Blair et al., 2010). Moreover, a rare subset of IL-10-producing memory CD24 ^{hi} CD27 $^{+}$ B cells that functions like murine B10 cells was also shown to exist in humans (Iwata et al., 2011). Humans also have IL-10 and TGFβ-producing CD25 ^{hi} CD27 ^{hi} CD86 ^{hi} CD1d ^{hi} B cells that

can suppress proliferation of autologous T cells and induce the generation of Foxp3 $^{+}$ CTLA-4 $^{+}$ Tregs (Kessel et al., 2012).

The majority of protective effects of Bregs requires IL-10 (Mizoguchi et al., 2002 ; Byrne and Halliday, 2005 ; Matsushita et al., 2008 ; Yanaba et al., 2008 ; Blair et al., 2010), a cytokine also utilized in other B cell-mediated suppression. For example, IL-10 is also abundantly produced and utilized by CD5 $^{+}$ B1 cells and MZ B cells to ameliorate collagen-induced arthritis in mice (O'Garra and Howard, 1992 ; Brummel and Lenert, 2005 ; Lenert et al., 2005 ; Evans et al., 2007) and by LPS-stimulated B cells to protect from autoimmune responses in mice by rendering T cells anergic (Parekh et al., 2003 ; Lampropoulou et al., 2008) and tolerogenic (Fuchs and Matzinger, 1992). The boundaries between Bregs and IL-10 producing B cells can often be obscure, raising question whether IL-10 is a primary mediator of suppressive activity or a factor that promotes homeostasis of Bregs. As for murine and human B1 cells (Balabanian et al., 2002 ; Gary-Gouy et al., 2002), IL-10 may promote survival and proliferation of Bregs. On the other hand, full suppressive power of Bregs and concomitant IL-10 production often requires activation, for example, by chronic inflammation or by engagement of their toll-like receptors (TLRs) or CD40 (Mizoguchi et al., 2002 ; Gray et al., 2007 ; Lampropoulou et al., 2008). This leads to production of other immunomodulatory factors (TGFβ and galectin-1) and upregulation of surface antigens, such as PD-1 and CTLA-4. As a result, activated Bregs can either directly induce apoptosis and anergy of effector Th1 cells and CD8 $^{+}$ T cells (Zuniga et al., 2001 ; Parekh et al., 2003 ; Frommer et al., 2008 ;

Tretter et al., 2008) or indirectly by converting Tregs (Reichardt et al., 2007 ; Sun et al., 2008 ; Sayi et al., 2011 ; Scapini et al., 2011) and modulating DCs (Byrne and Halliday, 2005 ; Watt et al., 2007).

Although cancer often uses homeostatic regulatory machinery to escape from immune surveillance, surprisingly the process seems does not involve Bregs that protect from autoimmune diseases. As such, the role of Bregs in cancer escape is poorly appreciated. Instead, B cells are mostly known for their “ pathogenic” antitumor properties (Lanzavecchia, 1985 ; Candolfi et al., 2011). The presence of CD20 $^{+}$ B cells in metastatic lymph nodes is a sign of favorable outcome in patients with head and neck cancer (Pretscher et al., 2009); and depletion of CD20-expressing B cells increases tumor burden in the lungs of mice intravenously injected with B16-F10 melanoma after (Sorrentino et al., 2011). Despite this, B cells also participate in carcinogenesis of methylcholanthrene-induced (Brodt and Gordon, 1978 , 1982) or transplanted tumors (Monach et al., 1993); and syngeneic tumors progress poorly in μMT mice deficient in B cells unless replenished with B220 $^{+}$ B cells (Qin et al., 1998 ; Olkhanud et al., 2011). Cancer-promoting B cells appear to exert a multitude of functions, such as production of immunoglobulins and cytokines (Townsend et al., 2010). As in autoimmunity, the immunoglobulin deposition induces FcR- and complement-mediated chronic inflammation needed for carcinogenesis (Zusman et al., 1996 ; de Visser et al., 2005). Activated B cells produce TGFβ (Parekh et al., 2003 ; Lampropoulou et al., 2008), and immunoglobulin can serve as a carrier for TGFβ and thereby mediate suppression of cellular immune responses (Stach and Rowley, 1993 ; Rowley and Stach, 1998).

