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# Effector and Memory T cell Responses to Commensal Bacteria

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## Abstract

Barrier surfaces are home to a vast population of commensal organisms that together encode millions of proteins, each of them possessing several potential foreign antigens. Regulation of immune responses to this enormous antigenic load represents a tremendous challenge for the immune system. Tissues exposed to commensals have developed elaborate systems of regulation including specialized populations of resident lymphocytes that maintain barrier function and limit potential responses to commensal antigens. However, in settings of infection and inflammation these regulatory mechanisms are compromised and specific effector responses against commensal bacteria can develop. This review discusses the circumstances controlling the fate of commensal specific T cells and how dysregulation of these responses could lead to severe pathological outcomes.

## Commensal microbiota shape T cell resident homeostasis

The body's epithelial surfaces act as a scaffold to sustain diverse communities of commensal organisms that include bacteria, archaea, fungi, protozoa, and viruses [1-5]. With an estimated composition of 100 trillion cells, commensals outnumber host cells by at least a factor of 10 and encode 100 fold more genes than their host's genome [6]. All barrier sites, including the genital mucosa, skin, airways and gut are constitutively colonized by highly diverse and site-specific flora. The GI tract represents the most abundant commensal niche with a population of more than 1000 individual strains that contain some 3 million unique genes [7,8]. While commensalism is defined in ecology as a relationship where only one party benefits while the other is neutral, many of the bacteria of the GI tract are better described as mutualists, adding tremendous enzymatic and protective capability to the host while taking advantage of the nutrient-rich environment the host provides [9,10]. In particular, commensal bacteria can prevent colonization by pathogenic organisms [11] and control many aspects of host physiology, not the least of which are lymphocytes of the immune system.

T cells are found in large numbers lining barrier surfaces where they are tasked with surveillance against infection while maintaining diplomatic relations with the resident commensal microbiota [12]. As a result, CD4 T cells at these surfaces can adopt multiple inter-related fates associated with the expression of characteristic cytokines and transcription factors [13,14]. Under steady state conditions, the GI tract and gut-associated lymphoid tissue (GALT) is dominated by IL-17A producing Th17 cells, IFNγ producing $Th_1$ cells and Foxp3+ regulatory T (Treg) cells [15,16]. The balance between these populations is tightly controlled by the cytokine milieu, which at barrier surfaces is in part dependent upon dietary

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elements and the microbiota [17-20]. $Th_{17}$ cells are largely limited to barrier surfaces and have been an area of particular interest in the study of mucosal immunology. The protective role of IL-17A is associated with its capacity to induce neutrophil granulopoiesis by stimulating epithelial cells to secrete granulocyte colony-stimulating factor (G-CSF) and drive the recruitment of neutrophils by local stromal cells [21,22]. Additionally, via their capacity to also produce both IL-22 and IL-17A, $Th_{17}$ cells can bolster innate epithelial defense mechanisms and reinforce tight junctions [23-26]. The function of mucosal $Th_1$ cells under steady state conditions remains unclear, but we might speculate that these cells also contribute to the promotion of various aspects of innate mucosal responses. Treg cells also represent a prominent population of resident cells at barrier sites. Treg cells are required for the maintenance of tolerance to both self antigens and innocuous antigens derived from food, commensal bacteria and other environmental sources [27]. Treg cells that line the GI tract can arise from the thymus or be induced locally in response to oral antigen, a process required for the acquisition of oral tolerance [28,29].

