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Cancer inflammation and regulatory T cells

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Abstract

Chronic inflammation is essential for cancer growth and metastasis. It follows that factors reducing inflammation would abrogate cancer and restore tissue health. However, roles for anti-inflammatory CD41 regulatory cells (T_{REG}) in cancer are enigmatic and controversial. Our recent data reveal that T_{REG} may function in cancer similarly to inflammatory bowel disease or multiple sclerosis, whereby T_{REG} accumulate but lack potency to restore tissue homeostasis under inflammatory conditions. Interestingly, early life exposures to diverse environmental organisms reinforce a protective T_{REG} phenotype that inhibits cancer. In contrast, hygienic individuals with few exposures earlier in life suffer from a dysregulated T_{REG} feedback loop. Consequently, hygienic subjects have increased risk of malignancy later in life. This cancer condition is reversible by blocking underlying inflammation. Taken together, these data help explain increased inflammation-associated cancer rates in hygienic societies and identify targets to abrogate cancer and restore overall health.

Keywords

gut bacteria; inflammation; TREG cells; cancer

The link between chronic inflammation and cancer has been recognized for many years.[1] However, the precise interaction between inflammatory cells and their elaborated factors and tumor cells remains unknown. Originally, inflammation was believed to be primarily a beneficial host response, representing the body's fight against invading tumor cells. More recent data however suggest just the opposite: that inflammation may be the cause of some cancers and a powerful stimulus for tumor growth and invasion.[1–4] Indeed, there are numerous data supporting the association of chronic long-standing inflammation and increased risk of tumors of the gastrointestinal (GI) tract.[5–8] In addition, systemic non-steroidal anti-inflammatory drug (NSAID) use has been linked with a significant decrease in cancer of the bowel, lung, liver and prostate.[9] However, therapies aimed at restoring homeostasis involve numerous challenges including immune suppression with increased risk

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of pathogenic infections, as well as removing beneficial antitumor effects of immune surveillance.

Balanced Activities of T Cells are Essential for Health

Overall health with ability to control inflammation and restore homeostasis requires adaptation to diverse challenges throughout life.[10,11] Pivotal roles for T lymphocytes in this process of homeostasis were first discovered in mouse models more than a decade ago.[12] A major component of immune tolerance and homeostasis is performed by CD4+ cells expressing CD25, namely regulatory T cells (T_{REG}). T_{REG} prevent immune disorders by suppressing proliferation of reactive T lymphocytes and other destructive immune responses.[11,13–15] Ability of CD4+ T_{REG} to offer protection from other destructive inflammatory sequelae has been convincingly demonstrated during inflammatory bowel disease (IBD) in humans and in mice.[10] However, maintenance of lower bowel homeostasis actually also depends on differentiation and specialization of proinflammatory T-effector cells (T_{EFF}). Along with corresponding anti-inflammatory T_{REG} cells, the CD4+ subsets function together to allow for effective immune responses to diverse GI tract insults in naïve animals. In the bowel, prior exposures to bacteria appear to impart increased T_{EFF} and T_{REG} efficiency to downregulate subsequent infectious challenges and IBD.[11,14–19] T_{REG} functions to inhibit pathology have also been well characterized in autoimmune disorders such as multiple sclerosis (MS).[20] Mouse models of experimental allergic encephalitis (EAE) mimic MS in human patients and show that equilibrium between activities of proinflammatory T_{EFF} cells and anti-inflammatory T_{REG} is needed to sustain overall health. Taken together, T_{REG} are widely viewed as essential to maintain immune homeostasis and restore tissue and whole body health.

T_{REG} are generally regarded as a homogeneous population of cells. However, recent data suggest that the same transcriptional factors involved in T_{EFF} antigen specific polarization may also be involved in programming of matching T_{REG}.[21] According to this paradigm, context-dependent T_{REG} suppress the corresponding type of host T_{EFF} responses, whether these may be dedicated to T helper (Th) type-1 for self or intracellular pathogens, to Th-2 for extracellular pathogens, to Th-17 for self or pathogens, or, finally, to lymphoid follicle stimulating events.[21] Thus, T_{REG} primed toward diverse classes of organisms earlier in life will more efficiently downregulate corresponding inflammation later in life, whether that inflammation is driven by Th-1, Th-2 or Th-17 (Fig. 1). This raises the possibility that T_{REG} may be re-directed and applied therapeutically during conditions involving uncontrolled inflammation such as IBD, MS and some types of inflammation-associated cancer.

Foxp3+ T_{REG} Cells Accumulate But Fail to Protect During Inflammatory Disorders

A large body of literature exists demonstrating the efficacy of T_{REG} to suppress inflammation in humans. However, an enigma arises: the presence of large numbers of T_{REG} cells does not assure anti-inflammatory potency, tissue restoration or health, even though properly functioning T_{REG} are clearly protective in humans and in mice. To address this paradox, molecular and cellular mechanisms for T_{REG} have been studied using animals

models with antibody-mediated blockade or adoptive transfer of T_{REG}.[22] One challenge to better understand T_{REG} biology has been the lack of T_{REG} identity markers. Transcription factor Foxp3 is critical for T_{REG} functions and therefore has been widely used for phenotyping and localization,[23] albeit with some limitations.

Although adoptive transfer of T_{REG} cures IBD in mice, Foxp3+ T_{REG} cells accumulate in colon and secondary lymphoid organs during IBD with low T_{REG} potency to restore tissue balance (Fig. 2).[24] This observation may be explained by insufficiently low levels of anti-inflammatory cytokine interleukin (IL)-10 in bowel that predispose to a break in immune tolerance. This undermines T_{REG} function and favors proinflammatory Th-17 T_{EFF} cells.[25,26] Likewise, in autoimmune disorders Viglietta *et al*.[27] found a significant decrease in functional efficacy of T_{REG} from blood of human patients with MS. Bettelli *et al*.[20] subsequently showed that Th-17 was crucial in the break in tolerance during MS. Large numbers of T_{REG} accumulate during MS but fail to suppress disease in humans or mice with chronically elevated levels of TNF-α and IL-6.[28]

> Under normal physiological conditions, T_{REG} responses are beneficial to the host to reinforce protective acute inflammation. Afterward, T_{REG} regain anti-inflammatory functions and restore tissue homeostasis and health.

Recent work from our laboratory found robust accumulations of Foxp3+ T_{REG} surrounding colonic polyps as well as rapidly growing mammary tumors in mice (Fig. 2).[29] In those mice, Foxp3+ cells accumulated in large numbers in the desmoplastic periphery and local lymph nodes, but failed to protect and instead exacerbated pathology. Blocking inflammation using anti-TNF-α antibody or by supplementation with gut bacteria-primed T_{REG} resulted in rapid tumor regression with commensurate reduction and redistribution of Foxp3+ T_{REG} to intratumoral locations.[29] Remarkably, Salama *et al*.[30] found that patients bearing colonic tumors with a high intratumoral density of FOXP3+ T_{REG} showed a significant improvement in survival. A better understanding of this process to harness potency of T_{REG} to restore normal tissue architecture and homeostasis may provide insight into novel therapies for inflammation- associated cancers.