Tumor-infiltrating B cells also produce lymphotoxin α/β and promote androgen-independent growth of prostate cancer cells by inducing the nuclear translocation of IKKα and activation of STAT3 (Ammirante et al., 2010). Pro-tumorigenic activity of B cells also requires production of IL-10 (Inoue et al., 2006) and TNFα (Schioppa et al., 2011) to presumably mediate Th2 polarization and inhibition of the cytotoxic activity of $CD8^+$ T and NK cells. Importantly, B cells isolated from tumor-bearing mice inhibit $CD4^+$ T cell-mediated help for CTLs (Qin et al., 1998).

To date, there are only two clearly defined examples of cancer escape-promoting Bregs are reported. First, murine B10 cells can abrogate monocyte activity and reduce surface expression of FcγR in IL-10-dependent fashion (Horikawa et al., 2011). As a result, the presence of B10 cells inhibits the therapeutic efficacy of anti-CD20 antibody against lymphoma. On the other hand, we recently discovered a unique subset of tumor-evoked Bregs (tBregs) that actively facilitates breast cancer escape and metastasis in BALB/C mice bearing 4T1 carcinoma cells (Olkhanud et al., 2011). In fact, the cancer cells themselves induce the generation of TGFβ-producing tBregs from normal B cells. As a result, tBreg then convert non-Treg $CD4^+$ T cells into metastasis-promoting $FoxP3^+$ Tregs (Olkhanud et al., 2011), which in turn inactivate antitumor NK cells and protect metastasizing cancer cells in the lungs (Olkhanud et al., 2009). We believe that the tBreg-like cells also exist in humans, as they can be readily generated *ex vivo* by treating normal human donor B cells with conditioned media of human cancer lines, such as breast, ovarian, and colon carcinomas (Olkhanud et al., 2011). tBregs differ

phenotypically and functionally from other Bregs involved in autoimmune responses (Mizoguchi et al., 2002 ; Matsushita et al., 2008 ; Yanaba et al., 2008) and LPS- or BCR-activated B cells (Fuchs and Matzinger, 1992 ; Hussain and Delovitch, 2007). tBregs resemble B2 cells (IgD ^{High}) but express constitutively active Stat3 and surface markers like CD25 ^{High} B7-H1 ^{High} CD81 ^{High} CD86 ^{High} CCR6 ^{High} and CD62L ^{Low} IgM $^{Int/Low}$ and poorly proliferate. They do not express CD27 and CD5 or up regulate CD1d, and their suppressive activity does not require IL-10 or other known suppressive pathways, such as B7-H1-PD-1, Fas-FasL, and IL27/IL35. Treatment with *S. aureus* Cowan 1 antigen can also generate suppressive CD25 $^{+}$ B cells that induce anergy of activated T cells by competing for IL-2 (Tretter et al., 2008). However, unlike them, tBregs regulate both resting and activated T cells (both CD4 $^{+}$ and CD8 $^{+}$ T cells) acting independently of IL-2 and without inducing cell death.

Since cancer actively converts tBregs from normal B cells, the clinical implication of this is that, as long as cancer persists, it will induce their generation and thereby initiate the chain of suppressive events. Thus, strategies that abrogate any step of this process are expected to inhibit cancer escape and metastasis, a primary cause of patients' bad disease outcome. However, the success of a strategy will also depend on the use of tailored approaches, ideally, ones that only inactivate tBregs, while protecting or promoting " good" B cells needed for optimal cancer eradication. For example, 4T1 breast cancer metastasis is abrogated by antibody that targets IL2Rα expressed on Tregs and tBregs (Olkhanud et al.,

2009 , 2011). Despite this, no clinical benefit was elicited in patients with renal cell carcinoma treated with B cell-depleting anti-CD20 antibody rituximab (Aklilu et al., 2004). Although this result questions the role of Bregs in human cancers, our recent data indicate that tBregs can escape anti-CD20 antibody due to low levels of CD20 expression. As a result, treatment with anti-CD20 antibody preferentially depletes “ good” and activated B cells, while enriching for tBregs and thereby enhancing cancer escape and metastasis (Bodogai et al., MS in preparation). Overall, although plethora of conventional B cells can often conceal and hamper analysis of small population of Bregs, the use of tailored and unique methodologies clearly indicates their existence and importance in mediation of cancer escape. It is time to unequivocally accept Bregs and tBregs as true members of the regulatory immune network.

