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Anne O'Garra *Editors*

Interleukin-10 in Health and Disease

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Interleukin-10 in Health and Disease

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Preface

The maintenance of immune homeostasis, as well as the induction and resolution of immune responses is tightly controlled by the balanced provision of stimulatory and regulatory signals. Interleukin (IL)-10 was identified in 1989, and is the best understood suppressive cytokines of the immune system. The studies described in this volume provide a large overview of our current knowledge on this factor. The topics discussed range from the implication of IL-10 in human intestinal inflammatory diseases, its role during infectious diseases, the regulation of its expression, the biology of IL-10-producing T and B cells, the molecular properties of its receptor, and the different functions of another cytokine of the IL-10 family, namely IL-22. These chapters emphasize how studies performed in animal models and human diseases complement each other to accelerate the inseparable progresses needed in fundamental and therapeutic concepts. Many questions remain to be answered on how this cytokine contributes to immune regulation, and we hope this book will help to tackle these issues.

Our thanks are due to the authors of this volume, and Anne Clauss as well as Andrea Schlitzberger for their constant support in this project.

Simon Fillatreau
Anne O'Garra

Contents

IL-10 in Humans: Lessons from the Gut, IL-10/IL-10 Receptor Deficiencies, and IL-10 Polymorphisms

Karin R. Engelhardt and Bodo Grimbacher

Abstract Inflammatory bowel disease (IBD) represents a heterogeneous group of gastrointestinal disorders, where commensal gut flora provokes an either (a) insufficient or (b) uncontrolled immune response. This results either in a lack of or in excessive inflammation mainly manifesting as Crohn's disease or ulcerative colitis. IBD commonly presents in adolescence and adulthood and often follows a chronic relapsing course. Genetic and/or environmental factors contribute to the failure of gut immune homeostasis. Genome-wide association studies have identified over 160 susceptibility loci associated with IBD, including polymorphisms in interleukin-10 (IL-10). The anti-inflammatory cytokine IL-10 dampens intestinal inflammation and is therefore a good candidate gene for IBD. Polymorphisms in the IL-10 receptor are also associated with ulcerative colitis presenting in early childhood. Moreover, severe infantile enterocolitis resembling Crohn's disease, caused by loss-of-function mutations in IL-10 and IL-10 receptor, is characterised by a very early onset (usually within the first 3 months of life), unresponsiveness to standard treatment including immunosuppressive therapy, and severe perianal disease with abscesses and fistulas. In these patients, inflammation and polymorphic infiltrates are mainly confined to the colon with very little involvement of the small intestine. Ulceration and granulomas, bloody diarrhoea and failure to thrive also occur. Furthermore, patients may suffer from recurrent fever and respiratory infections. Individuals with IL-10 receptor mutations also experience cutaneous folliculitis and arthritis. Hematopoietic stem cell transplantation is currently the only curative therapy.

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1 Introduction

1.1 The Gastrointestinal Tract (GIT)

In a healthy gastrointestinal tract (GIT), the intestinal microbiome, the intestinal epithelium and gut-associated lymphoid tissue interact with each other in perfect balance. The microbiome consists of 500–1000 different species of bacteria, protozoa and fungi that inhabit the GIT. They usually have a symbiotic coexistence with the host, supplying key nutrients, modulating the energy metabolism and initiating intestinal immune responses (Bäckhed et al. 2005). The intestinal epithelium is in itself crucial for the defence against invading microbes, and also plays a critical role in the development of the gastrointestinal immune response. It represents a physical barrier to limit pathogen entry into the circulation by forming intercellular tight junctions between epithelial cells (Turner 2006). A protective layer of mucus building over the epithelium further limits the interaction between pathogens and epithelial cells. Mucus is produced by goblet cells, which are tightly packed into villi and crypts covering a huge surface area. Goblet cells also produce factors contributing to epithelial repair and regulation of inflammation (Taupin and Podolsky 2003). Villi and crypts further contain Paneth cells, specialised epithelial cells secreting antimicrobial peptides, such as defensins. Thus, the cells of the intestinal epithelium provide both a physical barrier and antimicrobial products as a first line of defence against pathogens, and therefore help limit intestinal tissue damage, controlling and resolving inflammation.

Beneath the epithelium lies the lamina propria, which contains Peyer's patches and diffusely distributed immune cells. When intestinal microbes break through the epithelial barrier, they encounter dendritic cells (DCs) and other cells from the innate immune system, stimulate pattern recognition receptors such as TLRs, and activate antigen-presenting cells (APCs) and early defence mechanisms (Neufert et al. 2010). B- and T-lymphocytes are activated in Peyer's patches and mesenteric lymph nodes, and then home into intestinal tissues. To restrict tissue damage, intestinal APCs secrete only small amounts of proinflammatory cytokines, and T cells are rendered tolerogenic by mechanisms involving IL-10 and TGF-β. These

cytokines drive the development of IL-10-producing regulatory CD4+ T cells (Tregs), which represent the major regulatory cell type in the intestine and are important contributors to immune homeostasis (Barnes and Powrie 2009; Maynard et al. 2007). Dysregulation of immune homeostasis in the gut can lead to diseases such as inflammatory bowel disease (IBD) and colitis; in mouse models, transfer of Tregs can prevent the development of colitis and also cure established disease (Izcue et al. 2006). IL-10-dependent suppressive mechanisms of Tregs include inhibition of expansion and function of conventional T cells, downmodulation of innate immune responses, and suppression of pathogenic Th17 cell responses (Uhlig and Powrie 2005; Huber et al. 2011; Chaudhry et al. 2011). Th17 cells are crucial for host defence at mucosal surfaces, but, if not tightly controlled, can also play a role in chronic inflammatory diseases.

In IBD, the accumulation of innate and adaptive immune cells in intestinal tissues and the lamina propria results in an exaggerated inflammatory response with elevated levels of proinflammatory cytokines and cytokines of the IL-23/Th17 axis. This leads to continued epithelial damage, which, in turn, allows more intestinal microbes to cross the epithelial barrier and activate further immune responses, resulting in an amplification of the inflammatory response (Abraham and Cho 2009). On the other hand, the lack of an acute inflammatory response, including neutrophil recruitment and cytokine production, leads to failure of bacterial and fungal clearance from the intestinal tissue. Macrophages take up the foreign material and form giant cells and granulomas. Pathogen sequestration in granulomas and chronic granulomatous inflammation as a result of impaired acute inflammation and unresolved infections is seen in CD and chronic granulomatous disease (CGD) (Marks and Segal 2008). CGD is an inherited neutrophil disorder with mutations of the NADPH oxidase complex, where neutrophils show impaired bactericidal activity due to defective 'oxidative burst' (Segal et al. 2011). In addition to severe bacterial and fungal infections, these patients develop a CD-like granulomatous enterocolitis (Marks et al. 2009).

1.2 Inflammatory Bowel Disease (IBD)

IBD is a chronic, idiopathic, relapsing disorder of the GIT, mainly manifesting as Crohn's disease (CD) or ulcerative colitis (UC). Both diseases are characterised by chronic inflammation, severe diarrhoea with rectal bleeding, chronic abdominal pain and malabsorption leading to weight loss. They commonly present in adolescence and adulthood and have chronic recurring flare-ups, followed by remission (Podolsky 2002; Sawczenko and Sandhu 2003; Cho 2008). CD and UC differ in the site where the inflammation occurs. In CD, the terminal ileum is predominantly affected, showing transmural inflammation across the entire intestinal wall. In contrast, inflammation in UC spreads from the rectum to the colon and mostly affects mucosal layers (Maxwell and Viney 2009).

The aetiology of IBD is not fully understood. An abnormal intestinal immune response against the commensal gut microflora with an abundance of

inflammatory cytokines is thought to be crucially involved (Xavier and Podolsky 2007; Engel and Neurath 2010; Mizoguchi and Mizoguchi 2008; Elson et al. 2005; Goyette et al. 2007; Fava and Danese 2011). Inadequate control mechanisms leading to this excessive mucosal immune response might be caused by a range of genetic and environmental factors or, most likely, by a combination of both (Neuman and Nanau 2012; Ng et al. 2012). A genetic component to IBD is supported by the familial occurrence of the disease, higher disease prevalence in ethnic groups and concordance of monozygotic twins for IBD (Satsangi et al. 1994; Baumgart and Carding 2007). In adolescents and adults, IBD is probably a multigenetic disease. Over 160 susceptibility loci associated with CD and IBD were identified by genome-wide association studies (Jostins et al. 2012; Franke et al. 2010; Imielinski et al. 2009; Barrett et al. 2008). Genes in these regions are components of innate or adaptive immune responses (Abraham and Cho 2009; Van Limbergen et al. 2009) and have a function in autophagy (*ATG16L1, IRGM*) (Hampe et al. 2007; Parkes et al. 2007; Rioux et al. 2007), the intracellular sensing of bacteria (*NOD2*) (Hugot 2001; Abraham and Cho 2009), regulation of epithelial barrier integrity (Nlrp3 inflammasome, *DLG5*) (Zaki et al. 2011; Stoll et al. 2004) and the unfolded protein and ER stress response (*XBP1*) (Kaser et al. 2011, 2008).

On the side of the insufficient immune response, several monogenetic Mendelian diseases are associated with enterocolitis, such as Wiskott-Aldrich syndrome (mutations in *WASP*) (Thrasher and Burns 2010), NEMO deficiency (mutations in nuclear factor κB essential modulator) (Cheng et al. 2009), XIAP deficiency (mutations in X-linked inhibitor of apoptosis) (Pachlopnik Schmid et al. 2011), immunodysregulation-polyendocrinopathy-enteropathy-X-linked (IPEX) syndrome (mutations in *FOXP3*) (Moraes-Vasconcelos et al. 2008) and CGD (Marks et al. 2009).

As a monogenetic model for the above-described pathophysiology, severe very early- onset forms of IBD have recently been shown to be caused by mutations in interleukin-10 (IL-10) and IL-10 receptor subunits (Glocker et al. 2010, 2009; Begue et al. 2011; Kotlarz et al. 2012; Mao et al. 2012; Moran et al. 2013; Engelhardt et al. 2013).

1.3 Interleukin-10 and Its Receptor

IL-10 is a cytokine that counteracts excessive inflammatory immune responses (Moore et al. 2001). Produced by many cell types, it exerts its anti-inflammatory function by limiting the secretion of proinflammatory cytokines, such as IL-1, interferon γ (IFN-γ) and tumour necrosis factor α (TNF-α) (Fiorentino et al. 1991a, b), and by downregulating the function of APCs, thus providing feedback regulation for proinflammatory T cells (O'Garra and Murphy 2009). In particular, the restriction of inappropriate Th17 cell expansion (Huber et al. 2011; Chaudhry et al. 2011) and the maintenance of immune homeostasis in the GIT are dependent on intact IL-10 signalling (Uhlig and Powrie 2005).

IL-10 binds as a homodimer to its receptor, which is a tetramer formed of two α (IL-10R1) and two β (IL-10R2) chains (Kotenko et al. 1997; Pestka et al. 2004). IL-10R1 is the ligand-binding subunit, whereas IL-10R2 activates a downstream signalling cascade involving the Janus tyrosine kinases Jak1 and Tyk2, and the signal transducer and activator of transcription 3 (STAT3). Ultimately, phosphorylated STAT3 homodimerizes and translocates to the nucleus to promote the expression of IL-10-responsive genes (Donnelly et al. 1999; Weber-Nordt et al. 1996; Williams et al. 2004).

IL-10R1 specifically binds IL-10 and is unique for the IL-10 receptor. It is expressed on many cells of the innate and adaptive immune system (Liu et al. 1994). In contrast, IL-10R2 is also the signal transducing subunit of other cytokine receptors, such as IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 and IL-29 (Commins et al. 2008). In addition to cells of the immune system, it is expressed on epithelial cells, keratinocytes and other cell types (Spencer et al. 1998; Moore et al. 1993).

2 Defects in the IL-10 Signalling Pathway

The first suggestion that IL-10 controls immune responses to the intestinal microbiota was made 20 years ago by Kühn et al. by studying IL-10-deficient mice (Kühn et al. 1993). A few years later, this idea was reinforced by data from studies in the IL-10R2-deficient mouse model (Spencer et al. 1998). Both mouse strains developed severe intestinal inflammation, predominantly in the colon. In a germ-free environment, however, mice did not acquire enterocolitis, showing that uncontrolled immune responses to the gut microbiota were responsible. In recent years, support for the crucial role of IL-10 to provide negative-feedback signalling for the maintenance of intestinal immune homeostasis has also been found in humans. Loss-of-function mutations in *IL-10*, *IL-10RA,* or *IL-10RB* were found in children with severe, therapy-resistant, early onset enterocolitis with predominantly pancolitic inflammation and perianal involvement (Glocker et al. 2010, 2009).

2.1 IL-10 and IL-10 Receptor Polymorphisms in IBD

The first association of IL-10 polymorphisms with IBD in humans was made by a genome-wide association study (GWAS) published in 2008 (Franke et al. 2008b). Single nucleotide polymorphisms (SNPs) near the 3'-untranslated region of *IL-10* were significantly associated with UC. However, even though this region is involved in the regulation of IL-10 expression, no reduced IL-10 production could be shown in the patients. In 2009, Sanchez et al. investigated *IL-10* haplotypes in a paediatric CD cohort, and found six haplotypes that were only present in the patients. One haplotype (GCC) was associated with disease location in the colon, and another (ACC) with presentation in the terminal ileum (Sanchez et al. 2009).

Another study, a meta-analysis of six GWAS of a CD cohort, identified 30 new susceptibility loci, among which was the *IL-10* locus (Franke et al. 2010). Thus, genetic variants of *IL-10* are associated with both, UC and CD, and as such represent a generic IBD locus.