IL-10-Dependent Functions of T_{REG} Are Pivotal at Environmental Interfaces Such as Colon, Lung and Skin

Although Foxp3+ T_{REG} are best known for ability to suppress T_{EFF} proliferation,[31,32] cytokine-dependent suppression by IL-10 and transforming growth factor (Tgf)-β also appear essential to reduce inflammation and facilitate wound repair. IL-10 production by T_{REG} serves to maintain epithelial normalcy in tissues such as GI tract and lungs,[22] despite continual interactions with diverse infectious agents.[16,21] In addition, mice lacking IL-10 are also unable to sustain normal T_{REG} functions needed to mount tolerance.[33] Inter-related roles between T_{REG}, IL-10 and Tgf-β *in vivo* appear to be complexly interdependent and essential for epithelial integrity. Tgf-β normally induces functionally suppressive Foxp3+ T_{REG} from naive T cells in the periphery. However, under conditions of uncontrollable inflammation, IL-6 together with Tgf-β may instead inhibit T_{REG} and re-direct CD4+ cells toward a pathogenic Th-17 phenotype.[20] Likewise, uncontrollable elevations in other

inflammatory cytokines such as IL-4 (Th-2) also block the generation of Tgf-β-induced T_{REG} and instead induce a population of T_{EFF} that produce IL-9; interestingly, the IL-9+ T cells demonstrate no regulatory properties despite producing abundant IL-10.[34] Instead, IL-9 subverts IL-10 antineoplastic functions. Thus, it appears that resident T cells—including resident T_{REG}—may be re-directed toward proinflammatory phenotypes for "rescue" whenever Th-1 or Th-2 fails to restore balance. Taken together, these data from animal models show that uncontrollable inflammation, as in the developing cancer microenvironment, may modulate T_{REG} function. This may help explain apparent T_{REG} accumulation without restoration of immune or epithelial homeostasis.

T_{REG} Are Sufficient to Protect from Inflammation-Associated Carcinogenesis

Relationships between GI tract bacteria and cancer have been studied using mutant mice mimicking human cancers. *Recombinase activating gene 2 (Rag2)*-deficient mice lack functional lymphocytes[35] and are highly susceptible to microbial infections resulting in IBD.[10,36–38] IBD eventually progresses to colorectal carcinoma (CRC) in those animals[37] (Fig. 3). One well-established bacterial etiology for IBD in mice is *Helicobacter hepaticus*[39]— a bacterium also linked with carcinogenic inflammatory responses in both the liver and lower bowel of mice.[37] A closely related bacterial organism, *Helicobacter pylori*, is classified as a carcinogen by the World Health Organization (WHO) for stomach cancer in humans.[6–8] Taken together, *H. hepaticus* infection and innate immune inflammatory events were sufficient for carcinoma. However, strain-matched wild-type (WT) mice did not develop carcinoma after *H. hepaticus* infection, showing that effects of lymphocytes were sufficient to protect from cancer (Fig. 3).[37] In these models, IBD and carcinoma arose only when T_{REG} failed to suppress inflammation and restore epithelial health.

> IL-10-dependent activities of T_{REG} cells were essential to maintain mucosal integrity in the lower bowel. IL-10 served to normalize oncogenic K-ras within epithelia.

Poutahidis *et al*.[40] demonstrated rapid reversibility of established neoplastic epithelial invasion. This work showed that an imbalance between IL-10, Tgf-β and IL-6 modulated T_{REG} phenotype and predisposed to colorectal malignancy. Adoptive transfer of T_{REG} collected from IL-10-deficient mice exacerbated neoplastic peritoneal invasion, whereas T_{REG} from WT mice induced tumor remission. This loss of protection may have been due to decreased potency of IL-10 secretion by T_{REG}, or to an inability to sustain T_{REG} suppressive identity in the absence of IL-10.[29,33,40] In any event, supplementation with exogenous IL-10 served to downregulate IL-6 and oncogenic *K-ras* expression within epithelial cells. Interestingly, administration of exogenous IL-10 in *H. hepaticus*-infected mice increased frequency of IFN-γ-bearing T cells (unpublished data), suggesting that IL-10 reinforced Th-1-mediated tolerance in these murine models. In addition, treatment with IL-10 also significantly reduced Gr-1+ 7/4+ (neutrophils) cells proven necessary for cancer, as neoplastic invasion was reversible using anti-Ly-6G (Gr-1) antibody.[41] Taken together with earlier findings of Kullberg *et al*.,[25,29] we concluded that IL-10 protected against a bacteria-

triggered IL-6-mediated Th-17 host response contributing to IBD-associated cancer development and growth.

T_{REG} Cells Suppressed Established Adenomatous Polyps—Even in Mice Lacking Overt IBD

In most human patients, CRC is not directly linked with IBD. Instead, sporadic CRC arises from intestinal adenomatous polyps that undergo a well-characterized series of genetic mutations[42] rather than from IBD-associated premalignant foci.[43] Mice with a heterozygous mutation in *Apc* ($Apc^{Min/+}$) are predisposed to intestinal adenomas[44] mimicking 85% of sporadic colon cancers in patients. In humans, inactivation of the *Apc* gene and alterations in β-catenin and the *wnt* signaling pathway lead to cancer.[45–50] Amazingly, adoptive-transfer of highly purified CD4+CD25+ T_{REG} abrogated adenomatous intestinal polyps in aged $Apc^{Min/+}$ mice.[29,51–53] This was a T_{REG}-mediated effect, as transfer of purified T_{EFF} cells under the same conditions did *not* suppress polyps and instead exacerbated invasive adenocarcinoma within polyps.[53] T_{REG} under these conditions express IFN-γ but require IL-10 for antineoplastic efficacy. These data supported that purified T_{REG} may adopt hybrid phenotypes under conditions of uncontrollable inflammation.

> Adoptive transfer of T_{REG} cells induced regression of established intestinal polyps. However, T_{REG} from “hygienic” donor animals failed to protect from cancer and instead increased IL-17 levels and carcinogenesis in tumor-prone tissues.

At least some of the antineoplastic benefits of T_{REG} are attributable to reduced inflammation. Although *Min* mice entirely lacked overt IBD, intestinal polyp growth required proinflammatory cytokine TNF-α to sustain oncogenic c-myc levels and tumor growth.[52,53] It is possible that T_{REG} or IL-10 act directly to restore c-myc expression to baseline levels. Attempts at dissecting requirements for T_{REG} utilized targeted depletion of CD25+ cells in young adult mice leading to increased bowel and prostate malignancy commensurate with increased levels of TNF-α, IL-6 and IL-9. Lesions lacked overt inflammation except for mast cells[54] that were previously proven essential in tumorigenesis in *Apc* mutant mice.[55,56] Mast cells may also be important systemically in regional lymph nodes following microbial infections, but subsequent coordination of lymphocyte progenitor and neutrophil recruitment and activation remain poorly understood (Fig. 4).[57–59]

Gut Bacteria-Triggered T_{REG} Protect from Cancer in Extraintestinal Tissues

Surprisingly, earlier infection experiments with *H. hepaticus* in *Min* mice revealed that pathogenic gut bacteria significantly modulate carcinogenesis in extraintestinal tissues (Fig. 4). In those studies, *H. hepaticus* infection increased risk of mammary tumors in susceptible female *Min* mice when compared with uninfected counterparts. Mammary-associated lymph node enlargement after *H. hepaticus* infection may have been due to *H. hepaticus*-stimulated dendritic cells from either systemic activation or local exposure via teat mucosa. In this model, T cells from WT donors with earlier *H. hepaticus* infection had significantly increased potency to protect from not only adenomatous polyps but also mammary tumors.[52] Based on our earlier data, T_{REG} may require IL-10 exposure for cancer suppressive functions. Alternatively, secretion of IL-10 by T_{REG} may be needed for

epithelial balance. Kullberg *et al*.[16] showed *in vivo* and *in vitro* that ability of T cells to produce IL-10 led to their enhanced anti-inflammatory potency after infection with *H. hepaticus*. Taken together, these data suggested that IL-10-dependent functions of T_{REG} limited inflammation, restored epithelia and subsequently protected from cancer.