Acknowledgments

We are grateful to Ana Lustig (NIA/NIH) for helpful proofreading and comments. This research was supported by the Intramural Research Program of the National Institute on Aging, NIH.

References

Aklilu, M., Stadler, W. M., Markiewicz, M., Vogelzang, N. J., Mahowald, M., Johnson, M., and Gajewski, T. F. (2004). Depletion of normal B cells with rituximab as an adjunct to IL-2 therapy for renal cell carcinoma and melanoma. *Ann. Oncol.* 15, 1109–1114.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Ammirante, M., Luo, J. L., Grivennikov, S., Nedospasov, S., and Karin, M. (2010). B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 464, 302–305.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Balabanian, K., Foussat, A., Bouchet-Delbos, L., Couderc, J., Krzysiek, R., Amara, A., Baleux, F., Portier, A., Galanaud, P., and Emilie, D. (2002). Interleukin-10 modulates the sensitivity of peritoneal B lymphocytes to chemokines with opposite effects on stromal cell-derived factor-1 and B-lymphocyte chemoattractant. *Blood* 99, 427–436.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Blair, P. A., Norena, L. Y., Flores-Borja, F., Rawlings, D. J., Isenberg, D. A., Ehrenstein, M. R., and Mauri, C. (2010). CD19(+) CD24(hi) CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity* 32, 129–140.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Bouaziz, J. D., Yanaba, K., Venturi, G. M., Wang, Y., Tisch, R. M., Poe, J. C., and Tedder, T. F. (2007). Therapeutic B cell depletion impairs adaptive and autoreactive CD4+ T cell activation in mice. *Proc. Natl. Acad. Sci. U. S. A.* 104, 20878–20883.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Brodt, P., and Gordon, J. (1978). Anti-tumor immunity in B lymphocyte-deprived mice. I. Immunity to a chemically induced tumor. *J. Immunol.* 121, 359–362.

Pubmed Abstract | Pubmed Full Text

Brodt, P., and Gordon, J. (1982). Natural resistance mechanisms may play a role in protection against chemical carcinogenesis. *Cancer Immunol. Immunother.* 13, 125–127.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Brummel, R., and Lenert, P. (2005). Activation of marginal zone B cells from lupus mice with type A(D) CpG-oligodeoxynucleotides. *J. Immunol.* 174, 2429–2434.

Pubmed Abstract | Pubmed Full Text

Byrne, S. N., and Halliday, G. M. (2005). B cells activated in lymph nodes in response to ultraviolet irradiation or by interleukin-10 inhibit dendritic cell induction of immunity. *J. Invest. Dermatol.* 124, 570–578.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Candolfi, M., Curtin, J. F., Yagiz, K., Assi, H., Wibowo, M. K., Alzadeh, G. E., Foulad, D., Muhammad, A. K., Salehi, S., Keech, N., Puntel, M., Liu, C., Sanderson, N. R., Kroeger, K. M., Dunn, R., Martins, G., Lowenstein, P. R., and Castro, M. G. (2011). B cells are critical to T-cell-mediated antitumor

immunity induced by a combined immune-stimulatory/conditionally cytotoxic therapy for glioblastoma. *Neoplasia* 13, 947–960.

Pubmed Abstract | Pubmed Full Text

Dass, S., Vital, E. M., and Emery, P. (2007). Development of psoriasis after B cell depletion with rituximab. *Arthritis Rheum.* 56, 2715–2718.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

de Visser, K. E., Korets, L. V., and Coussens, L. M. (2005). De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 7, 411–423.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Ding, Q., Yeung, M., Camirand, G., Zeng, Q., Akiba, H., Yagita, H., Chalasani, G., Sayegh, M. H., Najafian, N., and Rothstein, D. M. (2011). Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J. Clin. Invest.* 121, 3645–3656.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Evans, J. G., Chavez-Rueda, K. A., Eddaoudi, A., Meyer-Bahlburg, A., Rawlings, D. J., Ehrenstein, M. R., and Mauri, C. (2007). Novel suppressive function of transitional 2 B cells in experimental arthritis. *J. Immunol.* 178, 7868–7878.