Polymorphisms in genes functionally related to IL-10 have also been found to be associated with UC and CD, such as variants in *STAT3* (Franke et al. 2008a; Barrett et al. 2008) and inducible T-cell co-stimulator ligand (*ICOS-L*) (Barrett et al. 2008). ICOS-L is upregulated in maturing plasmacytoid DCs and drives the generation of IL-10-producing Tregs with suppressive capabilities (Ito et al. 2007). STAT3 is a signalling molecule downstream of IL-10R and is crucial for Th17 cell differentiation, a cell type implicated in IBD (Elson et al. 2007). Furthermore, STAT3 is believed to play an important role in the process of mucosal wound healing and control of intestinal homeostasis, and patients with active CD have increased levels of activated STAT3 in the lamina propria and in intestinal epithelial cells (Neufert et al. 2010). However, patients with heterozygous dominant-negative *STAT3* mutations develop the autosomal-dominant hyper-IgE-syndrome, of which enterocolitis is not part of its phenotype (Holland et al. 2007; Yong et al. 2012).

Recently, polymorphisms in the IL-10 receptor were shown to contribute to the risk of developing IBD in the very young (Moran et al. 2013). Prompted by findings of IL-10R mutations in infantile IBD patients (Glocker et al. 2009), Moran et al. extended the age group and analysed polymorphisms in IL-10RA and IL-10RB by deep sequencing in a cohort of 188 paediatric IBD patients with disease onset <18 years of age or <6 years of age, respectively. In IL-10RB, one SNP was associated with an increased risk for developing CD in the group of <18 year-old individuals. In IL-10RA, two non-synonymous SNPs were found to be associated with an increased risk for developing UC in the group of <6 year-old children. These SNPs were heterozygous and the resulting amino acid changes were not predicted to be deleterious; hence, disease might only be caused together with another genetic variant(s) or upon environmental trigger.

Thus, polymorphisms in IL-10 and IL-10R are associated more generally with IBD and might represent a continuum from rare homozygous, loss-of-function mutations in infantile-onset IBD to polymorphisms that might merely decrease the efficacy of IL-10 signalling in individuals with disease onset later in life, requiring additional, (non-)genetic co-factors.

2.2 IL-10 Deficiency in Humans

To date, five individuals from consanguineous marriages have been identified with homozygous loss-of-function mutations in *IL-10*. Two unrelated patients from Pakistan carry a Gly113Arg mutation (Glocker et al. 2010), and three patients from Kuwait carry a Gly153Asp mutation (Kotlarz et al. 2012). All five patients presented at the age of 3 months or younger with severe, progressive, intractable Crohn's-like colitis with bloody diarrhoea and perianal disease that was refractory

to immunosuppression including steroids and anti-TNF-α antibodies. The two Pakistani patients had active ulcerating enteritis as shown by colonoscopy and biopsy. Perianal fistulae, abscesses, failure to thrive and recurrent feverish infections accompanied the colitis. The only female patient also had rectovaginal fistulae and oral ulcers, and she developed an undefined hearing loss at age 3.5 years. In contrast, the male probands did not have any extraintestinal manifestations.

Immunological workup showed no striking abnormalities in the patients, with the exception of increases in IgM and IgA in some.

2.3 IL-10 Receptor Deficiencies in Humans

Slightly more prevalent than the rare entity of IL-10 deficiency are deficiencies in either subunit of the IL-10 receptor. Mutations have been found in both *IL-10RA* and *IL-10RB,* the genes encoding IL-10R1 and IL-10R2, respectively. Glocker et al. were first to describe three distinct homozygous mutations in four patients with early onset enterocolitis (Glocker et al. 2009). Subsequently, more mutations in the IL-10 receptor were found by different groups (Begue et al. 2011; Kotlarz et al. 2012; Mao et al. 2012; Moran et al. 2013; Engelhardt et al. 2013). In addition to homozygous nonsense and missense mutations, compound heterozygous mutations (Kotlarz et al. 2012; Mao et al. 2012; Engelhardt et al. 2013) and a splice site mutations (Moran et al. 2013) were reported. From a total of 24 individuals with IL-10 receptor deficiency, 11 had mutations in *IL-10RA* and 13 had mutations in *IL-10RB* (Table 1).

Mutated IL-10 receptors were unable to transduce IL-10-induced anti-inflammatory signals, resulting in deficient STAT3 phosphorylation at tyrosine Tyr705. Consequently, patients' PBMCs secreted increased amounts of TNF-α and other proinflammatory cytokines after costimulation of with IL-10 and LPS (Glocker et al. 2009; Begue et al. 2011; Kotlarz et al. 2012; Mao et al. 2012; Moran et al. 2013; Engelhardt et al. 2013). Furthermore, in IL-10RA-mutated B cells, IL-10-induced induction of SOCS3 was reduced (Mao et al. 2012). Thus, immune cells lacking a functional IL-10 receptor were unresponsive to the negative feedback regulation provided by IL-10, leading to hyperinflammatory immune responses in the intestine. In addition, in a patient with a mutation in *IL-10RB*, defective responses to IL-22 in an intestinal tissue sample were found (Begue et al. 2011).

Like IL-10-deficient individuals, patients with a mutation in the IL-10 receptor have a phenotype reminiscent of Crohn's disease, with very early onset usually within the first 3 months of life. Severe enterocolitis and perianal disease characterised by granulomatous inflammation of the colon with little if any small bowel inflammation including the terminal ileum, proctitis, enterocutaneous fistulas and abscesses, mucosal ulcerations in the colon, as well as perianal abscesses, fistulas, ulcers, granulomas, and deep fissures are hallmarks of the disease. Non-specific inflammation with dense polymorphic infiltrates in the colon affects the rectum, sigmoid colon and descending colon (Mao et al. 2012). Bloody diarrhoea and severe

Table 1 Crohn's disease-like features and extraintestinal symptoms of IL-10- and IL-10R-deficient patients

Patient	# in original publication	Sex	Origin or ethnicity	Mutation	Crohn's disease-like features	Extraintestinal symptoms	Immunoglobulin levels
IL-10							
1	Pat 1 (Glocker et al. 2010); Pat 8 (Engelhardt et al. 2013)	F	Pakistan	IL-10 Gly113Arg	Severe colitis	Oral ulcers, moderate hearing loss	Normal
2	Pat 2 (Glocker et al. 2010); Pat 9 (Engelhardt et al. 2013)	M	Pakistan	IL-10 Gly113Arg	Severe colitis	None	Increased IgM and IgA
3	Pat 13 (Kotlarz et al. 2012)	M	Kuwait	IL-10 Gly153Asp	Severe colitis, bloody diarrhoea, perianal disease	None	N/A
4	Pat 14 (Kotlarz et al. 2012)	M	Kuwait	IL-10 Gly153Asp	Severe colitis, bloody diarrhoea, perianal disease	None	N/A
5	Pat 15 (Kotlarz et al. 2012)	M	Kuwait	IL-10 Gly153Asp	Severe colitis, bloody diarrhoea, perianal disease	None	N/A
IL-10RA							
6	Pat B-II-5 (Glocker et al. 2009); Pat 3 (Kotlarz et al. 2012)	F	Lebanon	IL-10RA Gly141Arg	Severe colitis, enteric fistulas, perianal abscesses, ulcerations	Folliculitis	Normal or increased
7	none (Glocker et al. 2009); Pat 4 (Kotlarz et al. 2012)	M	Germany	IL-10RA Thr84Ile	Severe colitis	Folliculitis	N/A
8	Pat 11 (Begue et al. 2011)	M	N/A	IL-10RA Arg262Cys	Pancolitis, perianal lesions, intestinal epithelioid cell granuloma	Folliculitis, bronchial infections	Enhanced

(continued)

Table1 (continued)

Patient	# in original publication	Sex	Origin or ethnicity	Mutation	Crohn's disease-like features	Extraintestinal symptoms	Immunoglobulin levels
9	Pat 5 (Kotlarz et al. 2012)	M	Turkey	IL-10RA Arg101Trp	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis	N/A
10	Pat 7 (Kotlarz et al. 2012)	M	Brazil	IL-10RA[a] Tyr57Tyr/Cys; Arg117Arg/Cys	Severe colitis, bloody diarrhoea, perianal disease	Arthralgia/arthritis, Kawasaki disease	N/A
11	Pat 12 (Kotlarz et al. 2012)	M	Black	IL-10RA Ile169Thr	Severe colitis, bloody diarrhoea, perianal disease	Atopic dermatitis, folliculitis	Enhanced
12	(Moran et al. 2013)	F	Canada	IL-10RA Pro206X	Severe colitis, colonic ulcerations, perianal and rectovaginal fistulas	Cellulitis, scalp eczema and pustules, generalised erythroderma, urinary tract infections, severe arthritis	Normal
13	(Mao et al. 2012)	M	China	IL-10RA[a] Thr84Thr/Ile; Arg101Arg/Trp	Severe colitis, perianal ulcerations and granulomas	Oral ulcers, intermittent pyoderma	Normal
14	Pat 1 (Engelhardt et al. 2013)	F	India	IL-10RA Arg117His	Severe colitis, toxic megacolon, bowel perforation, peritonitis	Recurrent ear infections, oral ulcers	Increased
15	Pat 5 (Engelhardt et al. 2013)	M	Arabic	IL-10RA Ex1_3del	Severe colitis, enteritis	Severe otitis and urinary tract infections, Candida spp. septicemia	Low IgG

(continued)

Table 1 (continued)

Patient	# in original publication	Sex	Origin or ethnicity	Mutation	Crohn's disease-like features	Extraintestinal symptoms	Immunoglobulin levels
16	Pat 6 (Engelhardt et al. 2013)	M	White	IL-10RA[a] Tyr57Tyr/Cys; Val23fsX31	Severe colitis, colon perforation requiring ileostomy	Sinusitis	Increased IgA
17	Pat 7 (Engelhardt et al. 2013)	F	Pakistan	IL-10RA Leu125Arg	Severe colitis, pancreatitis	Recurrent otitis and urinary tract infections	Low IgG
IL-10RB							
18	Pat A-II-3 (Glocker et al. 2009); Pat 1 (Kotlarz et al. 2012)	M	Turkey	IL-10RB Trp159X	Proctitis, perianal abscesses, enterocutaneous fistulas, multifocal ulcerations	Folliculitis, gonarthritis	Increased IgG, IgA and IgM
19	Pat A-II-4 (Glocker et al. 2009); Pat 2 (Kotlarz et al. 2012)	F	Turkey	IL-10RB Trp159X	Proctitis, rectovaginal fistulas	Folliculitis, gonarthritis, renal abscess (E.coli)	N/A
20	Pat 3 (Begue et al. 2011)	M	N/A	IL-10RB Glu141X	Pancolitis, perianal lesions, intestinal epithelioid cell granuloma	Folliculitis, bronchial infections, mastoiditis	Enhanced
21	Pat 6 (Kotlarz et al. 2012)	M	Turkey	IL-10RB Trp159X	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis, autoimmune hepatitis	N/A
22	Pat 8 (Kotlarz et al. 2012)	F	Bangladesh	IL-10RB Cys66Tyr	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis	N/A
23	Pat 9 (Kotlarz et al. 2012)	M	Israel	IL-10RB 3'UTR: c.C52T	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis, arthritis	N/A

(continued)

Table1 (continued)

Patient	# in original publication	Sex	Origin or ethnicity	Mutation	Crohn's disease-like features	Extraintestinal symptoms	Immunoglobulin levels
24	Pat 10 (Kotlarz et al. 2012)	F	Germany	IL-10RB[a] Trp204Trp/X; Ser230Ser/X	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis, dermatitis	N/A
25	Pat 11 (Kotlarz et al. 2012)	F	Poland	IL-10RB Trp204X	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis	N/A
26	Pat 16 (Kotlarz et al. 2012)	M	Turkey	IL-10RB c.331 + 907_574 del	Severe colitis, bloody diarrhoea, perianal disease	Folliculitis	N/A
27	Pat 2 (Engelhardt et al. 2013)	F	Arabic	IL-10RB	Severe colitis	Frequent bronchitis, oral ulcers, gingivitis, EBV- lymphoma, lymphadenopathy, hepatospleno-megaly, bilateral hydronephrosis	High IgA and IgG
28	Pat 3 (Engelhardt et al. 2013)	F	Pakistan	IL-10RB	Severe colitis, anal stenosis	Recurrent ear infecions, hearing loss, EBV-lymphoma	Increased IgA
29	Pat 4 (Engelhardt et al. 2013)	M	Turkey	IL-10RB Gly193Arg	Severe colitis, intestinal adhesions	Life-threatening bacterial infections, septicemia	N/A

[a]comound heterozygous mutation (all oter mutations are homozygous); m, male; f, female; *N/A*: information not available

failure to thrive are part of the colitis picture. Recurrent fever, respiratory and urinary tract infections are also frequently observed. Whether recurrent infections such as otitis media, bronchitis, and pneumonia are an underlying feature of the disease or are due to immunosuppressive treatment and/or malnutrition is unclear. In contrast to IL-10-deficient individuals, patients with mutations in the IL-10 receptor present with chronic cutaneous folliculitis, and some develop arthritis. Further complications reported include bowel perforation, toxic megacolon, oral ulcers, impaired wound healing, eczema, generalised erythroderma, cellulitis, mastoiditis, pancreatitis, peritonitis, a renal abscess caused by *Escherichia coli*, *Candida* spp. septicaemia, and Kawasaki disease (Table 1).

Immunological analyses are basically normal, with normal numbers and function of T cells, B cells, natural killer cells, and neutrophils in most patients. In some individuals, serum levels of IgG, IgM and/or IgA were increased, possibly reflecting the chronic active inflammation, but IgE was always normal. Two affected individuals had low levels of IgG (Table 1).