> Pathogenic gut microbial infections trigger an IL-6 and Th-17-associated break in immune tolerance resulting in IBD. Diverse microbial infections in youth stimulate protective immunity and tolerance and lower likelihood of IBD later in life.

Anti-inflammatory drugs significantly reduce risk of cancer in breast,[29] prostate,[54] lung and skin, even though these tissues may lack overt inflammatory disease. Supplementation with T_{REG} suppresses expression of COX-2[51] previously linked with several cancers in humans.[60] Likewise, T_{REG} also normalizes downstream expression of the *c-myc*[53] oncogene previously linked with breast cancer in women.[61] Indeed, uncontrolled inflammation increases relative risk for breast cancer in women.[62–67] One possible factor that lowers risk for breast cancer in women is prior exposures to microbial products found in soil, untreated water and unpasteurized foods. Exposure to these factors is widely believed to trigger innate immune responses that, in turn, encourage the development of a competent immune responses and subsequently promote constructive host responses to diverse inflammatory triggers during adulthood.[68–71] This is consistent with data from mouse models showing that gut bacteria-triggered T_{REG} are highly potent to suppress extraintestinal carcinogenesis.[52,54,72] In these studies protection from cancer was transferable between animals using purified T_{REG} from IL-10 competent donor mice. These data raise the possibility that T_{REG} may be modulated to protect from cancer.

Protective T_{REG} Functions Are Unified in Seemingly Divergent Disease Phenotypes: Allergies, Autoimmune Disease and Cancer

While improvements in societal cleanliness have reduced the incidence of many serious infections, with increased hygiene there has been a concomitant increase in ailments including autoimmune diseases and some types of cancers.[5,11,14,29,73] The “hygiene hypothesis” model suggests beneficial effects as a result of exposure to infectious agents earlier in life; those individuals have fewer aberrant immune reactions such as allergies and asthma later in life (Fig. 5).[73] Recent evidence suggests survival benefit due to efficient induction of tolerance during infancy.[74] This raises the possibility that periparturient and early life exposures denied during modern sanitization may increase vulnerability to some diseases later in life. Selectively re-introducing less pathogenic forms of microorganisms may protect against inflammation-associated pathology later in life due to infectious or noninfectious causes (Fig. 5).

> Hygienic individuals with a dysregulated IL-10 and T_{REG} feedback loop suffer from uncontrollable inflammation that redirects T_{REG} cells toward a T helper (Th)-17-driven procarcinogenic process.

Thus, efficient immune tolerance limits subsequent tissue damage[75,76] and prevents a host from “inflaming itself to death.” One possibility is that early life bacterial exposures result in more efficient IL-10-dependent T_{EFF} polarization toward Th-1 and robust immune

tolerance.[21] A naïve host with insufficient exposures earlier in life has weakened Th-1, uncontrolled TNF-α, subsequent upregulation of IL-1β and IL-6 and exuberant Th-17 host responses.[20,25,40] In this context, mice lacking IL-10 are unable to sustain Foxp3 expression and functional T_{REG} and unable to mount immune tolerance.[33] In modernized societies, hygienic individuals with a weakened IL-10 and T_{REG} feedback loop consequently suffer uncontrollable inflammatory disorders that re-direct T_{REG} toward a carcinogenic Th-17 phenotype. This cancer condition is readily reversible by blocking underlying inflammation.

> IL-10 stimulated by bacteria and parasites serve to enhance protective immune balance and reinforce epithelial repair and health.

These hypotheses are supported by evidence that risks of inflammation-associated cancer, such as CRC, are increased in societies with rigorous hygiene practices.[5] Murine models of colitis also support this “hygiene hypothesis” paradigm with dependency on IL-10 and T_{REG}[16,17,40,52,72] consistent with the observations of Belkaid and Rouse.[11] Specifically, beneficial cancer-suppressing effects of microbial infections were dependent on IL-10[40,51,52,77] similar to proposed roles for T_{REG} in allergies and many types of autoimmune responses.[14] This is displayed in mouse models of CRC whereby exogenous IL-10 administration is sufficient to restore T_{REG} anti-inflammatory and antineoplastic functions.[40,41] IL-10 arising after bacterial or parasitic challenges may serve to enhance protective immunity through-out the body *via* Th-1 versus Th-2.[5,40,52,54,72] This process may involve antigenic crossreactivity as described with heterologous immunity.[78,79] A robust IFN-γ response may explain greater T_{REG} anticancer potency after gut bacterial infections in mice.[52,72] Antigen specificity in T cells may be targeted against bacteria, even in extraintestinal sites. Using this strategy lifelong protection from cancer may be achieved using probiotic, non-pathogenic or sterile bacterial antigens. Probiotic *Lactobacillus reuteri* was previously shown to restore GI homeostasis in mice and humans.[80,81]

Plasticity and Reciprocal Relationships Between T_{REG} and Th-17

The immune system is designed to recognize and destroy foreign pathogens while preserving immune tolerance to self. T_{REG} cells in particular, play an essential role in maintaining immunological tolerance. A primary peripheral mechanism to adapt to novel immune challenges involves the Th-17 host response. Normally functioning T_{REG} efficiently protect against tissue injury to self or microbes in mouse models. However, under overwhelming proinflammatory conditions, T_{REG} may be redirected toward Th-17 response. Erdman *et al*.[29] showed that prior microbial exposures reinforced immune tolerance conveyed by T_{REG}; in contrast, hygienic rearing of donor mice yielded T_{REG} that exacerbated IL-6 and IL-17 in target tissue. A key role for IL-17 is demonstrable using neutralization with anti-IL17A antibody to inhibit tumorigenesis in mice (data not shown), and this has been shown by others using Min mice.[26] Targeting underlying IL-6 or STAT3 confers improved prognosis in colonic[26,40,82] and breast malignancies (Fig. 4).[83,84]

> Reciprocal roles exist between T_{REG} and Th-17 cells. Chronic inflammatory conditions subvert T_{REG} cell functions, recruit Th-17 cells and factors, and increase risk for malignancy.

Discovery of Foxp3 as a key transcription factor of T_{REG} has provided new insights into T_{REG} biology and revealed unexpected features of this lineage.[85] Recent studies suggest that differences in the ability of T_{REG} to suppress cancer may be due to downregulation of Foxp3. In hygienic donors, uncontrollable inflammation increases risk that T_{REG} will lose regulatory activity and become ex-T_{REG}. Under these conditions, T_{REG} become capable of T_{EFF} activities including the production of IL-17 (during high-ambient levels of IL-6) or perhaps IL-9 (with high levels of IL-4). Likelihood of these ex-T_{REG}[85] increases in hygienic, immunologically naïve animals. There is mounting evidence that the Foxp3-deficient ex-T_{REG} survive with considerable biologic function and converts to Th-17 cells.

During health, Foxp3 appears to maintain the suppressor cell program that allows a relatively small subset of T_{REG} to efficiently preserve immune tolerance.[86] If T_{REG} lose Foxp3 expression under inflammatory conditions, then these cells can develop into T_{EFF} thus explaining massive peritumoral accumulations of Foxp3 cells with only rare Foxp3+ cells within actual tumor parenchyma.[29] If T_{REG} subsets based on T-bet or IRF4 may selectively suppress Th-1 and Th-2 effector subsets,[87,88] then early life exposures to diverse microbiota may favor robust Th-1 that subsequently reduces likelihood of Th-17 or Th-2 responses associated with pathology later in life.