Pubmed Abstract | Pubmed Full Text

Frommer, F., Heinen, T. J., Wunderlich, F. T., Yogev, N., Buch, T., Roers, A., Bettelli, E., Müller, W., Anderton, S. M., and Waisman, A. (2008). Tolerance without clonal expansion: self-antigen-expressing B cells program self-reactive T cells for future deletion. *J. Immunol.* 181, 5748–5759.

Pubmed Abstract | Pubmed Full Text

Fuchs, E. J., and Matzinger, P. (1992). B cells turn off virgin but not memory T cells. *Science* 258, 1156–1159.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Gary-Gouy, H., Harriague, J., Bismuth, G., Platzer, C., Schmitt, C., and Dalloul, A. H. (2002). Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. *Blood* 100, 4537–4543.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Goetz, M., Atreya, R., Ghalibafian, M., Galle, P. R., and Neurath, M. F. (2007). Exacerbation of ulcerative colitis after rituximab salvage therapy. *Inflamm. Bowel Dis.* 13, 1365–1368.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Gray, M., Miles, K., Salter, D., Gray, D., and Savill, J. (2007). Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc. Natl. Acad. Sci. U. S. A.* 104, 14080–14085.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Horikawa, M., Minard-Colin, V., Matsushita, T., and Tedder, T. F. (2011). Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice. *J. Clin. Invest.* 121, 4268–4280.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Hussain, S., and Delovitch, T. L. (2007). Intravenous transfusion of BCR-activated B cells protects NOD mice from type 1 diabetes in an IL-10-dependent manner. *J. Immunol.* 179, 7225–7232.

Pubmed Abstract | Pubmed Full Text

Inoue, S., Leitner, W. W., Golding, B., and Scott, D. (2006). Inhibitory effects of B cells on antitumor immunity. *Cancer Res.* 66, 7741–7747.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Iwata, Y., Matsushita, T., Horikawa, M., Dilillo, D. J., Yanaba, K., Venturi, G. M., Szabolcs, P. M., Bernstein, S. H., Magro, C. M., Williams, A. D., Hall, R. P., St Clair, E. W., and Tedder, T. F. (2011). Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 117, 530–541.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Kessel, A., Haj, T., Peri, R., Snir, A., Melamed, D., Sabo, E., and Toubi, E. (2012). Human CD19(+) CD25(high) B regulatory cells suppress proliferation of CD4(+) T cells and enhance Foxp3 and CTLA-4 expression in T-regulatory cells. *Autoimmun. Rev.* 11, 670–677.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Lampropoulou, V., Hoehlig, K., Roch, T., Neves, P., Calderon Gomez, E., Sweenie, C. H., Hao, Y., Freitas, A. A., Steinhoff, U., Anderton, S. M., and Fillatreau, S. (2008). TLR-activated B cells suppress T cell-mediated autoimmunity. *J. Immunol.* 180, 4763–4773.

Pubmed Abstract | Pubmed Full Text

Lanzavecchia, A. (1985). Antigen-specific interaction between T and B cells. *Nature* 314, 537–539.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Lenert, P., Brummel, R., Field, E. H., and Ashman, R. F. (2005). TLR-9 activation of marginal zone B cells in lupus mice regulates immunity through increased IL-10 production. *J. Clin. Immunol.* 25, 29–40.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Matsushita, T., Yanaba, K., Bouaziz, J. D., Fujimoto, M., and Tedder, T. F. (2008). Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J. Clin. Invest.* 118, 3420–3430.