The differentiation of T cells at barrier sites into each of these different fates has been associated with the presence of signals derived from the commensal microbiota[30-33] .Notably, the capacity of T cells to produce IL-17A and IFN-γ is severely compromised in absence of commensals in both the gut and skin [16,32,34]. Germ-free mice also tend to harbor higher frequencies of Th2 cells and this too can be reversed by colonization of the mice with a single species of commensal bacteria [35]. In the GI tract, microbial products such as bacterial DNA or defined group of bacteria such as Segmented filamentous bacteria (SFB) can play a dominant role in the promotion of steady-state GI resident Th1 and Th17 cells [16,32]. In the skin reconstitution of germ free mice with *Staphylococcus epidermidis* restored dermal IL-17A[34]. The frequencies, origin and activation of $Foxp3^+$ Treg cells in the skin and gastrointestinal tract are also influenced by the microbiota. In the skin for instance, the absence of commensals is associated with enhanced frequencies of regulatory T cells [34]. In contrast, in the GI tract *B. fragilis* via expression of Polysaccharide A can expand IL-10 producing $CD4^+$T cells and Treg cells at the expense of the differentiation of $Th_{17}$ cells [33, 36-38]. Bacteria of the Clostridium cluster XIV species also promote Treg cell accumulation in the colon, but not small intestine via an increased capacity to promote TGF-β [31]. The gut microbiota can also set the tone of immune responses at distal sites during infection and in some cases contribute to the induction of autoimmune disorders. [31,39-42]

## Antigen-specific responses to commensal organisms at steady state

Independent of differentiation state and function, a critical question associated with T cells residing at barrier sites is their antigen specificity. The microbiota encodes millions of proteins each of them expressing several potential foreign antigens directly associated with inflammatory Pathogen-Associated Molecular Patterns. This enormous antigenic load represents a tremendous challenge for barrier immunity as unwanted responses against these antigens could lead to severe pathological consequences. A central strategy utilized by the mucosal immune system to maintain its homeostatic relationship with the microbiota is to limit contact between luminal microorganisms and the epithelial cell surface. This is accomplished by the establishment of a structural and immunological barrier resulting from the combined action of mucus, IgA, and antimicrobial proteins [12] (Figure 1, panel 1). Further, commensals can promote their own containment and thus mucosal tissue homeostasis by enhancing various aspects of this physical and immunological shield [43-45].

The physical segregation between the host and microbiota is not absolute and the GI epithelium is semi-permeable to allow for the passage of nutrients. Antigens that bypass the mucosal barrier are prevented from systemic traffic and confined to the GALT by the

'mucosal firewall' [46,47]. In the GALT, active mechanisms of tolerance regulate T cell responses and as a result the GI tract represents a privileged site for the induction of tolerance (Figure 1, panel 1).. The regulatory nature of the GI tract is perhaps best demonstrated by experiments examining oral tolerance to food [48]. This phenomenon has long been recognized in both rodent models and humans and is clinically important, as dysregulated T cell responses against food-derived antigens are associated with both Celiac disease and gastrointestinal allergies. Experiments have shown that under steady-state conditions, the predominant antigen-presenting cells of food-derived antigens are dendritic cells (DC) that express the integrin CD103 [28,29]. $CD103^+$ DCs drive the differentiation of $Foxp3^+$Treg cells via their capacity to produce TGF-β [ET1]and to convert retinol to retinoic acid (RA) [28,29,49-52]. Further $CD103^+$ DCs can also induce the expression of gut homing receptors on immune cells, including Tregs, favoring their migration to the GI tract [53]. Because commensal antigens are likely encountered in the context of these tolerogenic responses, it has been proposed that the GI tract also promotes the induction of Treg cells specific for commensal antigens (Figure 1, panel 1). Indeed, some types of commensal bacteria seem to support the differentiation and maintenance of colonic regulatory T cells, though whether these Tregs are specific to commensal-derived antigens is not known [31,54]. T cell receptor transgenic mice made specific to commensal-derived antigens can differentiate to become Treg cells after transfer to lymphopenic hosts [55,56] and $Foxp3^+$ Treg cells specific for commensals have been identified in the GI tract of healthy mice [55]. Aside from the direct maintenance of T cell tolerance, commensal-specific Treg cells may reinforce the mucosal firewall. Treg cells present in the Peyer's Patches of the small intestine have been shown to promote class-switching to IgA in an antigen-specific manner [45,57]. As IgA can directly modulate expression of commensal antigen and mucosal association, this implies that Treg cells may play multiple and complementary roles in controlling the host relationship with the microbiota [58,59]. Commensal-specific T cells transferred into lymphopenic hosts can also differentiate into Th1 and Th17 cells [56], but Th1, Th17 and Treg cells resident in the GI tract are by not by necessity specific to commensal-derived antigens [60,61]. For example, Th17 cells develop in the absence of cognate antigen in mice expressing a single TCR [60,61]. Further, Treg cells can be found in the GI tract of germ-free mice, and albeit significantly reduced, Th17 and Th1 cells are also present in the absence of live commensals [62]. It is worth noting that the diet of germ free mice contains microbial products, including antigens, that can provide surrogate signals to the ones normally provided by the flora and may be responsible for the presence of these cells without live commensals [63]. Alternatively, T cell differentiation and gut homing may occur at a low level independently of signals derived from the commensal flora. Although these data support the notion that the flora promotes the induction and/or maintenance of T cells in the GI tract independent of antigen specificity, they do not exclude that a significant portion of mucosal T cells recognize commensal antigens and the specificity of Th1, Th17 or Treg cells that reside at barrier site under physiological settings remains an important open question.