Harnessing the Homeostatic Potency of T_{REG} to Prevent or Treat Cancer

Underlying commonalities in diseases such as IBD and MS suggest that therapeutic approaches exploiting T-cell plasticity may be beneficial for a wide variety of immune-mediated diseases. Downregulating destructive inflammatory processes may be more effective and less toxic than more traditional chemotherapeutic approaches to cancer (Fig. 6). These approaches could be used collectively or alone:

1. Directly stimulate immune tolerance using GI bacteria
    a. probiotic bacteria such as *Lactobacillus reuteri*
    b. apathogenic commensal microbiota
    c. synthetic, sterile microbial antigen preparations
2. Downregulate underlying inflammation
    a. directly block key proinflammatory factors: TNF-α, IL-6 or STAT3 (chemotherapeutic or dietary)
    b. stimulate IL-10 in GI tract: block inflammation, induce immune tolerance and restore epithelial oncogenes to baseline levels
3. Preferentially stimulate beneficial T_{REG} *in vivo*
    a. vitamin A derivatives, *i.e.*, retinoic acid to induce T_{REG}
    b. vitamin D derivatives, *i.e.*, to stimulate T_{REG}
4. Re-program T_{REG} *ex vivo*
    a. “Condition” naïve CD4+ cells *ex vivo* and afterward return to host

Using gut microbiota to enforce an anti-inflammatory T_{REG} phenotype serves to protect from a wide variety of immune-mediated diseases. Certain types of bacteria may uniquely coordinate the intestinal T-cell profile, and thus may be utilized to repair dysfunctional immunity arising from the bowel. Population-based strategies such as probiotic bacteria in foods[81] may ultimately be used to reduce inflammation-associated cancer risk. Healthful benefits of routine yogurt consumption are well documented.

Directly targeting underlying inflammation would serve to block carcinogenic factors as well as to restore T_{REG} to a cancer-protective phenotype. Humans with elevated TNF-α levels have poor cancer outcomes,[1–3] and TNF-α is required for IBD-associated CRC and polyposis in mice.[29,40,52,54,55] Highly efficacious monoclonal antibodies against TNF (infliximab) and TNF-binding fusion proteins (etanercept) have been routinely used in human patients with IBD and arthritis,[89,90] albeit with reduced protection from serious bacterial infections, highlighting importance of overall immune balance for health. Restoration of homeostasis through suppression of TNF-α and reinforcement of T_{REG} cells has been proposed for human patients suffering from IBD and other uncontrolled inflammatory disorders.[89]

Enhanced nutrition or therapeutic delivery of vitamin derivatives may also be used to facilitate induction and recruitment of T_{REG}. Vitamin D promotes the differentiation of T_{REG} and inhibits the differentiation of Th-17 cells.[91] Increased serum levels of vitamin D have been associated with a decreased risk of MS, an autoimmune disorder characterized by a break in tolerance involving upregulation of Th-17. Vitamin D not only favors the induction of T_{REG} but also enhances recruitment to inflammatory sites by modulating dendritic cells. A potent anti-inflammatory T_{REG} phenotype could also be induced in resident T cells by administration of retinoic acid that favors anti-inflammatory Foxp3+ as described for IBD.[92]

Immediate benefits of T_{REG} could be achieved in humans using *ex vivo* stimulation with bacterial antigens to educate and tolerize CD4+ cells.[93] T_{REG} may then afterward be returned to a patient. Many unanswered questions remain to optimize this process for therapeutic purposes, in particular regarding T_{REG} identity and T_{REG} recruitment with polarization toward Th-1 rather than toward Th-17 later in life.

Conclusions

Mouse models that mimic diseases in humans provide evidence unifying immune cell biology in IBD, autoimmune diseases, asthma and cancer. Under normal physiological conditions, T_{REG} responses are beneficial to reinforce protective acute inflammation, and later regain protection from pathology.[72] In immune-mediated diseases, normal homeostatic mechanisms become dysregulated leading to chronic inflammatory disease. During a break in immune tolerance, resident and recruited T cells are redirected toward a pro-inflammatory host response. This inducible proinflammatory condition creates a feedback loop involving STAT-3, IL-6 and IL-17 that further contributes to neoplastic invasion and metastases. Under these conditions, T_{REG} accumulate locally, are not beneficial, and instead contribute to cancer growth. Interestingly, IL-10-dependent activities in the GI tract impart sustained

protection from aforementioned events. Taken together, these observations connect GI tract infections, the immune system, and diverse immune-mediated diseases including autoimmune diseases and cancer, and help explain paradoxical aspects of T_{REG} cell biology in these diseases.

Roles for T_{REG} cells in cancer appear contradictory on the surface.[94–97] We postulate that T_{REG} are beneficial—in fact, pivotal—in sustaining overall health and preventing cancer. Functional benefits of T_{REG} depend on the age and prior health status of the host, and are context within the cancer microenvironment. In immune-competent animals, T_{REG} constructively regulate inflammatory and epithelial growth factors that when uncontrolled lead to cancer growth. Thus, while it is true that T_{REG} accumulate in the vicinity of many types of tumors, T_{REG} also accumulate in large numbers during other uncontrolled inflammatory disease such as with IBD, but nonetheless may ultimately rescue pathology.[24,98] Conditions such as IBD are curable after restoring immune balance and relevant T_{REG} potency in mice. Under these conditions, T_{REG} redistribute within tumors with anti-neoplastic effect.[99–101] Likewise, supplementation with exogenous T_{REG} induces regression of inflammation-associated neoplastic conditions of colon, breast and prostate tissues in our mouse models.[40,52,53,72]

The interplay of carcinogenic host factors is showcased in *Apc*$^{Min/+}$ mice that are genetically susceptible to tumors arising from disruptions of the *wnt* signaling pathway. In these animals, growing intestinal tumor burden coincides with significantly increased levels of inflammatory cytokines IL-9, IL-6 and IL-17 predisposing to cancer growth.[54] Accumulation of improperly functioning T_{REG} under these conditions exacerbates cancer growth. These events accelerate a feedback loop that hastens cancer growth. In *Apc* mutant mice thymic depletion is coincident with polypogenesis[102–104] may be a result and a cause of widespread immune dysfunction impeding T_{REG} and further elevating cancer risk. Supplementary CD4+ T_{REG} collected from immune competent donor animals would, at least initially, be untainted by high levels of inflammation and provide rescue of homeostasis. In support of this, experimental depletion of CD25+ cells in young *Apc*$^{Min/+}$ mice rapidly induces intestinal and prostate carcinogenesis.[54]

The therapeutic strategy of blocking inflammation and redirecting T_{REG} would directly target and inhibit factors required to sustain cancer growth. Permanent health benefits could be achieved using combinations of diet, anti-inflammatory agents and probiotics. *Ex vivo* stimulation of T cells with bacterial antigens or other factors that stabilize T_{REG} phenotype may have utility to rescue individual cancer patients,[93] as demonstrated using adoptive transfer in murine models with IBD or CRC. Many unanswered questions remain to optimize this process, especially regarding T_{REG} identity and T_{REG} recruitment later in life with underlying immune instability. As this field evolves, knowledge of factors that promote terminal differentiation of T_{REG} may one day allow selective inhibition or exacerbation of polarized CD4+ T-cell responses in the clinics.