Pubmed Abstract | Pubmed Full Text

Mielke, F., Schneider-Obermeyer, J., and Dorner, T. (2008). Onset of psoriasis with psoriatic arthropathy during rituximab treatment of non-Hodgkin lymphoma. *Ann. Rheum. Dis.* 67, 1056–1057.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Mizoguchi, A., Mizoguchi, E., Smith, R. N., Preffer, F. I., and Bhan, A. K. (1997). Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. *J. Exp. Med.* 186, 1749–1756.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R. S., and Bhan, A. K. (2002). Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 16, 219–230.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Monach, P. A., Schreiber, H., and Rowley, D. A. (1993). CD4+ and B lymphocytes in transplantation immunity. II. Augmented rejection of tumor allografts by mice lacking B cells. *Transplantation* 55, 1356–1361.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Morris, A., and Moller, G. (1968). Regulation of cellular antibody synthesis effect of adoptively transferred antibody-producing spleen cells on cellular antibody synthesis. *J. Immunol.* 101, 439–445.

Pubmed Abstract | Pubmed Full Text

Newell, K. A., Asare, A., Kirk, A. D., Gisler, T. D., Bourcier, K., Suthanthiran, M., Burlingham, W. J., Marks, W. H., Sanz, I., Lechler, R. I., Hernandez-Fuentes, M. P., Turka, L. A., Seyfert-Margolis, V. L., Immune Tolerance

Network ST507 Study Group. (2010). Identification of a B cell signature associated with renal transplant tolerance in humans. *J. Clin. Invest.* 120, 1836–1847.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

O'Garra, A., and Howard, M. (1992). Cytokines and Ly-1 (B1) B cells. *Int. Rev. Immunol.* 8, 219–234.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Olkhanud, P. B., Baatar, D., Bodogai, M., Hakim, F., Gress, R., Anderson, R. L., Deng, J., Xu, M., Briest, S., and Biragyn, A. (2009). Breast cancer lung metastasis requires expression of chemokine receptor CCR4 and regulatory T cells. *Cancer Res.* 69, 5996–6004.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Olkhanud, P. B., Damdinsuren, B., Bodogai, M., Gress, R. E., Sen, R., Wejksza, K., Malchinkhuu, E., Wersto, R. P., and Biragyn, A. (2011). Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4 T cells to T-regulatory cells. *Cancer Res.* 71, 3505–3515.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Pallier, A., Hillion, S., Danger, R., Giral, M., Racape, M., Degauque, N., Dugast, E., Ashton-Chess, J., Pettré, S., Lozano, J. J., Bataille, R., Devys, A., Cesbron-Gautier, A., Braudeau, C., Larrose, C., Soulillou, J. P., and Brouard, S. (2010). Patients with drug-free long-term graft function display increased

numbers of peripheral B cells with a memory and inhibitory phenotype. *Kidney Int.* 78, 503–513.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Parekh, V. V., Prasad, D. V., Banerjee, P. P., Joshi, B. N., Kumar, A., and Mishra, G. C. (2003). B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J. Immunol.* 170, 5897–5911.

Pubmed Abstract | Pubmed Full Text

Pretscher, D., Distel, L. V., Grabenbauer, G. G., Wittlinger, M., Buettner, M., and Niedobitek, G. (2009). Distribution of immune cells in head and neck cancer: CD8+ T-cells and CD20+ B-cells in metastatic lymph nodes are associated with favourable outcome in patients with oro- and hypopharyngeal carcinoma. *BMC Cancer* 9, 292. doi: 10. 1186/1471-2407-9-292