## Environmentally-induced shifts in the microbiota

Active regulation of immune tolerance to the commensal microbiota is a life-long process, because, in contrast to the host genome, the commensal microbiota is not fixed. Recent studies have shown that while the core metagenome of the gut microbiota is quite stable, species composition and consequently antigenic composition of the flora varies over time in response to a variety of factors such as diet, sanitation, infection and antibiotic use [64,65]. Therefore, the adaptive immune system of the gastrointestinal mucosa must be flexible and constantly reset itself to take into account novel commensal antigens. Changes in the microbiota are particularly pervasive during gastrointestinal infection and inflammation, where the commensal microbiota becomes dominated by commensals with enhanced invasive properties [66]. For instance, the γ-proteobacteria class of bacteria are particularly

selected for growth during a diverse set of inflammatory conditions that ranges from Crohn's Disease (CD), colon cancer and Type 2 Diabetes in humans to *T. gondii* infections and IBD induction in mice [67-73]. Since the γ-proteobacteria family is mostly composed of genera that are opportunistic or obligate pathogens, this effect could be due to the unique ability of these organisms to colonize the inflamed gastrointestinal tract. Indeed, *E. coli* that dominates the GI tract in *T. gondii* infection are skewed toward inflammatory and invasive strains that can contribute to the pathological process during inflammatory responses and infection [70,71,74,75]. This phenomenon is not limited to γ-proteobacteria as many other clinically relevant species, such as *E. faecalis* and *C. difficile* can bloom during bouts of gastrointestinal dysbiosis [76,77]. How the immune system deals with these opportunistic residents and in particular whether tolerance to antigen associated with these bacteria is maintained during inflammation is of central importance to our understanding of mucosal inflammatory disorders.

## Environmentally-induced breach of the 'mucosal firewall'

At homeostasis and in highly controlled experimental settings, the mucosal firewall insures that bacterial translocation is strictly limited to the intestinal tissue and associated lymphoid structures [47]. In mouse models, this system was proven extraordinarily robust as only complete deficiency of key innate and adaptive mucosal immune mechanisms led to systemic commensal specific antibody responses [78]. Accordingly, in mice raised under pathogen free conditions, commensal-specific T cells present outside the mucosa remain naïve despite the presence of commensal antigens in the GI tract [45,79]. However, controlled environments that lack pathogens completely represent a highly artificial setting to understand tissue homeostasis and local immune responses. Indeed, we are now beginning to appreciate that under physiological conditions, systemic translocation of commensals and microbial products beyond the mucosa and GALT is a more common occurrence than initially postulated. Translocation of bacterial products to the systemic circulation has been associated clinically with a diverse set of circumstances such as alcohol abuse and cirrhosis, chronic NSAID use, malnutrition, chronic inflammation, extreme exercise regimens and in particular infections [80-86]. In a number of murine models of gastrointestinal infections, such as *Toxoplasma gondii* and *Yersinia pseudotuberculosis,* immunopathology can induce the translocation of commensal bacteria [70,79,87]. Chronic GI barrier dysfunction is also observed during HIV infection in humans and in SIV infection of rhesus macaques [88-90]. Barrier sites including the gut, skin and airway, are primary sites for infections. It is estimated that a child will suffer 10- 15 diarrheal episodes on average before the age of 5, all of them potentially associated with transient commensal translocation [91,92], which if added together with common skin and lung infections, provides ample opportunity for exposure of the immune system to commensal antigens under inflammatory conditions.