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Abbreviations

CRC	colorectal cancer
EAE	experimental allergic encephalitis
IBD	inflammatory bowel disease
IFN	interferon
IL	interleukin
MS	multiple sclerosis
T_{EFF}	proinflammatory CD4+ effector T cell
Th	T helper
Tgf	transforming growth factor
TNF	tumor necrosis factor
T_{REG}	anti-inflammatory CD4+ regulatory T cell
Rag	recombinase activating gene (lacking functional lymphocytes)
wt	wild type (genotype)

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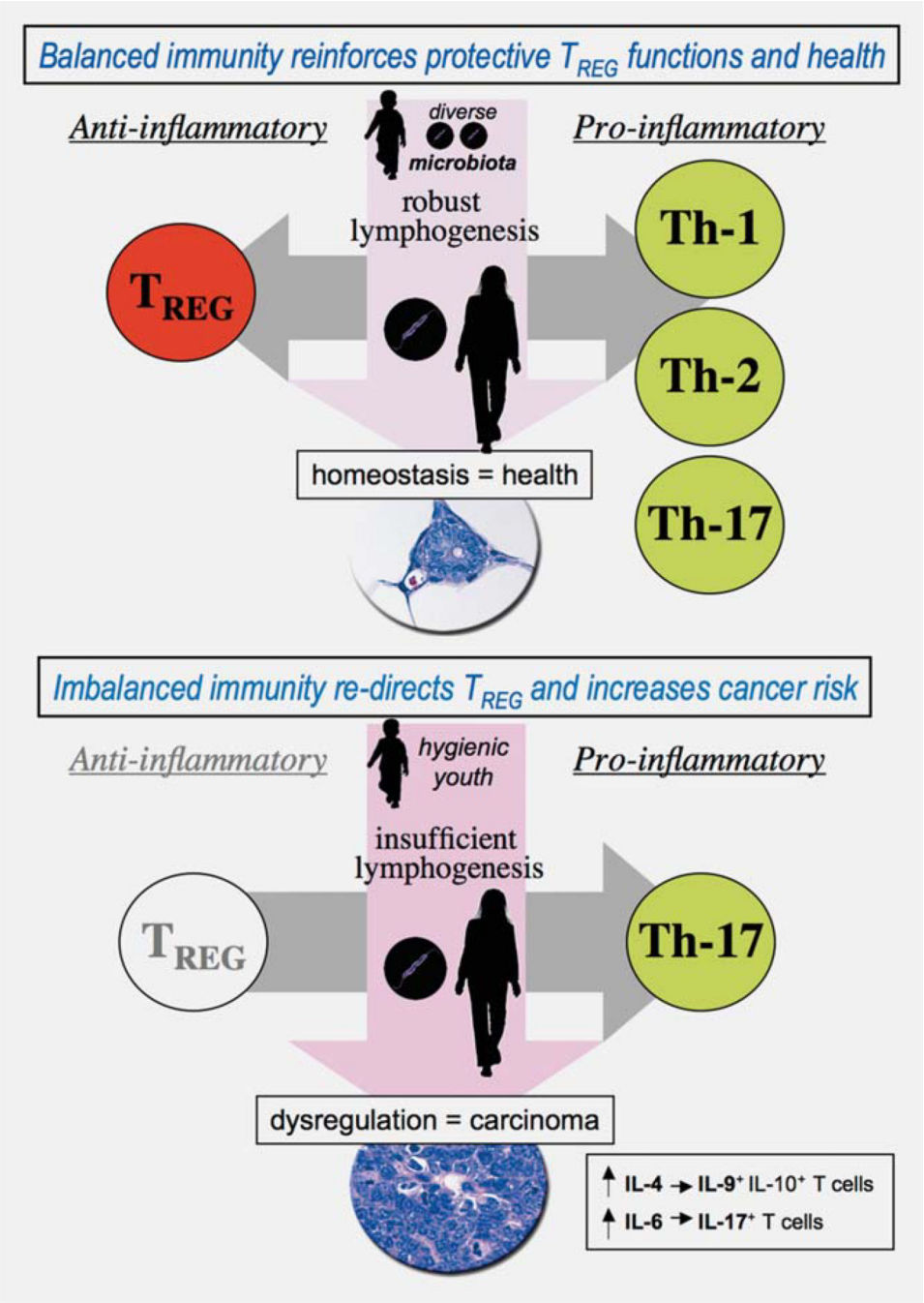

Figure 1.

Immunological profile is pivotal in cancer risk and outcome. Epidemiologic studies have shown that by midlife multiple preneoplastic lesions exist throughout the human body. However, only a few lesions will become cancerous. Data from mouse models suggest that interactions between infectious agents alter immunological profile and modulate cancer outcomes. Microbial exposures may either inhibit or exacerbate carcinogenesis by triggering immune cell activation or secretion of factors. The “hygiene hypothesis” theorizes that exposures to diverse microbiota reinforce innate immunity and T helper (Th)-1 host

responses that lead to balanced and efficient immune responses later in life. By contrast, stringent hygiene conditions deprive the immune system of essential priming rendering T_{REG} cells ineffectual and readily directed toward a carcinogenic Th-17 host response.