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Qin, Z., Richter, G., Schuler, T., Ibe, S., Cao, X., and Blankenstein, T. (1998). B cells inhibit induction of T cell-dependent tumor immunity. *Nat. Med.* 4, 627–630.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Ravetch, J. V., and Bolland, S. (2001). IgG Fc receptors. *Annu. Rev. Immunol.* 19, 275–290.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Reichardt, P., Dornbach, B., Rong, S., Beissert, S., Gueler, F., Loser, K., and Gunzer, M. (2007). Naive B cells generate regulatory T cells in the presence of a mature immunologic synapse. *Blood* 110, 1519–1529.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Rowley, D. A., and Stach, R. M. (1998). B lymphocytes secreting IgG linked to latent transforming growth factor-beta prevent primary cytolytic T lymphocyte responses. *Int. Immunol.* 10, 355–363.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Sagoo, P., Perucha, E., Sawitzki, B., Tomiuk, S., Stephens, D. A., Miqueu, P., Chapman, S., Craciun, L., Sergeant, R., Brouard, S., Rovis, F., Jimenez, E., Ballow, A., Giral, M., Rebollo-Mesa, I., Le Moine, A., Braudeau, C., Hilton, R., Gerstmayer, B., Bourcier, K., Sharif, A., Krajewska, M., Lord, G. M., Roberts, I., Goldman, M., Wood, K. J., Newell, K., Seyfert-Margolis, V., Warrens, A. N., Janssen, U., Volk, H. D., Soulillou, J. P., Hernandez-Fuentes, M. P., and Lechler, R. I. (2010). Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J. Clin. Invest.* 120, 1848–1861.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Sayi, A., Kohler, E., Toller, I. M., Flavell, R. A., Muller, W., Roers, A., and Müller, A. (2011). TLR-2-activated B cells suppress Helicobacter-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells. *J. Immunol.* 186, 878–890.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Scapini, P., Lamagna, C., Hu, Y., Lee, K., Tang, Q., DeFranco, A. L., and Lowell, C. A. (2011). B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 108, E823–E832.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Schioppa, T., Moore, R., Thompson, R. G., Rosser, E. C., Kulbe, H., Nedospasov, S., Mauri, C., Coussens, L. M., and Balkwill, F. R. (2011). B regulatory cells and the tumor-promoting actions of TNF-alpha during squamous carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 108, 10662–10667.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Shimamura, T., Hashimoto, K., and Sasaki, S. (1982). Feedback suppression of the immune response in vivo. I. Immune B cells induce antigen-specific suppressor T cells. *Cell. Immunol.* 68, 104–113.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Sorrentino, R., Morello, S., Forte, G., Montinaro, A., de Vita, G., Luciano, A., Palma, G., Arra, C., Maiolino, P., Adcock, I. M., and Pinto, A. (2011). B cells contribute to the antitumor activity of CpG-oligodeoxynucleotide in a mouse model of metastatic lung carcinoma. *Am. J. Respir. Crit. Care Med.* 183, 1369–1379.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Stach, R. M., and Rowley, D. A. (1993). A first or dominant immunization. II. Induced immunoglobulin carries transforming growth factor beta and suppresses cytolytic T cell responses to unrelated alloantigens. *J. Exp. Med.* 178, 841–852.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Sun, J. B., Flach, C. F., Czerkinsky, C., and Holmgren, J. (2008). B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit. *J. Immunol.* 181, 8278–8287.

Pubmed Abstract | Pubmed Full Text

Townsend, M. J., Monroe, J. G., and Chan, A. C. (2010). B-cell targeted therapies in human autoimmune diseases: an updated perspective. *Immunol. Rev.* 237, 264–283.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Tretter, T., Venigalla, R. K., Eckstein, V., Saffrich, R., Sertel, S., Ho, A. D., and Lorenz, H. M. (2008). Induction of CD4+ T-cell anergy and apoptosis by activated human B cells. *Blood* 112, 4555–4564.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Watt, V., Ronchese, F., and Ritchie, D. (2007). Resting B cells suppress tumor immunity via an MHC class-II dependent mechanism. *J. Immunother.* 30, 323–332.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Wolf, S. D., Dittel, B. N., Hardardottir, F., and Janeway, C. A. Jr. (1996). Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J. Exp. Med.* 184, 2271–2278.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Yanaba, K., Bouaziz, J. D., Haas, K. M., Poe, J. C., Fujimoto, M., and Tedder, T. F. (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 28, 639–650.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text

Zuniga, E., Rabinovich, G. A., Iglesias, M. M., and Gruppi, A. (2001). Regulated expression of galectin-1 during B-cell activation and implications for T-cell apoptosis. *J. Leukoc. Biol.* 70, 73–79.

Pubmed Abstract | Pubmed Full Text

Zusman, T., Lisansky, E., Arons, E., Anavi, R., Bonnerot, C., Sautes, C., Fridman, W. H., Witz, I. P., and Ran, M. (1996). Contribution of the intracellular domain of murine Fc-gamma receptor type IIB1 to its tumor-enhancing potential. *Int. J. Cancer* 68, 219–227.

Pubmed Abstract | Pubmed Full Text | CrossRef Full Text