## Commensal-specific T cell responses during infection and inflammation

Infections represent highly volatile situations for the mucosal immune system, as pathogens and commensals will share the same inflamed environment. The potential danger of this situation is illustrated by studies that suggest that oral tolerance to newly introduced food antigens breaks down during acute GI infection [93,94]. Recent evidence suggests that in similar manner, tolerance to commensal derived antigens may be lost during acute infections. In a mouse model of *T. gondii* infection, Treg cells are lost, commensals translocate and the immune system becomes unable to discriminate between commensals and pathogen-derived antigens [79] (Figure 1, panel 2). During this highly Th1 polarized infection, commensal specific T cells also develop as Th1 cells according to cues provided by the inflammatory milieu, rather than Th17 cells or Treg cells, as previous studies have associated with commensal-driven responses [31,32]. The idea that innate inflammatory cues drive the fate of commensal specific T cells rather than their specificity is further supported

by the observation that physical breakdown of the intestinal barrier via the administration of DSS resulted in the activation of commensal-specific T cells that differentiated towards the Th17 fate [79]. Thus, the immune response to gastrointestinal pathogens is associated with parallel responses against commensal-derived antigens that develop according to the inflammatory milieu. The CBir1 commensal antigen followed in this study, is expressed by the Clostridia cluster XIV class of bacteria, known to live in the mucus layer of the intestinal mucosa and is a dominant antigen in Crohn's Disease [31,95]. It will be important to determine with future studies whether these responses are directed against all commensal antigens or are limited to the most prevalent or accessible antigens.

## Commensal-specific memory T cell responses

One remarkable feature of all barrier sites is their ability to efficiently repair after inflammation or breach. In the GI tract, this implies that after acute tissue damage and transiently increased exposure to commensals, physical segregation between the flora and the immune system is rapidly restored. In absence of chronic exposure to antigen, activated lymphocytes can survive long–term as memory populations capable of rapid re-activation and proliferation. Indeed, following gastrointestinal infection with *T. gondii,* commensal-specific CD4 T cells can persist long-term in both the GI tract and secondary lymphoid tissue and maintain the ability to become activated, express Th1 inflammatory cytokines and proliferate upon secondary encounter with their cognate antigen [79].

Much like pathogen-specific CD4 T cells and in contrast to virus-specific CD8 memory T cells, commensal-specific memory T cells declined steadily over time [79,96,97]. As CD4 T cells carry out the complex task of discriminating pathogenic organisms from benign organisms in the face of a constantly changing environment, perhaps development of CD4 memory reflects this necessity for flexibility. An evolving pool of specificities within the Treg and CD4 effector compartment may allow for the maintenance of tolerance and barrier function in the context of fluctuating commensal populations and intermittent infection. Such flexible repertoire has been proposed for mucosal IgA responses that lack the memory characteristics associated with CD8 T cells and are able to respond to flux in the commensal microbiota composition. Indeed, established IgA producing clones are outcompeted by novel anti-bacterial responses allowing the mucosal immune system to respond to a constantly changing microbiota [98].

The physiological consequence of long-term CD4+T cell memory against commensals remains to be addressed. Due to the extraordinary antigenic diversity of the host microbiota at all body surfaces and the prevalence of infections, a significant fraction of memory cells are expected to be commensal specific and could develop over time in response to successive infections and/or various barrier breaches (Figure 2). In support of this hypothesis, healthy human serum contains antibodies specific to skin and intestinal microbiota [82]. Thus, primary exposure to a pathogen in the skin, lung and GI tract is likely to occur in the context of a much broader recall response against commensal bacteria. One possible consequence of these responses may be the induction of heterologous memory wherein antigen-specific responses against previously encountered commensal bacteria could drive the rapid production of inflammatory cytokines, leading to increased protection against secondary infection and associated translocation of commensal bacteria (Figure 2). Colonic resident Th17 cells have been shown to contribute to early protection against enteric pathogens, though whether these cells are specific to commensal antigens is not clear [99]. Further contributing to the possibility of heterologous protection against infection by commensal-specific T cells, a recent study suggests that CD4 T cell clones that are cross-reactive to commensals and viruses are common in healthy patients [100]. On the other hand, aberrant accumulation of commensal specific T cells under defined settings may lead to several pathogenic consequences, such as IBD and psoriasis [101]. Exploration of the antigen-