Several studies looked to see if FoxP3+ regulatory T cells (Tregs) were present in IL-10R-deficient patients (Begue et al. 2011; Mao et al. 2012; Moran et al. 2013). IL-10 secretion is critical for these cells to exert their suppressive function and is crucial for the maintenance of intestinal homeostasis and the control of intestinal inflammation (Izcue et al. 2006; Makita et al. 2004). However, conflicting data exist about the dependency on IL-10 for the maintenance of FoxP3+ Tregs in mice. One study found IL-10-dependent signals to be required for sustained FoxP3 expression and regulatory activity of Tregs (Murai et al. 2009), whereas another study using mice with a Treg lineage-specific IL-10R deletion showed that IL-10 signalling was dispensable for the maintenance, but not the function of Tregs (Chaudhry et al. 2011). IPEX patients with mutations in *FoxP3* lack regulatory T cells and present with multiple autoimmune disorders, including autoimmune enteropathy (Moraes-Vasconcelos et al. 2008; Torgerson and Ochs 2007). Thus, it is conceivable that FoxP3+ Treg cells are absent or reduced in individuals with defective IL-10 signalling. However, levels of FoxP3+ Treg cells were comparable to controls in all patients analysed (Begue et al. 2011; Mao et al. 2012; Moran et al. 2013), showing that development of FoxP3+ Treg cells in humans is independent of intact IL-10 signalling.

In contrast to IL-10R1, which is specific for IL-10, IL-10R2 is also the signal transducing subunit of the receptors for IL-22, IL-26, IL-28A, IL-28B, and IL-29 (Donnelly et al. 2004). Therefore, defective signalling of any of these cytokines due to IL-10R2 deficiency might have additional effects not seen in IL-10R1 deficiency. In particular, as IL-22 regulates innate immune responses and inflammatory processes in the skin (Wolk et al. 2004, 2006), skin disease, such as cutaneous folliculitis, might be a feature more common in patients with *IL-10RB* mutations. However, the frequency of skin disease is comparable in both groups; 7 out of 12 patients with mutations in *IL-10RA* have pyoderma, atopic dermatitis and/or folliculitis, whereas 9 out of 12 patients with *IL-10RB* defects have folliculitis. Another possibility for a difference between the two receptors subunit deficiencies lies in interferon-λ signalling. The λ-interferons IL-28A, IL-28B, and IL-29 are components of the antiviral

defence (Kotenko et al. 2003); thus, defective interferon-λ signalling together with immunosuppressive therapy might predispose to viral infections and EBV-associated lymphomas, as found in two patients with IL-10R2 deficiency. Yet, there is no overt genotype-phenotype correlation in IL-10 receptor deficiency.

2.4 Treatment of IL-10 and IL-10 Receptor Deficiencies

Attempts to ameliorate the symptoms in IL-10- and IL-10 receptor-deficient patients include exclusive enteral nutrition, treatments with immunosuppressive drugs (corticosteroids, azathioprine, methotrexate), immunomodulatory agents (anti-TNF-α monoclonal antibody, thalidomide) and surgical interventions (colectomy and ileostomy). However, despite multiple therapies, no sustained remission or long-term improvement can be achieved. The only curative treatment that has proven successful is allogeneic hematopoietic stem cell transplantation (HSCT), which was performed in 8 of the 26 living patients with mutations in the IL-10 signalling pathway (two patients with IL-10 deficiency, 4 with IL-10R1 deficiency, and 2 with IL-10R2 deficiency) (Glocker et al. 2009, 2010; Kotlarz et al. 2012; Engelhardt et al. 2013). Five patients received an immunosuppressive and myeloablative conditioning regimen, graft-versus-host disease (GvHD) prophylaxis and strict gut decolonisation prior to transplantation, whereas three patients had a milder conditioning regimen. Five individuals had an HLA-matched healthy relative as a donor and three an HLA-matched unrelated donor. Engraftment was achieved in all patients. Despite initial post-transplant complications, all patients improved greatly and seven achieved sustained clinical remission. Complications included skin and gut GvHD, viral infections or reactivations (Rotavirus, systemic HHV-6, RSV, CMV, Adenovirus, and EBV), *Pseudomonas aeruginosa*-induced sepsis, and graft rejection. The latter patient received a second transplant, which resulted in sustained engraftment. One patient with partial bone marrow failure was treated with a stem cell boost leading to bone marrow recovery and clinical improvement of colitis (Kotlarz et al. 2012). Transplanted patients were cured of colitis; ileal and colonic biopsies showed no signs of active inflammation; cutaneous folliculitis and inflammatory anal fistulas healed; patients accepted oral nutrition and gained weight; wound healing improved; and the need for hospitalisation was reduced. Thus, HSCT is a curative therapeutic approach that can provide the affected patients with a normal quality of life. Furthermore, the success of HSCT supports the hypothesis that the control of inflammation in the GIT is dependent on intact IL-10 signalling in hematopoietic cells.

3 Future Perspectives

The Paris Classification for IBD divides paediatric IBD into two groups: individuals diagnosed before the age of 10 years and individuals diagnosed between ages 10 and 18 years (Levine et al. 2011). With the discovery of IL-10/IL-10R

mutations and IL-10R polymorphisms associated with very early onset forms of IBD, it now seems necessary to further subdivide the younger group and create the subgroup of infantile IBD patients diagnosed younger than 1 year of age.

This group of infantile IBD patients should be genotyped for genetic defects in *IL-10* and *IL-10R*, particularly if the colitis has a severe and progressive course and is associated with severe perianal disease and resistance to standard treatment. Allogeneic HSTC should be offered promptly to these patients, as it is currently the only therapy inducing sustained remission without excessive toxicities or severe GvHD. Early treatment could avoid premature death or surgical interventions that might otherwise be necessary.

In future, other therapy options might become available, such as the use of living, genetically modified bacteria for mucosal delivery of immunomodulatory proteins (Neirynck and Steidler 2006). This approach has the advantage of avoiding systemic side effects and being suitable as long-term treatment for chronic intestinal disease. Genetically modified *Lactococcus lactis* that secrete human IL-10 in Crohn's disease were successful in a phase I clinical trial (Braat et al. 2006). Their efficacy could be tested in IL-10-deficient patients, who would benefit most from such an approach.

The rapid advancement of exome and whole genome sequencing in molecular diagnostics might lead to the identification of further mutations in the IL-10 signalling pathway causing chronic inflammation of the GIT.

References

Abraham C, Cho JH (2009) Inflammatory bowel disease. N Engl J Med 361:2066–2078. doi:10.1056/NEJMra0804647

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI (2005) Host-bacterial mutualism in the human intestine. Science 307:1915–1920

Barnes MJ, Powrie F (2009) Regulatory T cells reinforce intestinal homeostasis. Immunity 31:401–411. doi:10.1016/j.immuni.2009.08.011

Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD et al (2008) Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 40:955–962. doi:10.1038/ng.175

Baumgart DC, Carding SR (2007) Inflammatory bowel disease: cause and immunobiology. Lancet 369:1627–1640

Begue B, Verdier J, Rieux-Laucat F, Goulet O, Morali A, Canioni D et al (2011) Defective IL-10 signaling defining a subgroup of patients with inflammatory bowel disease. Am J Gastroenterol 106:1544–1555. doi:10.1038/ajg.2011.112

Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP et al (2006) A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol 4:754–759

Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM et al (2011) Interleukin-10 signaling in regulatory T cells is required for suppression of th17 cell-mediated inflammation. Immunity 34:566–578. doi:10.1016/j.immuni.2011.03.018

Cheng LE, Kanwar B, Tcheurekdjian H, Grenert JP, Muskat M, Heyman MB et al (2009) Persistent systemic inflammation and atypical enterocolitis in patients with NEMO syndrome. Clin Immunol 132:124–131. doi:10.1016/j.clim.2009.03.514

Cho JH (2008) The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol 8:458–466. doi:10.1038/nri2340

Commins S, Steinke JW, Borish L (2008) The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. J Allergy Clin Immunol 121:1108–1111. doi:10.1016/j.jaci.2008.02.026

Donnelly RP, Dickensheets H, Finbloom DS (1999) The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. J Interferon Cytokine Res 19:563–573

Donnelly RP, Sheikh F, Kotenko SV, Dickensheets H (2004) The expanded family of class II cytokines that share the IL-10 receptor-2 (IL-10R2) chain. J Leukoc Biol 76:314–321

Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT (2005) Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev 206:60–76

Elson CO, Cong Y, Weaver CT, Schoeb TR, McClanahan TK, Fick RB, Kastelein RA (2007) Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. Gastroenterology 132:2359–2370

Engel MA, Neurath MF (2010) New pathophysiological insights and modern treatment of IBD. J Gastroenterol 45:571–583. doi:10.1007/s00535-010-0219-3

Engelhardt KR, Shah N, Faizura-Yeop I, Kocacik Uygun DF, Frede N, Muise AM et al (2013) Clinical outcome in IL-10- and IL-10 receptor-deficient patients with or without hematopoietic stem cell transplantation. J Allergy Clin Immunol 131:825–830. doi:10.1016/j.jaci.2012.09.025

Fava F, Danese S (2011) Intestinal microbiota in inflammatory bowel disease: friend or foe? World J Gastroenterol 17:557–566. doi:10.3748/wjg.v17.i5.557

Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A (1991a) IL-10 inhibits cytokine production by activated macrophages. J Immunol 147:3815–3822

Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A (1991b) IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 146:3444–3451

Franke A, Balschun T, Karlsen TH, Hedderich J, May S, Lu T et al (2008a) Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. Nat Genet 40:713–715. doi:10.1038/ng.(a)

Franke A, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G et al (2008b) Sequence variants in *IL10, ARPC2* and multiple other loci contribute to ulcerative colitis susceptibility. Nat Genet 40:1319–1323. doi:10.1038/ng.221.(b)

Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T et al (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 42:1118–1125. doi:10.1038/ng.717

Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F et al (2009) Inflammatory bowel disease and mutations affecting the Interleukin-10 Receptor. N Engl J Med 361: 2033–2045. doi:10.1056/NEJMoa0907206

Glocker EO, Frede N, Perro M, Sebire N, Elawad M, Shah N et al (2010) Infant colitis—it's in the genes. Lancet 376:1272. doi:10.1016/S0140-6736(10)61008-2

Goyette P, Labbé C, Trinh TT, Xavier RJ, Rioux JD (2007) Molecular pathogenesis of inflammatory bowel disease: genotypes, phenotypes and personalized medicine. Ann Med 39:177–199

Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K et al (2007) A genome-wide association scan of non-synonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 39:207–211

Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N et al (2007) STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 357:1608–1619

Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, Chaudhry A et al (2011) Th17 cells express interleukin-10 receptor and are controlled by Foxp3(−) and Foxp3(+) regulatory CD4(+) T cells in an interleukin-10-dependent manner. Immunity 34:554–565. doi:10.1016/j.immuni.2011.01.020

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J et al (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411:599–603

Imielinski M, Baldassano RN, Griffiths A, Russell RK, Annese V, Dubinsky M et al (2009) Common variants at five new loci associated with early-onset inflammatory bowel disease. Nat Genet 41:1335–1340. doi:10.1038/ng.489

Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA et al (2007) Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J Exp Med 204:105–115

Izcue A, Coombes JL, Powrie F (2006) Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. Immunol Rev 212:256–271

Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY et al (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 491:119–124. doi:10.1038/nature11582

Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, Tilg H et al (2008) XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 134:743–756. doi:10.1016/j.cell.2008.07.021

Kaser A, Flak MB, Tomczak MF, Blumberg RS (2011) The unfolded protein response and its role in intestinal homeostasis and inflammation. Exp Cell Res 317:2772–2779. doi:10.1016/j.yexcr.2011.07.008

Kotenko SV, Krause CD, Izotova LS, Pollack BP, Wu W, Pestka S (1997) Identification and functional characterization of a second chain of the interleukin-10 receptor complex. EMBO J 16:5894–5903

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK et al (2003) IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4:69–77

Kotlarz D, Beier R, Murugan D, Diestelhorst J, Jensen O, Boztug K et al (2012) Loss of interleukin-10 signaling and infantile inflammatory bowel disease—implications for diagnosis and therapy. Gastroenterology 143:347–355. doi:10.1053/j.gastro.2012.04.045

Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274

Levine A, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK et al (2011) Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis 17:1314–1321. doi:10.1002/ibd.21493

Liu Y, Wei SH, Ho AS, de Waal R, Malefyt, Moore KW (1994) Expression cloning and characterization of a human IL-10 receptor. J Immunol 152:1821–1829

Makita S, Kanai T, Oshima S, Uraushihara K, Totsuka T, Sawada T et al (2004) CD4 + CD25bright T cells in human intestinal lamina propria as regulatory cells. J Immunol 173:3119–3130

Mao H, Yang W, Lee PP, Ho MH, Yang J, Zeng S et al (2012) Exome sequencing identifies novel compound heterozygous mutations of IL-10 receptor 1 in neonatal-onset Crohn's disease. Genes Immun 13:437–442. doi:10.1038/gene.2012.8

Marks DJ, Segal AW (2008) Innate immunity in inflammatory bowel disease: a disease hypothesis. J Pathol 214:260–266

Marks DJ, Miyagi K, Rahman FZ, Novelli M, Bloom SL, Segal AW (2009) Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. Am J Gastroenterol 104:117–124. doi:10.1038/ajg.2008.72

Maxwell JR, Viney JL (2009) Overview of mouse models of inflammatory bowel disease and their use in drug discovery. Curr Protoc Pharmacol Chapter 5: Unit5.57. doi:10.1002/0471141755.ph0557s47

Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, Weaver CT (2007) Regulatory T cells expressing interleukin 10 develop from Foxp3 + and Foxp3- precursor cells in the absence of interleukin 10. Nat Immunol 8:931–941

Mizoguchi A, Mizoguchi E (2008) Inflammatory bowel disease, past, present, and future: lessons from animal models. J Gastroenterol 43:1–17. doi:10.1007/s00535-007-2111-3

Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR (1993) Interleukin-10. Annu Rev Immunol 11:165–90

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Moraes-Vasconcelos D, Costa-Carvalho BT, Torgerson TR, Ochs HD (2008) Primary immune deficiency disorders presenting as autoimmune diseases: IPEX and APECED. J Clin Immunol 28(Suppl 1):S11–S19. doi:10.1007/s10875-008-9176-5

Moran CJ, Walters TD, Guo CH, Kugathasan S, Klein C, Turner D et al (2013) IL10R polymorphisms are associated with very-early-onset ulcerative colitis. Inflamm Bowel Dis 19:115–123

Murai M, Turovskaya O, Kim G, Madan R, Karp CL, Cheroutre H et al (2009) Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. Nat Immunol 10:1178–1184. doi:10.1038/ni.1791

Neirynck S, Steidler L (2006) Delivery of therapeutic proteins through Lactococcus lactis. Biotechnol Genet Eng Rev 22:253–266

Neufert C, Pickert G, Zheng Y, Wittkopf N, Warntjen M, Nikolaev A et al (2010) Activation of epithelial STAT3 regulates intestinal homeostasis. Cell Cycle 9:652–655

Neuman MG, Nanau RM (2012) Inflammatory bowel disease: role of diet, microbiota, life style. Transl Res 160:29–44. doi:10.1016/j.trsl.2011.09.001

Ng SC, Woodrow S, Patel N, Subhani J, Harbord M (2012) Role of genetic and environmental factors in British twins with inflammatory bowel disease. Inflamm Bowel Dis 18:725–736. doi:10.1002/ibd.21747

O'Garra A, Murphy KM (2009) From IL-10 to IL-12: how pathogens and their products stimulate APCs to induce T(H)1 development. Nat Immunol 10(9):929–932. doi:10.1038/ni0909-929

Pachlopnik Schmid J, Canioni D, Moshous D, Touzot F, Mahlaoui N, Hauck F et al (2011) Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) vs type 2 (XLP-2/XIAP deficiency). Blood 117:1522–1529. doi:10.1182/blood-2010-07-298372

Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA et al(2007) Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet 39:830–832

Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB (2004) Interleukin-10 and related cytokines and receptors. Annu Rev Immunol 22:929–979

Podolsky DK (2002) Inflammatory bowel disease. N Engl J Med 347:417–429

Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A et al (2007) Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 39:596–604

Sanchez R, Levy E, Costea F, Sinnett D (2009) IL-10 and TNF-alpha promoter haplotypes are associated with childhood Crohn's disease location. World J Gastroenterol 15:3776–3782

Satsangi J, Jewell DP, Rosenberg WM, Bell JI (1994) Genetics of inflammatory bowel disease. Gut 35:696–700

Sawczenko A, Sandhu BK (2003) Presenting features of inflammatory bowel disease in Great Britain and Ireland. Arch Dis Child 88:995–1000

Segal BH, Veys P, Malech H, Cowan MJ (2011) Chronic granulomatous disease: lessons from a rare disorder. Biol Blood Marrow Transplant 17(1 Suppl):S123–S131. doi:10.1016/j.bbmt.2010.09.008

Spencer SD, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM et al (1998) The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. J Exp Med 187:571–578

Stoll M, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA et al (2004) Genetic variation in DLG5 is associated with inflammatory bowel disease. Nat Genet 36:476–480

Taupin D, Podolsky DK (2003) Trefoil factors: initiators of mucosal healing. Nat Rev Mol Cell Biol 4:721–732

Thrasher AJ, Burns SO (2010) WASP: a key immunological multitasker. Nat Rev Immunol 10:182–192. doi:10.1038/nri2724

Torgerson TR, Ochs HD (2007) Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells. J Allergy Clin Immunol 120:744–750

Turner JR (2006) Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. Am J Pathol 169:1901–1909

Uhlig HH, Powrie F (2005) The role of mucosal T lymphocytes in regulating intestinal inflammation. Springer Semin Immunopathol 27:167–180

Van Limbergen J, Wilson DC, Satsangi J (2009) The genetics of Crohn's disease. Annu Rev Genomics Hum Genet 10:89–116. doi:10.1146/annurev-genom-082908-150013

Weber-Nordt RM, Riley JK, Greenlund AC, Moore KW, Darnell JE, Schreiber RD (1996) Stat3 recruitment by two distinct ligand-induced, tyrosine-phosphorylated docking sites in the interleukin-10 receptor intracellular domain. J Biol Chem 271:27954–27961

Williams L, Bradley L, Smith A, Foxwell B (2004) Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. J Immunol 172:567–576

Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R (2004) IL-22 increases the innate immunity of tissues. Immunity 21:241–254

Wolk K, Witte E, Wallace E, Döcke WD, Kunz S, Asadullah K et al (2006) IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol 36:1309–1323

Xavier RJ, Podolsky DK (2007) Unraveling the pathogenesis of inflammatory bowel disease. Nature 448:427–434

Yong PF, Freeman AF, Engelhardt KR, Holland S, Puck JM, Grimbacher B (2012) An update on the hyper-IgE syndromes. Arthritis Res Ther 14:228

Zaki MH, Lamkanfi M, Kanneganti TD (2011) The Nlrp3 inflammasome: contributions to intestinal homeostasis. Trends Immunol 32:171–179. doi:10.1016/j.it.2011.02.002

Control of Intestinal Inflammation by Interleukin-10

Abhisake Kole and Kevin J. Maloy

Abstract Twenty years ago, the observation that mice genetically deficient in IL-10 spontaneously developed severe intestinal inflammation, revealed an essential role for IL-10 in the maintenance of intestinal homeostasis. In the intervening period much has been learned about the cellular and molecular factors that are involved in IL-10-mediated regulatory pathways. Elegant experiments with conditional cell-type specific knockout strains have illustrated that IL-10 acts on both myeloid cells and T cells within the intestine to suppress innate and adaptive inflammatory responses and enhance regulatory circuits. Although several distinct cellular sources of IL-10 have been identified in the gut, $CD4^+$ T cells are a crucial non-redundant source of IL-10 for the regulation of intestinal inflammation. Induction of IL-10 may represent an important means through which intestinal microbiota establishes mutually beneficial commensalism with mammalian hosts, but can be exploited by certain pathogens to facilitate infection. Recent genetic studies in humans have confirmed the essential role of IL-10 in preventing deleterious inflammation in the gut. A better understanding of the molecular pathways involved in IL-10 induction and function in the intestine may facilitate the development of novel therapies for inflammatory bowel disease (IBD).

Contents

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1 Introduction

Interleukin (IL)-10 was originally identified as a factor secreted from $CD4^+$ T helper type 2 (Th2) cells, with a potent ability to inhibit cytokine secretion from Th1 cells (Fiorentino et al. 1989). Since then, IL-10 has been found to be secreted from a wide variety of T cell populations, including Th1 cells (Del Prete et al. 1993; Jankovic et al. 2007), $Foxp3^-$ regulatory T (Tr1) cells (Groux et al. 1997), $Foxp3^+$ regulatory T (Treg) cells (Rubtsov et al. 2008) and $CD8^+$ intestinal intraepithelial T cells (Das et al. 2003). In addition, many other leukocytes have been reported to be able to secrete IL-10, including B cells (O'Garra et al. 1992), macrophages (Chirdo et al. 2005; Denning et al. 2007; Rivollier et al. 2012), dendritic cells (DCs) (Chirdo et al. 2005; de Saint-Vis et al. 1998), eosinophils (Kayaba et al. 2001) and neutrophils (Romani et al. 1997), as well as certain non-haematopoietic cells like epithelial cells (Cella et al. 2009; Jarry et al. 2008).

IL-10 acts not only to control Th1 cell function, but also to suppress the proinflammatory activities of a wide variety of other cell types. In the absence of IL-10, mice developed spontaneous inflammation at a variety of environmental surfaces including the skin, lungs and intestines (Kuhn et al. 1993; Rubtsov et al. 2008), suggesting that IL-10 is critical in suppressing aberrant immune responses to innocuous environmental antigens. In this chapter, we review the essential role of IL-10 in intestinal homeostasis, summarise the key cellular sources of IL-10 in the gut and discuss the multiple mechanisms through which IL-10 prevents intestinal inflammation.

2 IL-10 Inhibits Chronic Intestinal Inflammation

The mammalian intestinal tract contains the highest bacterial load in the body, particularly the large intestine, which harbours up to 10^{14} bacteria per gram of faecal content (Hooper et al. 2012). The intestinal immune system engages in a constant and dynamic dialogue with the intestinal microbiota to maintain a mutually beneficial state of intestinal homeostasis (Hooper et al. 2012). However, a breakdown of the regulatory immune networks that control responsiveness to the microbiota can result in aberrant inflammatory responses that in humans may manifest as inflammatory bowel diseases (IBD)—chronic inflammatory disorders that are extremely debilitating and have no current cure (Maloy and Powrie 2011). The first demonstration that IL-10 played an essential role in preventing immunopathology against

the commensal microflora was the observation that *Il10*$^{-/-}$ mice developed a spontaneous, progressive enterocolitis starting in the cecum, ascending colon and transverse colon, that subsequently extended to the descending colon and rectum and eventually also affected the small intestine (Berg et al. 1996; Kuhn et al. 1993). Genetic deletion of *Il10rb*, which encodes an essential subunit of the IL-10R (Moore et al. 2001), also led to spontaneous colitis similar to that seen in *Il10*$^{-/-}$ mice (Spencer et al. 1998). Conversely, treatment of *Il10*$^{-/-}$ mice with exogenous IL-10 prevented disease development when given to weanlings (Berg et al. 1996), although IL-10 treatment of adult *Il10*$^{-/-}$ mice with established disease attenuated, but could not fully reverse, clinical pathology. Intestinal inflammation in *Il10*$^{-/-}$ mice was mediated by Th1 cells, as adoptive transfer of Th1 cells isolated from the inflamed colons of *Il10*$^{-/-}$ mice into IL-10-sufficient mice recapitulated the disease seen in *Il10*$^{-/-}$ mice (Davidson et al. 1996). Furthermore, blockade of IFN-γ, the signature cytokine of Th1 cells, ameliorated disease in *Il10*$^{-/-}$ mice (Berg et al. 1996).

Intestinal inflammation in *Il10*$^{-/-}$ mice is dependent on the presence of intestinal bacteria, although not all bacteria are equally able to induce colitis (Sellon et al. 1998). The opportunistic gram-negative bacterial pathogen, *Helicobacter hepaticus*, was shown to trigger the onset of inflammation in *Il10*$^{-/-}$ mice, although its presence was not strictly necessary (Devkota et al. 2012; Dieleman et al. 2000; Kullberg et al. 1998). *H. hepaticus*-induced typhlocolitis in *Il10*$^{-/-}$ mice, was dependent on Th1 cells and could be inhibited by blockade of IFN-γ (Kullberg et al. 1998). Similarly, *H. hepaticus*-infected wild type (WT) mice also developed typhlocolitis when treated with an anti-IL-10R antibody, and this was associated with robust Th1 and Th17 cell responses in the inflamed intestine (Kullberg et al. 2006). Surprisingly, germ-free (GF) *Il10*$^{-/-}$ mice that were monoassociated with *H. hepaticus* did not develop colitis. However, following colonisation with commensal *Lactobacillus reuteri*, *H. hepaticus* gained the ability to trigger colitis in GF *Il10*$^{-/-}$ mice, suggesting that interactions between microbial species enhanced the virulence of *H. hepaticus* (Whary et al. 2011). It is known that GF mice have underdeveloped mucosal immune systems (Macpherson and Harris 2004) and, since the immune response in *H. hepaticus*-infected *Il10*$^{-/-}$ mice was characterised by potent *H. hepaticus*-specific B and T cell responses (Kullberg et al. 2001, 1998; Whary et al. 2011), colonisation with bacteria capable of inducing lymphoid structures may be a prerequisite for *H. hepaticus*-induced inflammatory responses. In addition, colonization with *L. reuteri* greatly increased intestinal expression of the LPS receptor, TLR4, potentially facilitating increased detection of *H. hepaticus* upon subsequent infection (Whary et al. 2011). Alternatively, in specific pathogen-free (SPF) *Il10*$^{-/-}$ mice, colonization with *H. hepaticus* led to strain-specific blooms of distinct bacterial families, which correlated with the susceptibility of the strain to colitis development (Buchler et al. 2012). However, it remains to be tested whether *H. hepaticus* triggers inflammatory responses against other commensal bacteria.

Despite a strong link between inflammation in *Il10*$^{-/-}$ mice and *H. hepaticus* infection, *Il10*$^{-/-}$ mice maintained in certain *Helicobacter*-free facilities also

developed colitis (Dieleman et al. 2000), indicating that other constituents of the intestinal microbiota can also elicit inflammatory responses in the absence of IL-10. The gram-negative anaerobic bacterium, *Bilophila wadsworthia*, was recently identified as another colitogenic bacterium in *Il10*$^{-/-}$ mice (Devkota et al. 2012). *B. wadsworthia* bloomed in mice fed a diet rich in saturated milk-derived fats, but only caused colitis in the absence of IL-10. Furthermore, unlike *H. hepaticus*, monoassociation with *B. wadsworthia* in GF *Il10*$^{-/-}$ mice led to development of a Th1 cell-mediated colitis (Devkota et al. 2012). Taken together, these results suggest that IL-10 is an essential regulatory cytokine for suppressing inflammation due to dysregulation of microbial communities.