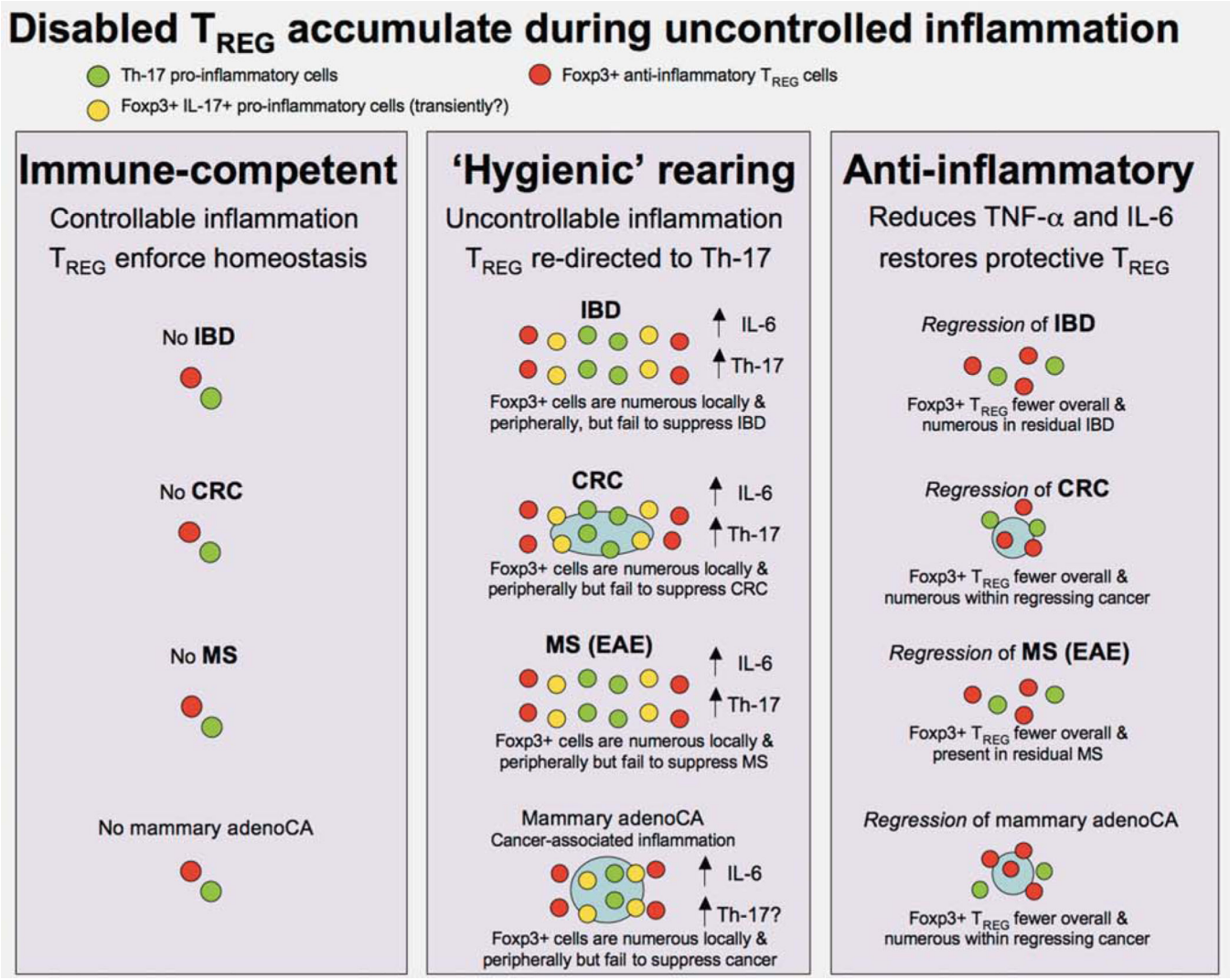

Figure 2.

Reciprocal roles of Th-17 and T_{REG} cells emerge in seemingly diverse diseases: multiple sclerosis (MS), inflammatory bowel disease (IBD) and some types of cancer. Improvements in societal cleanliness have reduced many serious infections but led to an increase in allergies, asthma and autoimmune diseases including multiple sclerosis (MS) and inflammatory bowel disease (IBD). Research using mouse models with experimental allergic encephalitis (EAE), mimicking patients with MS, displays an interleukin (IL)-6-mediated shift in T cells toward pathogenic Th-17. EAE is remedied by downregulation of inflammation and restoration of properly functioning T_{REG} cells. Some types of inflammation-associated cancers such as colorectal carcinoma (CRC) demonstrate similar immunologic underpinnings. Most surprising were our discoveries that cancers lacking overt inflammation in extraintestinal sites, such as prostate and mammary tissue, display similar T_{REG} cell distribution and clinical responses after anti-inflammatory therapy. Broader relevancy of these findings is highlighted by observations that non-steroidal anti-inflammatory drugs (NSAIDs) lower the risk of many cancers including nor only CRC but

also cancers of prostate, lung and breast in humans. Hygienic rearing increases risk of uncontrollable Th-17 host response.

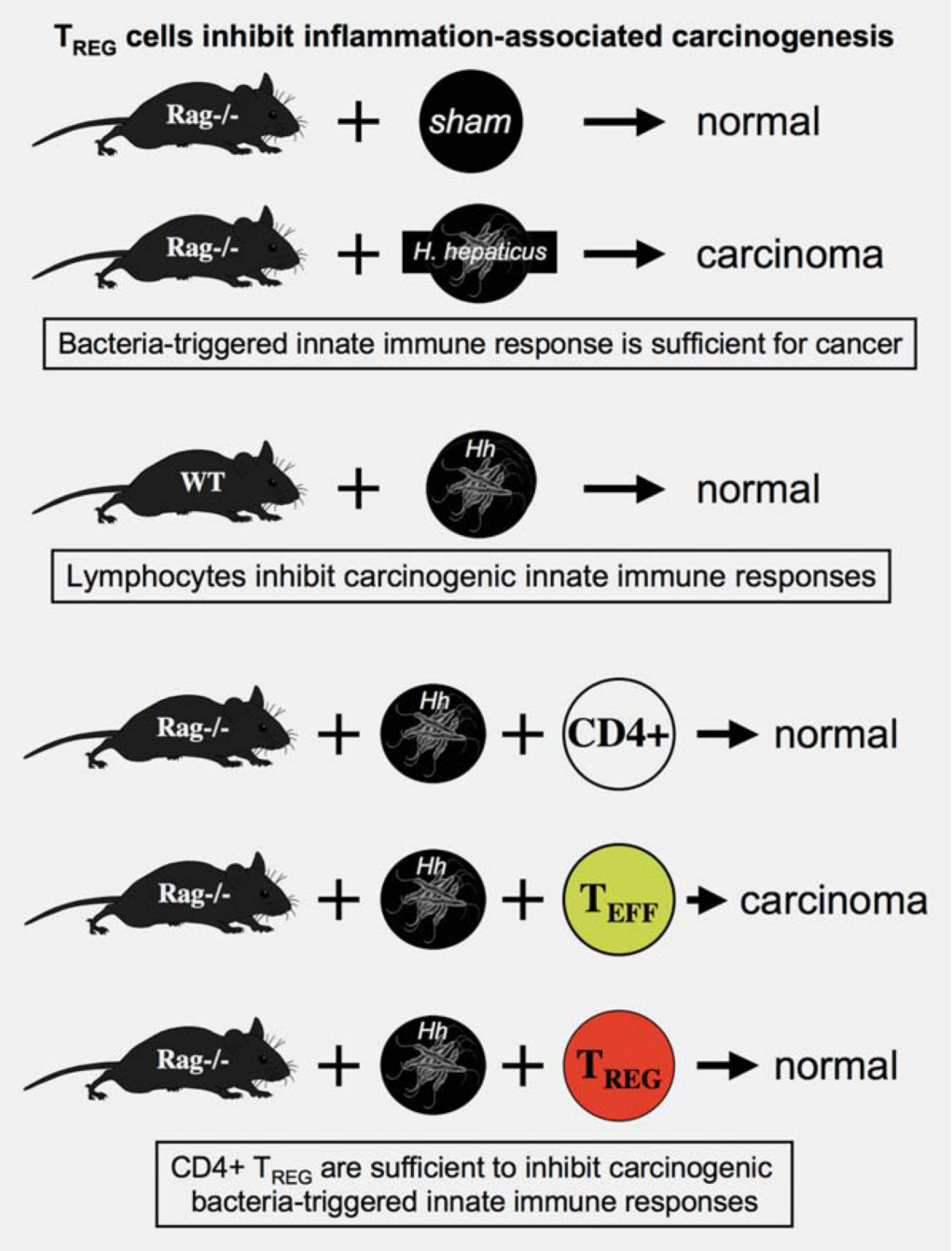

Figure 3.

T_{REG} cells suppress deleterious immune-mediated pathology and afterward restore epithelial homeostasis. Similarities in roles for T_{REG} in inflammation and carcinogenesis emerge during bacteriatriggered inflammatory diseases of the gastrointestinal (GI) tract. Gastritis-associated stomach cancer in humans, for example, is caused by infection with *Helicobacter pylori*, which is classified as a carcinogen by the World Health Organization (WHO). Transgenic mouse models have been widely employed to mimic GI tract inflammation and cancer in humans. Rag2-deficient mice entirely lacking functional lymphocytes are highly

susceptible to inflammation-associated carcinoma after infection with closely related bacteria, *H. hepaticus*. These data from mice indicated that innate immune inflammatory events were sufficient for carcinoma. However, wild-type animals did not develop carcinoma after *H. hepaticus* infection, showing that effects of lymphocytes were sufficient to protect from cancer. Adoptive transfer of highly purified T_{REG} cells was sufficient to prevent cancer in Rag−/− mice. Inflammatory bowel disease (IBD) and carcinoma arose only when T_{REG} failed to suppress inflammation and restore epithelial homeostasis.