specificity of the memory cell compartment of lymphocytes residing at all barrier sites would inform us of the potential impact of these commensal-specific T cell responses on tissue physiology and subsequent pathologies. It would be of particular interest to address how responses to conserved bacterial antigens across barrier surfaces impact local and systemic tissue responses over time.

## Commensal-specific T cells and gastrointestinal disorders

Inflammatory Bowel Diseases (IBD) refers to a group of chronic inflammatory disorders affecting the gastrointestinal tract [102]. There are two main clinical forms of IBD: Crohn's disease (CD) that can affect any part of the gastrointestinal tract and ulcerative colitis (UC), in which pathology is restricted to the colonic mucosa [102]. The etiology of these disorders is complex and believed to be the consequence of genetic factors, the host immune system and environmental factors such as the microbiota [103]. Individual genome-wide association studies have revealed that a large number of risk factors are associated with active immune responses and altered barrier function. [104-108]. In light of recent findings, commensal-specific T cells may represent an important component of the disease. In mouse models of colitis, it is well known that the commensal microbiota is necessary for the induction of disease via activation of both innate and adaptive immune mechanisms. Commensal-specific CD4 T cells have been identified in murine models of colitis and in some models are required to drive disease [109-111]. For example, immortalized commensal-specific CD4 T cell clones derived from colitis-prone mice are capable of transferring disease to wild-type mice [112]. Activated T cells can also induce colitis in NOD2 deficient mice in response to antigens carried by commensal bacteria [113]. Studies comparing TCR transgenic cells in the Rag/SCID model of colitis indicate that although commensal specificity is unnecessary for the proliferation and accumulation of CD4 T cells at mucosal sites, cognate antigen responses are required for the induction of colitis [114]. Additionally, germ-free IL-10 knockout mice monocolonized with either *E.coli* or *E. faecalis* were shown to develop CD4 T cell responses against the colonizing bacteria [109]. Further, feeding IL-10 knockout mice a diet high in milk fat is associated with increased $Th_1$ T cell responses to a particular bacteria, *B. wadsworthia,* though whether these are strictly antigen-specific remains unclear [115]., A single genus of bacteria, *Helicobacter,* is sufficient for the induction of colitis in both the IL-10 and Rag/SCID models of colitis and T cells specific to *Helicobacter* are present and sufficient for disease in animal models of IBD [116-118]. Finally, in mice where both IL-10 and TGFβ signaling is deficient in T cells spontaneous colitis occurs that is directly dependent upon the presence of bacteria of the genus *Bacteroides* [119]. One key point taken from these studies is that multiple bacterial types comprising several distantly related bacterial phyla can promote colitis, possibly via the induction of specific CD4 T cell responses, supporting the idea that IBD may develop as a consequence of a broad loss of tolerance to the commensal microbiota [101]. In support of this hypothesis, higher titers of commensal-specific antibodies are found in the serum of Crohn's patients compared to healthy donors and the measurement of antibody responses to a panel of seemingly unrelated commensal-derived antigens is commonly used as a diagnostic for IBD [82,120]. However, commensal specific responses are observed in healthy individuals suggesting that on its own, immunity to commensals is not sufficient for the induction of diseases [100,121]. Thus, under normal settings, effector and memory responses against commensals that have been induced by infections or injuries are likely held in check by the combined effect of the mucosal firewall and active mechanisms of tolerance. IBD, on the other hand, could be the result of the environmental activation of commensal-specific T cells in the context of a genetic predisposition for intestinal pathology and in particular defects in repair and immune regulation. This multiple hit mechanism of disease induction is supported by data that shows mucosal viral infection, the commensal microbiota and diminished Paneth cell function due to reduced expression of ATG16L1, converge to increase disease severity in experimental

colitis [122]. However, despite clear connections between commensal-specific T cell responses and IBD, more remains to be done to understand how immunity to commensals could be causative in disease and how commensal-specific effector T cells are regulated under homeostatic conditions (Figure 1, Panel 3).