IL-10 was also shown to have protective roles in other non-spontaneous models of colitis. For example, in vivo activation of T cells with anti-CD3 mAb leads to massive induction of T cell-derived TNF-α and IFN-γ, and marked enteropathy (Zhou et al. 2004). However, anti-CD3 mAb treatment also led to induction of IL-10 (Durez et al. 1993; Ferran et al. 1994) that limited the enteropathy, as anti-CD3 treatment of *Il10*$^{-/-}$ mice led to increased levels of both TNF-α and IFN-γ, and to increased epithelial cell apoptosis and intestinal tissue damage, relative to that seen in IL-10-sufficient mice (Zhou et al. 2004). In another model, administration of piroxicam, a non-steroidal anti-inflammatory drug (NSAID) and inhibitor of prostaglandin synthesis, led to rapid, acute colitis in *Il10*$^{-/-}$ mice but not WT mice (Berg et al. 2002). Treatment with NSAIDs that did not inhibit prostaglandin synthesis did not induce colitis, suggesting that prostaglandins and IL-10 provide redundant anti-inflammatory effects. Finally, treatment with exogenous IL-10 has been shown to ameliorate the severity of disease in several diverse models of colitis, including the naive T cell transfer model of colitis (Powrie et al. 1994), acute colitis driven by dextran sulphate sodium (DSS)-induced disruption of the epithelial barrier (Qiu et al. 2013; Steidler et al. 2000), and granulomatous colitis induced by streptococcal peptidoglycan–polysaccharide polymers (Herfarth et al. 1996).

The role of IL-10 in human IBD, including Crohn's disease and ulcerative colitis, is currently under investigation and will be discussed elsewhere in this issue. Briefly, mutations and single nucleotide polymorphisms (SNP) in the genes for human IL-10 receptor led to development of paediatric IBD and were associated with ulcerative colitis (Franke et al. 2008; Glocker et al. 2009). However, not all loss-of-function SNPs in the IL-10R genes led to IBD. For example, a SNP in *IL-10R1* resulting in increased TNF-α production by monocytes, was found in equal frequency in both controls and IBD patients (Gasche et al. 2003). Additionally, IL-10 levels, as well as the frequency of IL-10^{+} myeloid cells, were equal in the colons of controls and IBD patients (Hart et al. 2005; Schreiber et al. 1995). However, IL-10 is induced upon treatment of IBD with steroids (Santaolalla et al. 2011) and steroid-refractory patients may benefit from treatment with exogenous IL-10 (Schreiber et al. 1995; van Deventer et al. 1997). Thus, these results suggest that IL-10 may be an effective molecule for dampening intestinal inflammation in humans. The mechanisms of action and sources of IL-10 in the intestine will be discussed in the following sections.

3 Regulatory Activities of IL-10 in the Intestine

3.1 Effects on Innate Immune Cells

The IL-10 receptor, composed of *Il10ra* and *Il10rb*, is expressed on both innate and adaptive immune cells as well as non-hematopoietic cells (Moore et al. 2001). Upon engagement of IL-10R, most cells use the Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway to control the transcription of IL-10-responsive genes. STAT3 is an essential protein downstream of IL-10 in this pathway, although interactions with other STAT proteins may mediate cell- or tissue-specific roles (Moore et al. 2001).

STAT3 is indispensable for both the anti-proliferative and anti-inflammatory effects of IL-10 on macrophages (Takeda et al. 1999). Mice with a myeloid cell-specific deletion of STAT3 (LysM-cre/*STAT3*$^{fl/fl}$) developed a spontaneous enterocolitis characterised by excessive Th1 cell activity similar to that seen in *Il10*$^{-/-}$ mice (Takeda et al. 1999). Peritoneal macrophages isolated from these conditional STAT3 mutants were unresponsive to IL-10. IL-10 failed to reduce LPS-induced TNF-α and IL-6 secretion, induce expression of the anti-inflammatory suppressor of cytokine signalling 3 (SOCS3) or inhibit macrophage cell growth in conditional STAT3 mutants (Kobayashi et al. 2003; Takeda et al. 1999). In addition, CD11c^{+} cells from the colon lamina propria (LP) of LysM-cre/*STAT3*$^{fl/fl}$ mice secreted significantly greater amounts of IL-12p40, both spontaneously and upon ex vivo LPS stimulation, which was required for development of spontaneous colitis (Kobayashi et al. 2003). Furthermore, TLR4$^{-/-}$ conditional STAT3 mutants did not produce IL-12p40 and were also protected from spontaneous colitis and LysM-cre/*STAT3*$^{fl/fl}$ mice also failed to develop colitis when crossed onto the RAG$^{-/-}$ background (Kobayashi et al. 2003). Taken together, these results suggest that in the absence of IL-10 signalling, colon myeloid cells secrete excess IL-12p40 in response to LPS and stimulate IFN-γ production from CD4^{+} T cells. IFN-γ signals via STAT1, and STAT1/STAT3 double mutants were partially protected from spontaneous colitis (Kobayashi et al. 2003), suggesting that IFN-γ may feed back onto colon myeloid cells to further exacerbate colitis in the absence of IL-10-mediated counter-regulation.

Other cytokines, such as IL-6, also signal through STAT3 and thus, phenotypes observed in conditional STAT3 mutants may not be fully attributable to a lack of IL-10 signalling. However, myeloid cell-specific deletion of IL-10R also led to increased secretion of IL-12p40, TNF-α and IL-1β upon stimulation with LPS (Pils et al. 2010). Intestinal macrophages are normally anergic to TLR stimulation in both the human and mouse and produced IL-10, but no detectable IL-12 or IL-23, upon LPS stimulation (Monteleone et al. 2008; Rivollier et al. 2012; Smythies et al. 2005). Hyporesponsiveness to TLR stimulation was dependent on constitutive IL-10 secretion by the colonic macrophages, as when stimulated with either LPS or CpG in the presence of an anti-IL-10R neutralising antibody, they regained the ability to produce IL-12p40 (Monteleone et al. 2008). Similarly, LP

macrophages isolated from *Il10*$^{-/-}$ mice produced IL-12 and IL-23, even without exogenous stimulation (Rivollier et al. 2012). In addition to suppressing production of IL-12 family cytokines by colonic macrophages and DC, in the Peyer's patches, IL-10 was one of the host factors responsible for suppressing pro-inflammatory type I interferon secretion from plasmacytoid DCs (Contractor et al. 2007).

Intestinal inflammation in *Il10*$^{-/-}$ mice was dependent on MyD88 signalling in colon myeloid cells. MyD88 signalling is downstream of most TLRs, which further supports the notion that IL-10 suppresses intestinal inflammation by promoting the anergic phenotype of LP macrophages and DCs. By crossing *Il10*$^{-/-}$*MyD88*$^{fl/fl}$ mice to mice expressing Cre recombinase in various cellular compartments, Hoshi et al. were able to assess whether TLR signalling on specific cells was necessary for the development of colitis in *Il10*$^{-/-}$ mice (Hoshi et al. 2012). When MyD88 was conditionally ablated in either LysM-expressing cells (monocytes, macrophages, neutrophils and some DCs) or CD11c^{+} cells (DCs and some macrophages), *Il10*$^{-/-}$ mice were protected from the development of spontaneous colitis (Hoshi et al. 2012). Furthermore, the spontaneous production of IL-1β, IL-6, IL-12p40 and TNF-α in the colon LP of *Il10*$^{-/-}$ mice was completely abrogated when myeloid cells were deficient in MyD88 signalling (Hoshi et al. 2012). Secretion of IFN-γ and IL-17 were also abrogated, suggesting that MyD88-dependent myeloid cell activation in the absence of IL-10 promotes the differentiation of pathogenic CD4^{+} T cells in the LP. Similarly, in human colon explant cultures, blockade of IL-10 also led to increased secretion of IFN-γ and IL-17 (Jarry et al. 2008).

IL-10 also reduces the expression of co-stimulatory molecules on macrophages (Ding et al. 1993; Ding and Shevach 1992). After initial priming in the lymph nodes, CD4^{+} T cells receive secondary signals in the tissue that fully activate them and promote cytokine secretion (McLachlan et al. 2009). In the intestines, however, IL-10 suppressed expression of CD80 and CD86 on CX_3CR1^{hi} macrophages, rendering them unable to activate effector CD4^{+} T cells (Kayama et al. 2012). Thus, CX_3CR1^{hi} cells, which comprise approximately 70 % of the MHC II^{+} myeloid cells in the LP (Rivollier et al. 2012), sequestered CD4^{+} T cells from CX_3CR1^{-} DCs, which expressed higher levels of CD80 and CD86. Accordingly, transfer of CX_3CR1^{hi} cells from WT, but not STAT3$^{-/-}$ mice, ameliorated T cell-dependent colitis (Kayama et al. 2012). In vitro, CX_3CR1^{hi} cells inhibited DC-driven CD4^{+} T cell proliferation by a mechanism that was dependent on IL-10 signalling in CX_3CR1^{hi} cells, but not on IL-10 secretion by CX_3CR1^{hi} cells.

The studies above highlight the important role of IL-10 in suppressing pro-inflammatory cytokine production from normally anergic myeloid cells. In conjunction with reducing the co-stimulatory and antigen-presenting capacity of these cells (de Waal Malefyt et al. 1991; Ding et al. 1993; Ding and Shevach 1992), IL-10 has profound effects on controlling T cell expansion and T cell-mediated immunopathology. However, IL-10 has also been shown to be involved in regulation of innate immune-mediated colitis. Infection of RAG$^{-/-}$ mice on a 129 background with *H. hepaticus* leads to chronic IL-23-dependent typhlocolitis that is driven by the accumulation and activation of innate lymphoid cells (ILC)

(Buonocore et al. 2010; Maloy et al. 2003). Transfer of $CD4^+CD25^+$ T cells inhibited the development of *H. hepaticus*-induced innate typhlocolitis and was fully dependent on IL-10 production by this regulatory T cell subset (Maloy et al. 2003). However, it is still unclear whether IL-10 acts directly on ILCs to suppress their inflammatory potential or indirectly via myeloid cells to suppress secretion of IL-23, a key cytokine in ILC activation (Buonocore et al. 2010).

3.2 Effects on Adaptive Immune Cells

Though IL-10 can clearly control adaptive immune responses indirectly via signalling on antigen-presenting cells, IL-10 also signals directly onto $CD4^+$ T cells. Although naïve $CD4^+$ T cells and many effector $CD4^+$ T cells do not express IL-10R, $Foxp3^+$ regulatory T (Treg) cells and $IL\text{-}17A^+$ $CD4^+$ T cells both expressed IL-10R on the cell surface (Chaudhry et al. 2011; Huber et al. 2011).

IL-10 promotes the maintenance, expansion and function of Treg cells. In the T cell adoptive transfer model of colitis, $CD4^+$ $CD45RB^{hi}$ naïve T cells transferred into lymphopenic hosts expand in response to IL-23 and microbiota-derived signals, seed the LP, and induce severe colitis (Ahern et al. 2010; Feng et al. 2010; Powrie et al. 1993). Co-transfer of Treg cells prevents immunopathology caused by $CD45RB^{hi}$ T cells (Powrie et al. 1993). The ability of Treg cells to inhibit development of colitis was dependent on IL-10 signalling on the Treg cells (Murai et al. 2009). Thus, *Il10rb*$^{-/-}$ Treg cells failed to prevent development of colitis as did WT Treg cells transferred into *Il10*$^{-/-}$*/RAG*$^{-/-}$ mice (Murai et al. 2009). This study further demonstrated that *Il10rb*$^{-/-}$ Treg cells and WT Treg cells transferred into *Il10*$^{-/-}$*/RAG*$^{-/-}$ both lost their suppressive function due to a failure to maintain expression of Foxp3 (Murai et al. 2009). Continued expression of Foxp3 is required for Treg cells to maintain their suppressor function (Williams and Rudensky 2007).

Another example illustrating the importance of intrinsic IL-10 signals for Treg cell activity in the gut was the observation that Treg cell-specific ablation of *Il10ra* in lymphoreplete mice resulted in the development of severe, spontaneous colitis (Chaudhry et al. 2011). In this case, however, IL-10R-dependent Treg cell function was not due to maintenance of Foxp3 expression, as *Il10ra*$^{-/-}$ Treg cells maintained Foxp3 expression for up to 3 months after *Il10ra* was deleted (Chaudhry et al. 2011). In this model, IL-10R was functional in naïve $CD4^+$ T cells and only deleted upon commitment to the $Foxp3^+$ Treg cell lineage. Thus, it is possible that IL-10R signalling at the time of T cell priming is necessary for Treg cell commitment, and that in its absence, Treg cells become vulnerable to plasticity due to transient or impermanent expression of Foxp3. Consistent with this hypothesis, a recent study found that a subset of non-Treg cells transiently express Foxp3 (Miyao et al. 2012). Thus, it is also possible that in lymphopenic settings, IL-10 promotes the expansion of committed Treg cells over non-Treg cells transiently expressing Foxp3.

Though not required for the maintenance of Foxp3 expression in lymphoreplete mice, IL-10 signalling on Treg cells was necessary for their control of $CD4^+$ T cell

accumulation (Chaudhry et al. 2011). As in myeloid cells, the effects of IL-10 were dependent on STAT3 phosphorylation as Treg cell-specific ablation of STAT3 also resulted in spontaneous intestinal inflammation (Chaudhry et al. 2009). Intestinal inflammation in Treg cell-specific IL-10R or STAT3 conditional mutants was accompanied by a selective increase in the frequency of IL-17A-producing $CD4^+$ T cells, but not IFN-γ-producing $CD4^+$ T cells (Chaudhry et al. 2009, 2011).

IL-10 signalling on Treg cells induced their secretion of IL-10, which was critical for the control of Th17 cells during intestinal inflammation (Chaudhry et al. 2011; Huber et al. 2011). Th17 cells, but not naïve or Th1 cells, expressed the IL-10R and responded to IL-10 with a reduction in their rate of proliferation. Accordingly, $CD4^+$ T cell-specific dysfunction in IL-10 signalling led to increased frequencies of IL-$17A^+$ cells (Chaudhry et al. 2011; Huber et al. 2011). Thus, although IL-10 controls Th1 cells indirectly via suppression of pro-inflammatory cytokine secretion from myeloid cells, IL-10 is able to control Th17 cell-mediated intestinal inflammation via direct signalling on the T cell.