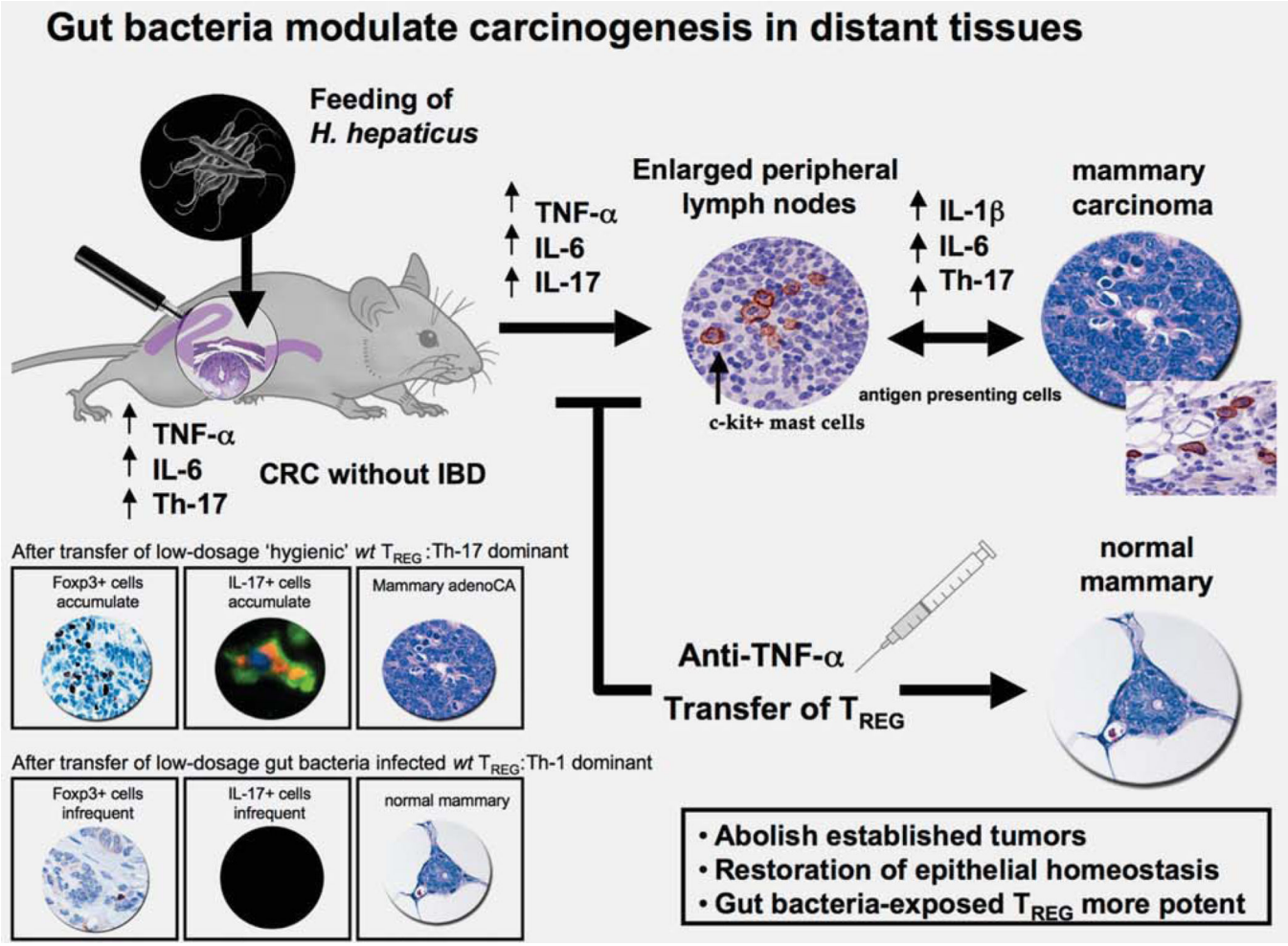

Figure 4.

Gut microbial infections modulate systemic immune events and outcome of carcinogenesis throughout the body. *Helicobacter hepaticus* colonizes the large intestine of C57BL/6 $Apc^{Min\backslash-}$ mice genetically prone to adenomatous intestinal polyps without causing overt typhlocolitis. Nonetheless, malignant transformation of adenomatous polyps is enhanced after infection with *H. hepaticus*, even in the absence of overt IBD. Surprisingly, *H. hepaticus* colonization in the lower bowel greatly accelerated carcinogenesis in extraintestinal tissues such as the mammary gland. The tumorigenic effect coincided with a generalized enlargement of the lymph nodes and an elevation of proinflammatory cytokines including TNF-α, interleukin (IL)-6 and IL-17. Interestingly, innate immunity was sufficient for this carcinogenic effect. Mast cells were a feature of intestinal polyps, as well as mammary and prostate carcinogenesis in murine models.

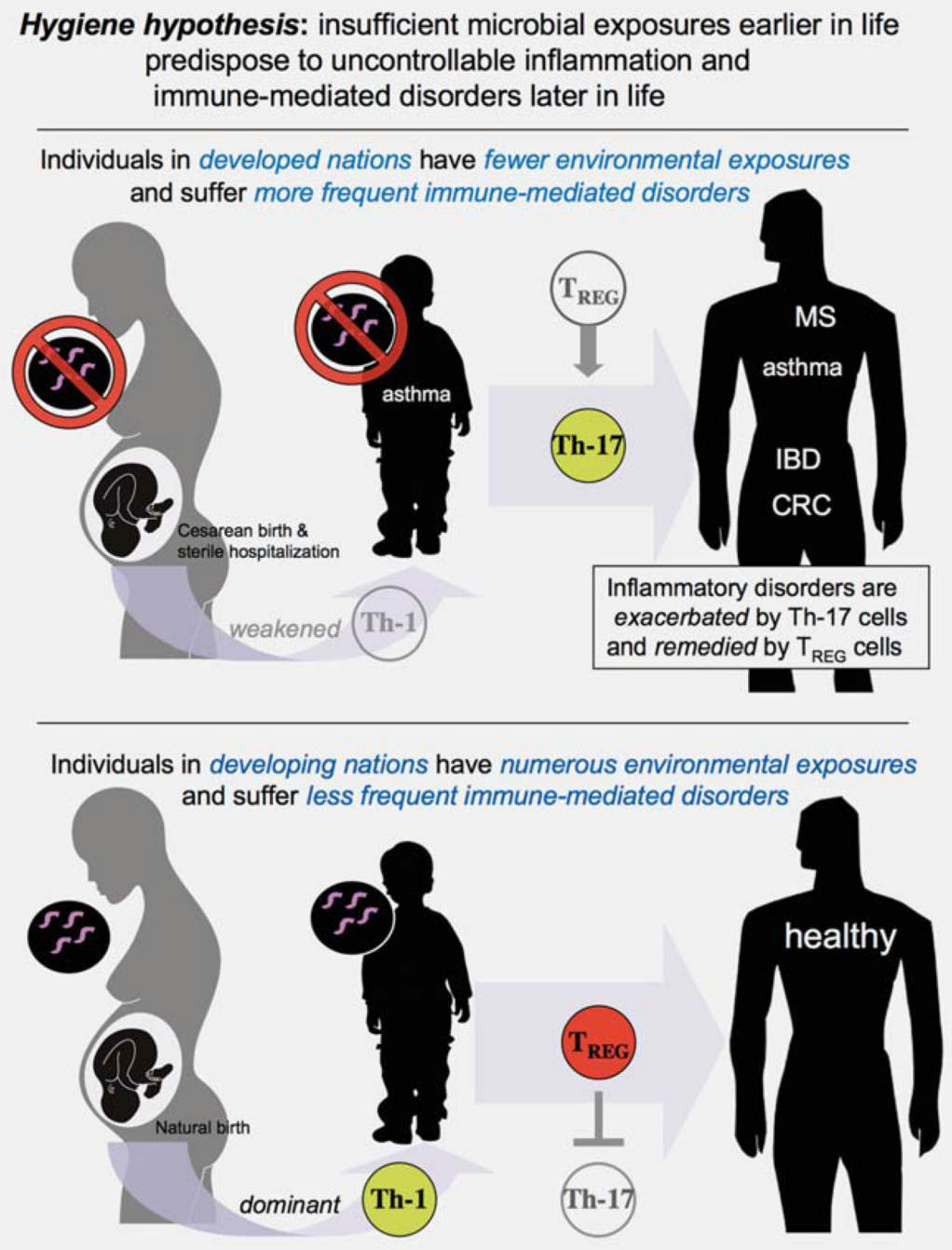

Figure 5.