### Concluding remarks

Adaptive immunity, as defined by the presence of lymphocytes with re-arranged antigen receptors of near infinite specificity, is a characteristic of organisms that carry complex populations of microbial symbionts upon their mucosal surfaces. One might speculate that the co-evolution between the adaptive immune system and commensal microbiota was primarily driven by the difficulty of maintaining and controlling such a complex relationship. However, barrier surfaces are not static and are often perturbed by environmental or infectious challenges, causing changes to the commensal microbiota and increasing tissue permeability. In Westernized countries, increased used of antibiotics, reduced worm infections and drastic changes in nutrition have imposed massive changes in our relationship with these organisms. Our understanding of commensal-immune interactions under these highly fluctuating circumstances is still in its infancy and much remains to be understood about commensal-specific responses and their consequences for human health.

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## Highlights

- The GI tract prevents systemic T cell responses against commensal bacteria
- Infection can induce changes in the composition of commensals and translocation
- Infection induces differentiation of effector/memory commensal-specific T cells
- Commensal-specific T cells are present and may contribute to IBD

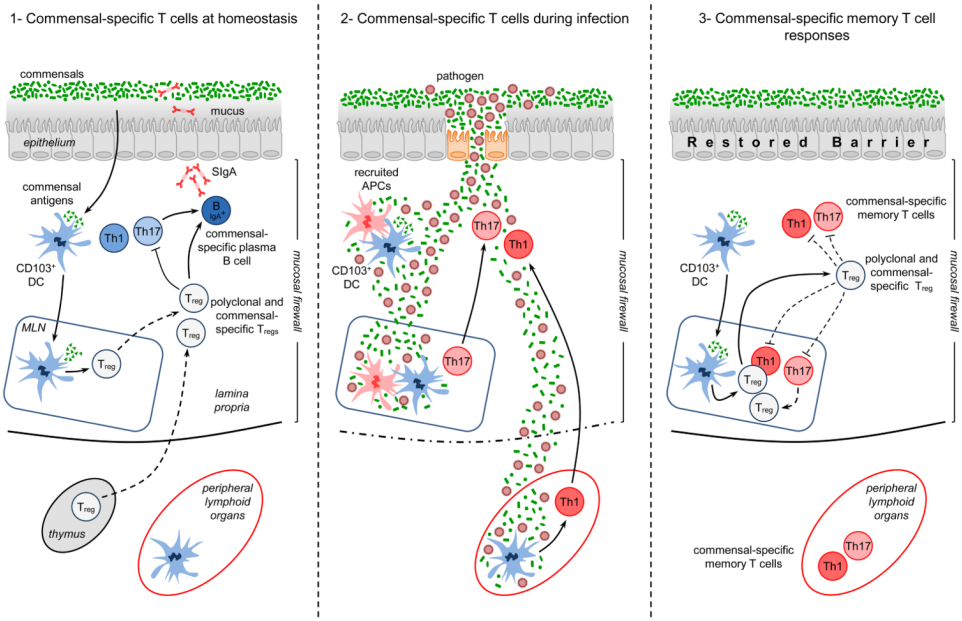


**Figure 1.**

Commensal-specific T cells at homeostasis and during infection: 1) At homeostasis – commensal and food antigens are presented to T cells by $CD103^+$ T cells that have trafficked to the mLN from the lamina propria. Presentation by these DCs to commensal-specific T cells may lead to the differentiation of commensal-specific T regulatory cells (Tregs). Commensal-specific Treg cells traffic to the lamina propria and Peyer's Patches, where they, along with polyclonal Tregs, can regulate effector T cell responses and induce class switching and IgA production from resident commensal-specific B cells, reinforcing the commensal barrier. Critically, the combination of the epithelial barrier, mucus layer, IgA and regulatory DCs and T cells comprises the 'mucosal firewall', which limits the passage of commensal and food-derived antigens to the Gut-Associated Lymphoid Tissue (GALT), preventing untoward activation and pathology. 