IL-10 has been shown to potentiate not only the function of Treg cells, but also their expansion. In a model of oral tolerance, it was shown that after a priming phase in the mesenteric lymph nodes (MLNs), Treg cells homed to the intestinal LP, where they expanded in response to IL-10 from CX_3CR1^{hi} macrophages (Hadis et al. 2011). Similarly, in human gut sections, $Foxp3^+$ Treg cells were found to be in close contact with IL-10-producing non-T cells (Uhlig et al. 2006). In T cell transfer colitis, treatment with anti-IL-10R completely abrogated the ability of Treg cells to cure colitis induced by $CD45RB^{hi}$ $CD4^+$ T cells (Liu et al. 2003; Uhlig et al. 2006). This was partly due to the inability of Treg cell-derived IL-10 to signal onto non-Treg cells, but *Il10*$^{-/-}$ Treg cells also partially suppressed colitis, suggesting that anti-IL-10R treatment inhibited some Treg cell-mediated mechanisms of immune suppression that were independent of their ability to secrete IL-10.

4 Sources of IL-10 in the Intestine

As noted above, many different cells are capable of producing IL-10. Their anatomical location and interactions with other cells determine which cell type is the critical source of IL-10 under various circumstances. In the second section, we summarise recent work elucidating the factors governing IL-10 production from intestinal immune cells and the relative contributions of each cell type to IL-10-mediated regulation of intestinal inflammation.

4.1 Innate Sources of IL-10

Intestinal macrophages, characterised by high expression of CD11b, F4/80 and CX_3CR1, are robust producers of IL-10 (Chirdo et al. 2005; Denning et al. 2007;

Rivollier et al. 2012). In contrast, DCs, characterised by CD11c expression and intermediate to low expression of F4/80 and CX_3CR1, are not believed to be major producers of IL-10 in the LP (Denning et al. 2007; Rivollier et al. 2012). Recent data from a human IL-10 transgenic mouse also confirmed that macrophages, and not DCs, were the principal source of innate immune cell-derived IL-10 in the intestine (Ranatunga et al. 2012). Steady-state macrophage production of IL-10 was partially dependent on the presence of commensal bacteria since germ-free mice showed an approximately 50 % reduction in IL-10 (Rivollier et al. 2012). Consistent with this finding, it was recently shown that the commensal bacterium, *Clostridium butyricum*, specifically induced IL-10 from intestinal macrophages during DSS-induced colitis (Hayashi et al. 2013). Production of steady-state IL-10 was MyD88-independent, while induction of IL-10 by *C. butyricum* during inflammation was dependent on TLR2 and MyD88 (Hayashi et al. 2013; Rivollier et al. 2012). This discrepancy can be explained by the finding that most steady-state macrophages do not express TLRs, while monocyte-derived cells recruited during inflammation express TLR2 (Platt et al. 2010). Infiltrating monocyte-derived cells normally exhibit an inflammatory phenotype (Rivollier et al. 2012), but signals from *C. butyricum* may attenuate their colitogenic potential. Indeed, colonization with *C. butyricum* lessened the severity of DSS-induced colitis by a mechanism dependent on myeloid cell-derived IL-10 (Hayashi et al. 2013).

Commensal-dependent but MyD88-independent production of constitutive IL-10 may instead be downstream of the TRIF signalling pathway. Downstream of TLR3, TLR4 and cytosolic DNA sensors, TRIF connects microbiota-derived signals to the production of interferon (IFN)-β. We recently found that myeloid cells from mice lacking the IFN-α/β receptor (IFNAR) produced significantly lower amounts of IL-10 both with and without stimulation with TLR ligands (Kole et al. 2013). Similarly, ex vivo treatment of LP myeloid cells with an anti-IFNAR neutralising antibody also inhibited IL-10 production (Kole et al. 2013). Furthermore, type I interferons enhanced CD40L-induced IL-10 secretion (Luft et al. 2002). These result position type I interferons as an essential regulator of IL-10 production by intestinal macrophages under both steady-state and inflammatory conditions.

As mentioned above, CX_3CR1^{hi} LP macrophages were an important source of IL-10 for Treg cell expansion in the gut (Hadis et al. 2011). CX_3CR1, the receptor for fractalkine, was not only a marker of intestinal macrophages, but was also necessary for their secretion of IL-10. Expansion of Treg cells by IL-10-producing CX_3CR1^{hi} macrophages was a prerequisite for dissemination of Treg cells to peripheral sites and the establishment of oral tolerance (Hadis et al. 2011). Thus, $CX_3CR1^{-/-}$ mice were unable to mount tolerance against ingested antigens, but could be rescued by the adoptive transfer of CX_3CR1^{+} antigen-presenting cells (Hadis et al. 2011). Similarly, adoptive transfer of LP $CD11b^{+}CD11c^{+}F4/80^{+}$ cells, which also express CX_3CR1 (Rivollier et al. 2012), rescued Treg cells transferred into $Il10^{-/-}/RAG^{-/-}$ mice from loss of Foxp3 expression (Murai et al. 2009).

The importance of innate immune cell-derived IL-10 is questionable, however. For example, myeloid cell-specific ablation of IL-10 (LysM-cre/$Il10^{fl/fl}$) did not

result in the development of spontaneous colitis (Siewe et al. 2006) and *Il10*$^{-/-}$/*RAG*$^{-/-}$ mice did not develop worse colitis upon transfer of naive CD45RB^{hi} CD4^{+} T cells (Murai et al. 2009). In contrast, transfer of bulk splenic CD4^{+} T cells into *Il10*$^{-/-}$/*RAG*$^{-/-}$ recipients did lead to worse colitis, that was characterised by increased levels of IL-12p40 and concomitant increases in IFN-γ and IL-17 secretion (Liu et al. 2011). Innate sources of IL-10 were also necessary for TGF-β signalling and SMAD3 phosphorylation on CD4^{+} T cells (Liu et al. 2011). Thus, although dispensable for controlling the colitogenic potential of naïve CD4^{+} T cells, innate leukocyte-derived IL-10 may restrain the inflammatory phenotype of effector and/or memory CD4^{+} T cells.

4.2 Adaptive Sources of IL-10

Unlike myeloid cell-specific deletion of IL-10, CD4^{+} T cell-specific deletion of IL-10 (CD4-cre/*Il10*$^{fl/fl}$) resulted in development of spontaneous colitis (Roers et al. 2004). It is important to note, however, that CD4-cre/*Il10*$^{fl/fl}$ mice were positive for *Helicobacter ganmani* while the *Helicobacter* status of LysM-cre/*Il10*$^{fl/fl}$ mice was not divulged (Roers et al. 2004; Siewe et al. 2006). As mentioned earlier, *Il10*$^{-/-}$ mice administered piroxicam develop an acute colitis due to inhibition of prostaglandin synthesis (Berg et al. 2002). *RAG*$^{-/-}$ mice reconstituted with bulk CD4^{+} T cells from *Il10*$^{-/-}$ mice, but not WT mice, were also susceptible to piroxicam-induced colitis (Blum et al. 2004), providing further evidence that CD4^{+} T cells are a crucial source of IL-10 for the control of intestinal inflammation.

IL-10 can be produced by many different subsets of CD4^{+} T cells in the gut, including Foxp3^{+} and Foxp3^{-} cells. In the small intestine, IL-10 was produced by Foxp3^{-} intraepithelial cells, whereas in the colon and small intestine LP, IL-10 was produced by both Foxp3^{+} and Foxp3^{-} LP cells (Kamanaka et al. 2006; Maynard et al. 2007). Human IL-10 in a transgenic mouse model was also expressed highly in CD4^{+} Foxp3^{+} T cells from the colon LP (Ranatunga et al. 2012). Cell tracking studies demonstrated that Foxp3^{+} IL-10-producing cells were derived from both Foxp3^{+} and Foxp3^{-} thymic precursors, while Foxp3^{-} IL-10-producing cells were derived only from Foxp3^{-} precursors (Maynard et al. 2007).

When IL-10^{-} splenic CD4^{+} T cells were transferred into *RAG*$^{-/-}$ mice, they gained the ability to produce IL-10 after homing to either the small intestinal epithelium or the LP of the large intestine, suggesting that T cells receive local signals that stimulate their production of IL-10 (Kamanaka et al. 2006). Although capable of inducing IL-10 from Treg cells, IL-10 signalling was not strictly required for the development of IL-10^{+} CD4^{+} T cells in the intestine (Chaudhry et al. 2011; Maynard et al. 2007). Instead, TGF-β, a cytokine constitutively expressed in the LP (Babyatsky et al. 1996), was required for IL-10 expression in CD4^{+} T cells (Maynard et al. 2007). In contrast, both retinoic acid and IL-23 produced by mucosal DCs inhibited IL-10 production by intestinal Treg cells (Ahern et al. 2010; Maynard et al. 2009).

Intestinal $CD4^+$ T cells also receive antigenic stimulation from the resident microbiota. IL-10^+ Treg cells in the LP were characterised by high surface expression of CD44 and low expression of CD62L, consistent with antigen-experienced effector or memory cells (Ranatunga et al. 2012). Several distinct types of intestinal bacteria, including both commensals and pathogens, have been shown to promote IL-10^+ Treg cell development in the intestine. Indigenous *Clostridium* species induced IL-10 production from colonic $FoxP3^+$ Treg cells, which may be partially dependent on their induction of TGF-β from intestinal epithelial cells (Atarashi et al. 2011). Persistent colonization of WT mice with opportunistic pathogen, *H. hepaticus*, led to the induction of both $CD25^+$ and $CD25^-$ IL-10-producing $CD45RB^{lo}$ Treg cells that suppressed *H. hepaticus*-induced intestinal inflammation (Kullberg et al. 2002). The pathogen, *Yersinia enterocolitica*, also induced IL-10^+ Treg cells by a mechanism dependent on TLR2/6 signalling (DePaolo et al. 2012). TLR2/6 was previously shown to recognise LcrV from *Yersinia* to induce IL-10 from myeloid cells and $Foxp3^-$ $CD4^+$ T cells (Depaolo et al. 2008). Finally, polysaccharide A from the commensal bacterium, *Bacteroides fragilis*, directly engaged TLR2 on $Foxp3^+$ $CD4^+$ T cells to induce IL-10 production (Round et al. 2011). Taken together, these studies suggest that induction of IL-10^+ Treg cells in the intestine may represent an important pathway through which commensal microbiota establishes beneficial mutualism with their mammalian host, but that certain pathogens may exploit this mechanism to facilitate infection.

In normal healthy mice, the antigen-experienced $CD45RB^{lo}$ $CD4^+$ T cell fraction contains several regulatory T cell populations that can suppress $CD4^+$ T cell-induced colitis via an IL-10-dependent mechanism (Asseman et al. 1999; Powrie et al. 1993). However, $CD4^+CD25^+$ Treg cells within the $CD45RB^{lo}$ population did not require IL-10 to suppress naive $CD45RB^{hi}$ $CD4^+$ T cell transfer colitis in $RAG^{-/-}$ hosts (Murai et al. 2009). However, adoptively transferred $Il10^{-/-}$ $CD45RB^{lo}$ T cells themselves elicited colitis in $RAG^{-/-}$ recipients, suggesting that IL-10 from $CD45RB^{lo}$ Treg cells is required for the suppression of colitogenic cells also contained within the antigen-experienced $CD45RB^{lo}$ population (Asseman et al. 1999). Accordingly, inflammatory $Foxp3^-$ $CD45RB^{lo}$ $CD4^+$ T cells were controlled by Treg cell-derived IL-10, while control of naive $CD45RB^{hi}$ $CD4^+$ T cells was IL-10 independent (Kamanaka et al. 2011). In addition, $CD45RB^{lo}$ $CD4^+$ T cell-induced colitis was driven by IL-22, a cytokine expressed by Th17 cells (Liang et al. 2006), providing further evidence that IL-10 directly inhibits Th17 cell pathogenicity.

Although dispensable for the prevention of $CD45RB^{hi}$ $CD4^+$ T cell-mediated colitis, IL-10 was required for Treg cell cure of established colitis in this model (Liu et al. 2003; Uhlig et al. 2006). IL-10 was also required for Treg cell control of immunopathology induced by *H. hepaticus* in a T cell-independent colitis model, or after infection with *Toxoplasma gondii* (Hall et al. 2012; Maloy et al. 2003). Thus, Treg cells preventing colitis by inhibiting T cell priming in the lymph nodes (Schneider et al. 2007) do so independently of IL-10, while Treg cell-mediated immunosuppression at sites of inflammation require IL-10. Consistent with this

hypothesis, IL-10$^+$ Treg cells accumulate in the colon LP during the cure of colitis (Uhlig et al. 2006). Furthermore, Treg cell-specific ablation of IL-10 resulted in spontaneous inflammation not only in the colon, but also in the lungs and skin (Rubtsov et al. 2008). Importantly, however, systemic, multi-organ, fatal autoimmunity, as observed in Foxp3$^{-/-}$ mice (Brunkow et al. 2001), did not develop in mice containing IL-10-deficient Treg cells, indicating that Treg cell-derived IL-10 was specifically important for the suppression of inflammation at sites where immune cells directly interact with environmental antigens.