Insufficient microbial exposures earlier in life predispose to uncontrollable inflammatory disorders later in life. Modern sanitization has reduced the incidence of many serious infections. However, individuals living in developed countries with more stringent hygiene practices also suffer from increased incidence of allergies, asthma, autoimmune disorders and some types of inflammation-associated cancer. This is due, at least in part, to dysregulated immune tolerance. Inability of T_{REG} to inhibit autoimmune diseases such as MS depends on levels of IL-6 that redirect toward a Th-17 host response. Likewise, an IL-10

deficiency during IBD leads to break in immune tolerance and a proinflammatory Th-17 response. Hygienic individuals with a weakened IL-10 and T_{REG} feedback loop suffer from uncontrollable inflammation that subsequently redirects T_{REG} cells toward a Th-17-driven procarcinogenic process. In contrast, gut microbial exposures earlier in life may reinforce Th-1 host responses and homeostasis. Taken together, these observations link the immune system, gastrointestinal infections and seemingly divergent downstream phenotypes: IBD, MS and other autoimmune disease and cancer.

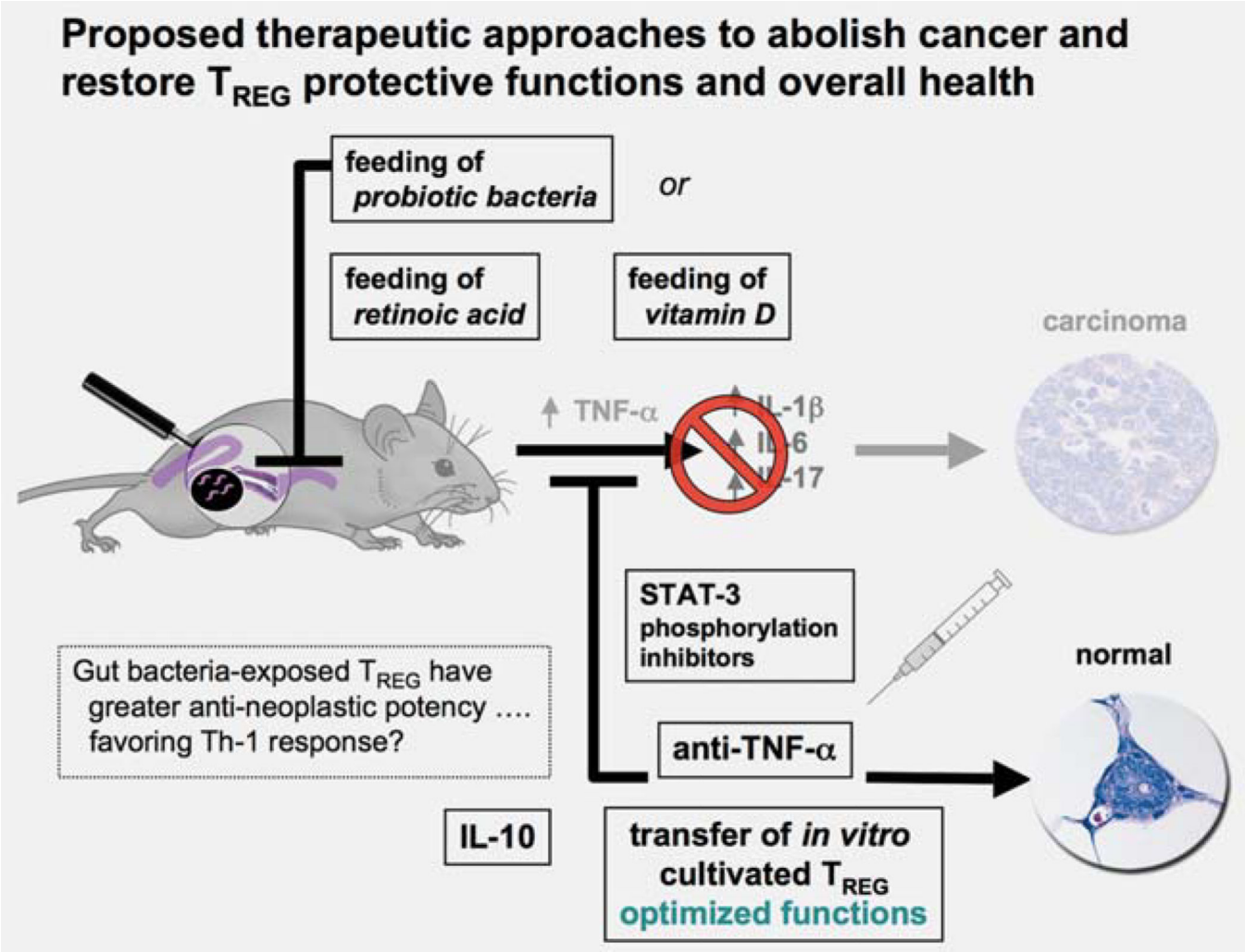

Figure 6.

Proposed strategies to reinforce protective T_{REG} functions to abolish cancer and restore overall health. Cancer immunotherapy strategies to date focus primarily on boosting the antitumoral immunological responses of the cancer patient. In that way, T_{REG} are viewed as an obstacle in tumor immunotherapy and a target for elimination. Recent data suggests that tumor survival and progression are instead vitally dependent on elevated and sustained local and systematic proinflammatory signaling. This raises the possibility that interrupting this proinflammatory loop of signals may be a more direct and constructive route to tumor eradication and restoration of overall health. Thus blocking TNF-α or the STAT3 inflammatory signaling pathway have been shown to rapidly and efficiently abrogate growing cancers in mouse models. Blocking these proinflammatory factors rapidly restores beneficial T_{REG} functions and epithelial homeostasis. An emerging concept of overall health is systemic benefit of gut microbiota to reinforce beneficial effects of IL-10-dependent anti-inflammatory T_{REG} cells. Adoptive-transfer studies in mice have shown the unsurpassed ability of gut bacteria-primed T_{REG} to rapidly restore homeostasis in tissues of the GI tract and also in extraintestinal sites. It was surprising that transfer of T_{REG} actually suppressed established carcinoma in mice. One therapeutic challenge will be to overcome redirection of

T_{REG} in the proinflammatory tumor environment. Harnessing antineoplastic potential may involve *ex vivo* cultivation, expansion and stabilization of human T_{REG} for individualized therapies using autologous transfusions. Broader population-based reinforcement of T_{REG} could be achieved by dietary habits undertaken earlier in life including probiotics or other bacterialbased modalities, or supplementation with retinoic acid and vitamin D.