2) During GI infection – Invasion of the intestinal barrier can cause inflammation and tissue damage. This pathology can disrupt the mucosal firewall and allows for systemic translocation of commensal organisms and their associated antigens. During times of translocation, the host immune system becomes unable to discriminate between commensal and pathogen-derived antigens and therefore commensal-specific T cell responses mimic responses to the invasive pathogen. 3) After clearance of the infection –the intestinal barrier reforms and the mucosal firewall is restored perhaps preventing the chronic activation of commensal-specific memory T cells. Treg cells may regulate commensal specific responses either directly or via the modulation of resident dendritic cells.

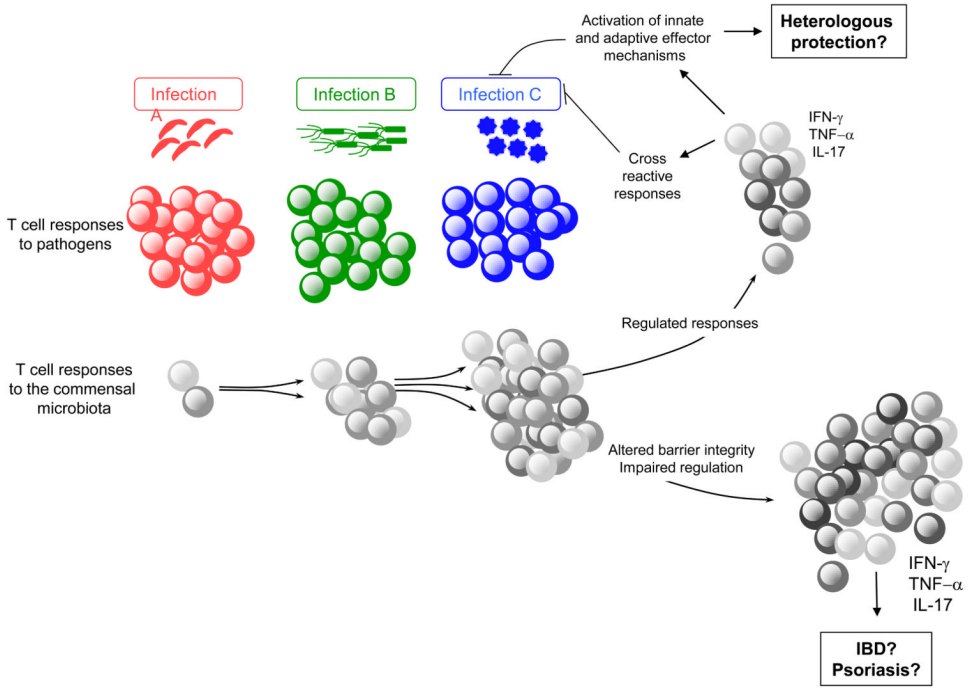


**Figure 2.**

Potential consequences of commensal-specific memory T cells: During infection at barrier sites (gut, skin, airway, genital mucosa) immune responses against the invading agent can be associated with specific T cell responses against a large number of coincident commensal antigens. These commensal-specific effector T cell responses can persist as memory cells that upon subsequent infection will be recalled as secondary commensal-specific effectors, alongside the priming of a novel immune response to the invasive pathogen. Therefore, each infection at barrier surfaces represents an additional opportunity for the reactivation of commensal-specific T cells. Given the extraordinary number of commensal antigens, these responses may represent a significant proportion of memory T cells. If properly controlled, commensal-specific effector/memory cells could contribute to protection against infections by promoting innate and adaptive effector mechanisms that assist in the clearance of the pathogen. Further, commensal memory responses could be protective due to cross reactivity with pathogen-derived antigens. In contrast, in situations where commensal-specific T cells become dysregulated due to impaired regulatory pathways and/or barrier function, these T cells could drive chronic pathology such as IBD or psoriasis.