Foxp3$^-$ IL-10$^+$ CD4$^+$ T (Tr1) cells are another regulatory subset with the potential to suppress colitis (Groux et al. 1997). Tr1 cells could be found amongst the intestinal epithelial lymphocytes as well as in the colon LP (Kamanaka et al. 2006; Maynard et al. 2007). Induction of Tr1 cells is dependent on chronic T cell activation (Groux et al. 1997) and is influenced by several cytokines including IL-27, type I interferons and IL-10. DC-derived IL-10 and IL-27 were both shown to induce differentiation of naïve CD4$^+$ T cells into a Foxp3$^-$ IL-10-producing phenotype (Awasthi et al. 2007; Groux et al. 1997; Wakkach et al. 2003). Type I interferons potentiated the TCR stimulation-dependent and IL-10-dependent differentiation of Tr1 cells (Corre et al. 2013; Levings et al. 2001), either via direct signalling on CD4$^+$ T cells or indirectly via signalling on antigen-presenting cells (Dikopoulos et al. 2005).

IL-27 also acted on effector Th1, Th2 or Th17 cells to induce their transition into an IL-10-producing self-regulating CD4$^+$ T cell (Fitzgerald et al. 2007; Stumhofer et al. 2007). Infection with *Toxoplasma gondii* resulted in fatal Th1 cell-mediated necrosis of the small intestine in the absence of IL-10 (Suzuki et al. 2000) and a subsequent study showed that Th1 cells themselves were a critical source of protective IL-10 during *T. gondii* infection (Jankovic et al. 2007). Although it was not determined in this study whether IL-27 was required for induction of IL-10 during toxoplasmosis, *Il27*$^{-/-}$ mice also succumbed to fatal immunopathology (Hall et al. 2012).

Mucosal myeloid cells are specialised for the induction of Foxp3$^-$ IL-10$^+$ CD4$^+$ T cells. They were shown to constitutively produce type I interferons and type I interferon signalling was essential for optimal production of both IL-10 and IL-27 (Kole et al. 2013). However, the main producers of these three cytokines were resident non-migratory LP macrophages, suggesting that they may be more important in adapting the phenotype of CD4$^+$ T cells locally in the LP rather than priming a distinct lineage. In contrast, migratory DCs presenting antigens from apoptotic intestinal epithelial cells were shown to be able to prime IL-10$^+$ CD4$^+$ T cell responses in the MLNs (Jang et al. 2006).

CD8$^+$ T cells can also be induced to produce IL-10. It was recently reported that naïve CD8$^+$ T cells cultured in the presence of IL-4 acquired an IL-10-producing phenotype and adoptive transfer of IL-10$^+$ CD8$^+$ T cells ameliorated colitis induced by 2,4,6-trinitrobenzene sulphonic acid, a hapten that elicits severe, acute colitis and diarrhoea (Zhao et al. 2013). Furthermore, previous studies had identified a population of CD8$^+$ T cells expressing the CD8αα homodimer that were specifically located in the small intestinal intraepithelial layer, which

recognised self-antigen and responded via the production of IL-10 (Saurer et al. 2004). Other studies indicated that both $CD4^+$ and $CD4^-$ subsets of $CD8\alpha\alpha^+$ intra-epithelial T cells were able to suppress Th1-mediated colitis in an IL-10-dependent manner (Das et al. 2003; Poussier et al. 2002). As was shown in vitro, expression of IL-10 in $CD4^+$ $CD8\alpha\alpha^+$ T cells was dependent on IL-4 and expression of the transcription factor, GATA3 (Das et al. 2003). Remarkably, $CD8\alpha\alpha^+$ T cells retained their tolerogenic phenotype even upon recognition of viral antigens (Saurer et al. 2004). Taken together, these data suggest that intraepithelial $CD8\alpha\alpha^+$ T cells constitute a discrete population of intestinal lymphocytes that may also mediate immunoregulatory activity through the secretion of IL-10.

Finally, B cells are also a functional source of IL-10. Although B cell-specific deletion of IL-10 did not result in spontaneous colitis (Madan et al. 2009), $IL\text{-}10^+$ B cells accumulated in the MLNs and LP of mice with chronic intestinal inflammation (Mizoguchi et al. 2002). Furthermore, several studies have provided in vivo evidence that IL-10-secreting B cells can regulate intestinal inflammation. Thus, adoptive transfer of WT B cells, but not $Il10^{-/-}$ B cells, suppressed spontaneous development of colitis in genetically susceptible strains (Mizoguchi et al. 2002), inhibited T cell transfer colitis (Schmidt et al. 2012) and attenuated DSS-induced colitis (Yanaba et al. 2011). As with macrophages and $CD4^+$ T cells, B cell secretion of IL-10 was partially dependent on signals derived from the microbiota (Schmidt et al. 2012). Thus, B cells may be a non-essential source of IL-10 that can contribute to intestinal immunoregulation.

5 Concluding Remarks

The diversity of cells that can produce and respond to IL-10 shows its central role in immune regulation. Development of spontaneous colitis in mice with Treg cell-specific deletion of IL-10 (Rubtsov et al. 2008) positions Treg cells as the critical source of IL-10 for maintenance of intestinal homeostasis. Similarly, spontaneous colitis in mice with defects in myeloid cell or Treg cell IL-10R signalling identifies them as the critical responders to IL-10 (Chaudhry et al. 2011; Takeda et al. 1999). Other sources of IL-10, though not essential for homeostasis, can also suppress intestinal inflammation.

Mice with defects in IL-10 and IL-10 signalling develop colitis primarily at sites of interaction with environmental antigens such as the resident microbiota, suggesting that IL-10 functions to maintain commensalism. Furthermore, spontaneous colitis is dependent on the presence of commensal bacteria, which elicit immunopathology in the absence of IL-10. Accordingly, several commensal bacterial species induce IL-10 production from innate and adaptive immune cells, as well as expression of the IL-10R in the intestines (Mirpuri et al. 2012).

The use of IL-10 for the treatment of IBD has not been as successful as hoped. However, this may be due to a hyporesponsiveness to IL-10 conferred by genetic mutations in the IL-10R gene (Glocker et al. 2009), or to ineffective delivery of

IL-10 to sites of inflammation such as the intestinal LP. Further work must be done to properly harness the immunosuppressive potential of IL-10 observed in multiple animal models of intestinal inflammation.

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References

Ahern PP, Schiering C, Buonocore S, McGeachy MJ, Cua DJ, Maloy KJ, Powrie F (2010) Interleukin-23 drives intestinal inflammation through direct activity on T cells. Immunity 33:279–288

Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 190:995–1004

Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y et al (2011) Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331:337–341

Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 8:1380–1389

Babyatsky MW, Rossiter G, Podolsky DK (1996) Expression of transforming growth factors alpha and beta in colonic mucosa in inflammatory bowel disease. Gastroenterology 110:975–984

Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D (1996) Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and $CD4^{(+)}$ TH1-like responses. J Clin Investig 98:1010–1020

Berg DJ, Zhang J, Weinstock JV, Ismail HF, Earle KA, Alila H, Pamukcu R, Moore S, Lynch RG (2002) Rapid development of colitis in NSAID-treated IL-10-deficient mice. Gastroenterology 123:1527–1542

Blum AM, Metwali A, Elliott DE, Berg DJ, Weinstock JV (2004) $CD4^{+}$ T cells from IL-10-deficient mice transfer susceptibility to NSAID-induced Rag colitis. Am J Physiol Gastrointest Liver Physiol 287:G320–G325

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27:68–73

Buchler G, Wos-Oxley ML, Smoczek A, Zschemisch NH, Neumann D, Pieper DH, Hedrich HJ, Bleich A (2012) Strain-specific colitis susceptibility in IL10-deficient mice depends on complex gut microbiota-host interactions. Inflamm Bowel Dis 18:943–954

Buonocore S, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ, Powrie F (2010) Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature 464:1371–1375

Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M (2009) A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 457:722–725

Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, Rudensky AY (2009) $CD4^{+}$ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science 326:986–991

Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, Jack RS, Wunderlich FT, Bruning JC, Muller W et al (2011) Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity 34:566–578

Chirdo FG, Millington OR, Beacock-Sharp H, Mowat AM (2005) Immunomodulatory dendritic cells in intestinal lamina propria. Eur J Immunol 35:1831–1840

Contractor N, Louten J, Kim L, Biron CA, Kelsall BL (2007) Cutting edge: Peyer's patch plasmacytoid dendritic cells (pDCs) produce low levels of type I interferons: possible role for IL-10, TGF beta, and prostaglandin E2 in conditioning a unique mucosal pDC phenotype. J Immunol 179:2690–2694

Corre B, Perrier J, El Khouri M, Cerboni S, Pellegrini S, Michel F (2013) Type I interferon potentiates T-cell receptor mediated induction of IL-10-producing CD4 T cells. Eur J Immunol 43:2730–2740

Das G, Augustine MM, Das J, Bottomly K, Ray P, Ray A (2003) An important regulatory role for $CD4^+$ CD8 alpha alpha T cells in the intestinal epithelial layer in the prevention of inflammatory bowel disease. Proc Natl Acad Sci USA 100:5324–5329

Davidson NJ, Leach MW, Fort MM, Thompson-Snipes L, Kuhn R, Muller W, Berg DJ, Rennick DM (1996) T helper cell 1-type $CD4^+$ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. J Exp Med 184:241–251

de Saint-Vis B, Fugier-Vivier I, Massacrier C, Gaillard C, Vanbervliet B, Ait-Yahia S, Banchereau J, Liu YJ, Lebecque S, Caux C (1998) The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol 160:1666–1676

de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries JE (1991) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 174:915–924

Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S (1993) Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 150:353–360

Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol 8:1086–1094

DePaolo RW, Kamdar K, Khakpour S, Sugiura Y, Wang W, Jabri B (2012) A specific role for TLR1 in protective T(H)17 immunity during mucosal infection. J Exp Med 209:1437–1444

Depaolo RW, Tang F, Kim I, Han M, Levin N, Ciletti N, Lin A, Anderson D, Schneewind O, Jabri B (2008) Toll-like receptor 6 drives differentiation of tolerogenic dendritic cells and contributes to LcrV-mediated plague pathogenesis. Cell Host Microbe 4:350–361

Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB (2012) Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in $Il10^{-/-}$ mice. Nature 487:104–108

Dieleman LA, Arends A, Tonkonogy SL, Goerres MS, Craft DW, Grenther W, Sellon RK, Balish E, Sartor RB (2000) Helicobacter hepaticus does not induce or potentiate colitis in interleukin-10-deficient mice. Infect Immun 68:5107–5113

Dikopoulos N, Bertoletti A, Kroger A, Hauser H, Schirmbeck R, Reimann J (2005) Type I IFN negatively regulates $CD8^+$ T cell responses through IL-10-producing $CD4^+$ T regulatory 1 cells. J Immunol 174:99–109

Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM (1993) IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. J Immunol 151:1224–1234

Ding L, Shevach EM (1992) IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. J Immunol 148:3133–3139

Durez P, Abramowicz D, Gerard C, Van Mechelen M, Amraoui Z, Dubois C, Leo O, Velu T, Goldman M (1993) In vivo induction of interleukin 10 by anti-CD3 monoclonal antibody or bacterial lipopolysaccharide: differential modulation by cyclosporin A. J Exp Med 177:551–555

Feng T, Wang L, Schoeb TR, Elson CO, Cong Y (2010) Microbiota innate stimulation is a prerequisite for T cell spontaneous proliferation and induction of experimental colitis. J Exp Med 207:1321–1332

Ferran C, Dautry F, Merite S, Sheehan K, Schreiber R, Grau G, Bach JF, Chatenoud L (1994) Anti-tumor necrosis factor modulates anti-CD3-triggered T cell cytokine gene expression in vivo. J Clin Investig 93:2189–2196

Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170:2081–2095

Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, Saris CJ, Gran B, Ciric B, Rostami A (2007) Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat Immunol 8:1372–1379

Franke A, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, Domingues FS, Albrecht M, Nothnagel M, Ellinghaus D et al (2008) Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. Nat Genet 40:1319–1323

Gasche C, Grundtner P, Zwirn P, Reinisch W, Shaw SH, Zdanov A, Sarma U, Williams LM, Foxwell BM, Gangl A (2003) Novel variants of the IL-10 receptor 1 affect inhibition of monocyte TNF-alpha production. J Immunol 170:5578–5582

Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, Perro M, Diestelhorst J, Allroth A, Murugan D et al (2009) Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med 361:2033–2045

Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG (1997) A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389:737–742

Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, Muller W, Sparwasser T, Forster R, Pabst O (2011) Intestinal tolerance requires gut homing and expansion of $FoxP3^+$ regulatory T cells in the lamina propria. Immunity 34:237–246

Hall AO, Beiting DP, Tato C, John B, Oldenhove G, Lombana CG, Pritchard GH, Silver JS, Bouladoux N, Stumhofer JS et al (2012) The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. Immunity 37:511–523

Hart AL, Al-Hassi HO, Rigby RJ, Bell SJ, Emmanuel AV, Knight SC, Kamm MA, Stagg AJ (2005) Characteristics of intestinal dendritic cells in inflammatory bowel diseases. Gastroenterology 129:50–65

Hayashi A, Sato T, Kamada N, Mikami Y, Matsuoka K, Hisamatsu T, Hibi T, Roers A, Yagita H, Ohteki T et al (2013) A single strain of Clostridium butyricum induces intestinal IL-10-producing macrophages to suppress acute experimental colitis in mice. Cell Host Microbe 13:711–722

Herfarth HH, Mohanty SP, Rath HC, Tonkonogy S, Sartor RB (1996) Interleukin 10 suppresses experimental chronic, granulomatous inflammation induced by bacterial cell wall polymers. Gut 39:836–845

Hooper LV, Littman DR, Macpherson AJ (2012) Interactions between the microbiota and the immune system. Science 336:1268–1273

Hoshi N, Schenten D, Nish SA, Walther Z, Gagliani N, Flavell RA, Reizis B, Shen Z, Fox JG, Iwasaki A et al (2012) MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice. Nat Commun 3:1120

Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, Chaudhry A, Kamanaka M, Kobayashi Y, Booth CJ, Rudensky AY et al (2011) Th17 cells express interleukin-10 receptor and are controlled by $Foxp3^{(-)}$ and $Foxp3^+$ regulatory $CD4^+$ T cells in an interleukin-10-dependent manner. Immunity 34:554–565

Jang MH, Sougawa N, Tanaka T, Hirata T, Hiroi T, Tohya K, Guo Z, Umemoto E, Ebisuno Y, Yang BG et al (2006) CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. J Immunol 176:803–810

Jankovic D, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, Wilson M, Wynn TA, Kamanaka M, Flavell RA, Sher A (2007) Conventional T-bet$^{(+)}$Foxp3$^{(-)}$ Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J Exp Med 204:273–283

Jarry A, Bossard C, Bou-Hanna C, Masson D, Espaze E, Denis MG, Laboisse CL (2008) Mucosal IL-10 and TGF-beta play crucial roles in preventing LPS-driven, IFN-gamma-mediated epithelial damage in human colon explants. J Clin Investig 118:1132–1142

Kamanaka M, Huber S, Zenewicz LA, Gagliani N, Rathinam C, O'Connor W Jr, Wan YY, Nakae S, Iwakura Y, Hao L et al (2011) Memory/effector (CD45RB(lo)) CD4 T cells are controlled directly by IL-10 and cause IL-22-dependent intestinal pathology. J Exp Med 208:1027–1040

Kamanaka M, Kim ST, Wan YY, Sutterwala FS, Lara-Tejero M, Galan JE, Harhaj E, Flavell RA (2006) Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. Immunity 25:941–952

Kayaba H, Dombrowicz D, Woerly G, Papin JP, Loiseau S, Capron M (2001) Human eosinophils and human high affinity IgE receptor transgenic mouse eosinophils express low levels of high affinity IgE receptor, but release IL-10 upon receptor activation. J Immunol 167:995–1003

Kayama H, Ueda Y, Sawa Y, Jeon SG, Ma JS, Okumura R, Kubo A, Ishii M, Okazaki T, Murakami M et al (2012) Intestinal CX3C chemokine receptor 1(high) (CX3CR1(high)) myeloid cells prevent T-cell-dependent colitis. Proc Natl Acad Sci USA 109:5010–5015

Kobayashi M, Kweon MN, Kuwata H, Schreiber RD, Kiyono H, Takeda K, Akira S (2003) Toll-like receptor-dependent production of IL-12p40 causes chronic enterocolitis in myeloid cell-specific Stat3-deficient mice. J Clin Investig 111:1297–1308

Kole A, He J, Rivollier A, Silveira DD, Kitamura K, Maloy KJ, Kelsall BL (2013) Type I IFNs regulate effector and regulatory T cell accumulation and anti-inflammatory cytokine production during T cell-mediated colitis. J Immunol 191:2771–2779

Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274

Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, Cua DJ, Powrie F, Cheever AW, Maloy KJ et al (2006) IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. J Exp Med 203:2485–2494

Kullberg MC, Jankovic D, Gorelick PL, Caspar P, Letterio JJ, Cheever AW, Sher A (2002) Bacteria-triggered CD4$^{(+)}$ T regulatory cells suppress Helicobacter hepaticus-induced colitis. J Exp Med 196:505–515

Kullberg MC, Rothfuchs AG, Jankovic D, Caspar P, Wynn TA, Gorelick PL, Cheever AW, Sher A (2001) Helicobacter hepaticus-induced colitis in interleukin-10-deficient mice: cytokine requirements for the induction and maintenance of intestinal inflammation. Infect Immun 69:4232–4241

Kullberg MC, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A (1998) Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. Infect Immun 66:5157–5166

Levings MK, Sangregorio R, Galbiati F, Squadrone S, de Waal Malefyt R, Roncarolo MG (2001) IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. J Immunol 166:5530–5539

Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 203:2271–2279

Liu B, Tonkonogy SL, Sartor RB (2011) Antigen-presenting cell production of IL-10 inhibits T-helper 1 and 17 cell responses and suppresses colitis in mice. Gastroenterology 141:653–662, 662 e651–654

Liu H, Hu B, Xu D, Liew FY (2003) $CD4^+$ $CD25^+$ regulatory T cells cure murine colitis: the role of IL-10, TGF-beta, and CTLA4. J Immunol 171:5012–5017

Luft T, Luetjens P, Hochrein H, Toy T, Masterman KA, Rizkalla M, Maliszewski C, Shortman K, Cebon J, Maraskovsky E (2002) IFN-alpha enhances CD40 ligand-mediated activation of immature monocyte-derived dendritic cells. Int Immunol 14:367–380

Macpherson AJ, Harris NL (2004) Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 4:478–485

Madan R, Demircik F, Surianarayanan S, Allen JL, Divanovic S, Trompette A, Yogev N, Gu Y, Khodoun M, Hildeman D et al (2009) Nonredundant roles for B cell-derived IL-10 in immune counter-regulation. J Immunol 183:2312–2320

Maloy KJ, Powrie F (2011) Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature 474:298–306

Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F (2003) $CD4^+$ $CD25^+$ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. J Exp Med 197:111–119

Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, Weaver CT (2007) Regulatory T cells expressing interleukin 10 develop from $Foxp3^+$ and $Foxp3^-$ precursor cells in the absence of interleukin 10. Nat Immunol 8:931–941

Maynard CL, Hatton RD, Helms WS, Oliver JR, Stephensen CB, Weaver CT (2009) Contrasting roles for all-trans retinoic acid in TGF-beta-mediated induction of Foxp3 and Il10 genes in developing regulatory T cells. J Exp Med 206:343–357

McLachlan JB, Catron DM, Moon JJ, Jenkins MK (2009) Dendritic cell antigen presentation drives simultaneous cytokine production by effector and regulatory T cells in inflamed skin. Immunity 30:277–288

Mirpuri J, Sotnikov I, Myers L, Denning TL, Yarovinsky F, Parkos CA, Denning PW, Louis NA (2012) Lactobacillus rhamnosus (LGG) regulates IL-10 signaling in the developing murine colon through upregulation of the IL-10R2 receptor subunit. PLoS ONE 7:e51955

Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, Huehn J, Hori S (2012) Plasticity of $Foxp3^{(+)}$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. Immunity 36:262–275

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

Monteleone I, Platt AM, Jaensson E, Agace WW, Mowat AM (2008) IL-10-dependent partial refractoriness to Toll-like receptor stimulation modulates gut mucosal dendritic cell function. Eur J Immunol 38:1533–1547

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Murai M, Turovskaya O, Kim G, Madan R, Karp CL, Cheroutre H, Kronenberg M (2009) Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. Nat Immunol 10:1178–1184

O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M (1992) Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. Eur J Immunol 22:711–717

Pils MC, Pisano F, Fasnacht N, Heinrich JM, Groebe L, Schippers A, Rozell B, Jack RS, Muller W (2010) Monocytes/macrophages and/or neutrophils are the target of IL-10 in the LPS endotoxemia model. Eur J Immunol 40:443–448

Platt AM, Bain CC, Bordon Y, Sester DP, Mowat AM (2010) An independent subset of TLR expressing CCR2-dependent macrophages promotes colonic inflammation. J Immunol 184:6843–6854

Poussier P, Ning T, Banerjee D, Julius M (2002) A unique subset of self-specific intraintestinal T cells maintains gut integrity. J Exp Med 195:1491–1497

Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL (1993) Phenotypically distinct subsets of $CD4^+$ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int Immunol 5:1461–1471

Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL (1994) Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi $CD4^+$ T cells. Immunity 1:553–562

Qiu ZB, Chen J, Chen JJ, Rong L, Ding WQ, Yang HJ, Zhong L (2013) Effect of recombinant Lactobacillus casei expressing interleukin-10 in dextran sulfate sodium-induced colitis mice. J Dig Dis 14:76–83

Ranatunga DC, Ramakrishnan A, Uprety P, Wang F, Zhang H, Margolick JB, Brayton C, Bream JH (2012) A protective role for human IL-10-expressing $CD4^+$ T cells in colitis. J Immunol 189:1243–1252

Rivollier A, He J, Kole A, Valatas V, Kelsall BL (2012) Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. J Exp Med 209:139–155

Roers A, Siewe L, Strittmatter E, Deckert M, Schluter D, Stenzel W, Gruber AD, Krieg T, Rajewsky K, Muller W (2004) T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. J Exp Med 200:1289–1297

Romani L, Mencacci A, Cenci E, Spaccapelo R, Del Sero G, Nicoletti I, Trinchieri G, Bistoni F, Puccetti P (1997) Neutrophil production of IL-12 and IL-10 in candidiasis and efficacy of IL-12 therapy in neutropenic mice. J Immunol 158:5349–5356

Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK (2011) The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332:974–977

Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, Treuting P, Siewe L, Roers A, Henderson WR Jr et al (2008) Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 28:546–558

Santaolalla R, Mane J, Pedrosa E, Loren V, Fernandez-Banares F, Mallolas J, Carrasco A, Salas A, Rosinach M, Forne M et al (2011) Apoptosis resistance of mucosal lymphocytes and IL-10 deficiency in patients with steroid-refractory Crohn's disease. Inflamm Bowel Dis 17:1490–1500

Saurer L, Seibold I, Rihs S, Vallan C, Dumrese T, Mueller C (2004) Virus-induced activation of self-specific TCR alpha beta CD8 alpha alpha intraepithelial lymphocytes does not abolish their self-tolerance in the intestine. J Immunol 172:4176–4183

Schmidt EG, Larsen HL, Kristensen NN, Poulsen SS, Claesson MH, Pedersen AE (2012) B cells exposed to enterobacterial components suppress development of experimental colitis. Inflamm Bowel Dis 18:284–293

Schneider MA, Meingassner JG, Lipp M, Moore HD, Rot A (2007) CCR7 is required for the in vivo function of $CD4^+$ $CD25^+$ regulatory T cells. J Exp Med 204:735–745

Schreiber S, Heinig T, Thiele HG, Raedler A (1995) Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. Gastroenterology 108:1434–1444

Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB (1998) Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 66:5224–5231

Siewe L, Bollati-Fogolin M, Wickenhauser C, Krieg T, Muller W, Roers A (2006) Interleukin-10 derived from macrophages and/or neutrophils regulates the inflammatory response to LPS but not the response to CpG DNA. Eur J Immunol 36:3248–3255

Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, Orenstein JM, Smith PD (2005) Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Investig 115:66–75

Spencer SD, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, Sordat B, Gibbs VC, Aguet M (1998) The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. J Exp Med 187:571–578

Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, Fiers W, Remaut E (2000) Treatment of murine colitis by Lactococcus lactis secreting interleukin-10. Science 289:1352–1355

Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'Shea JJ, Hunter CA (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8:1363–1371

Suzuki Y, Sher A, Yap G, Park D, Neyer LE, Liesenfeld O, Fort M, Kang H, Gufwoli E (2000) IL-10 is required for prevention of necrosis in the small intestine and mortality in both genetically resistant BALB/c and susceptible C57BL/6 mice following peroral infection with Toxoplasma gondii. J Immunol 164:5375–5382

Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I, Akira S (1999) Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 10:39–49

Uhlig HH, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, Tannapfel A, Fontenot JD, Ramsdell F, Powrie F (2006) Characterization of Foxp3$^+$ CD4$^+$ CD25$^+$ and IL-10-secreting CD4$^+$ CD25$^+$ T cells during cure of colitis. J Immunol 177:5852–5860

van Deventer SJ, Elson CO, Fedorak RN (1997) Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's disease study group. Gastroenterology 113:383–389

Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H (2003) Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity 18:605–617

Whary MT, Taylor NS, Feng Y, Ge Z, Muthupalani S, Versalovic J, Fox JG (2011) Lactobacillus reuteri promotes Helicobacter hepaticus-associated typhlocolitis in gnotobiotic B6.129P2-IL-10(tm1Cgn) (IL-10$^{(-/-)}$) mice. Immunology 133:165–178

Williams LM, Rudensky AY (2007) Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol 8:277–284

Yanaba K, Yoshizaki A, Asano Y, Kadono T, Tedder TF, Sato S (2011) IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. Am J Pathol 178:735–743

Zhao Y, Zhao H, Sun Y, Hao J, Qi X, Zhou X, Wu Z, Wang P, Kaech SM, Weaver CT et al (2013) IL-4 induces a suppressive IL-10-producing CD8$^+$ T cell population via a Cdkn2a-dependent mechanism. J Leukoc Biol 94:1103–1112

Zhou P, Streutker C, Borojevic R, Wang Y, Croitoru K (2004) IL-10 modulates intestinal damage and epithelial cell apoptosis in T cell-mediated enteropathy. Am J Physiol Gastrointest Liver Physiol 287:G599–G604

Tr1 Cells and the Counter-Regulation of Immunity: Natural Mechanisms and Therapeutic Applications

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Abstract T regulatory Type 1 (Tr1) cells are adaptive T regulatory cells characterized by the ability to secrete high levels of IL-10 and minimal amounts of IL-4 and IL-17. Recently, CD49b and LAG-3 have been identified as Tr1-cell-specific biomarkers in mice and humans. Tr1 cells suppress T-cell- and antigen-presenting cell- (APC) responses primarily via the secretion of IL-10 and TGF-β. In addition, Tr1 cells release granzyme B and perforin and kill myeloid cells. Tr1 cells inhibit T cell responses also via cell-contact dependent mechanisms mediated by CTLA-4 or PD-1, and by disrupting the metabolic state of T effector cells via the production of the ectoenzymes CD39 and CD73. Tr1 cells were first described in peripheral blood of patients who developed tolerance after HLA-mismatched fetal liver hematopoietic stem cell transplant. Since their discovery, Tr1 cells have been proven to be important in maintaining immunological homeostasis and preventing T-cell-mediated diseases. Furthermore, the possibility to generate and expand Tr1 cells in vitro has led to their utilization as cellular therapy in humans. In this chapter we summarize the unique and distinctive biological properties of Tr1 cells, the well-known and newly discovered Tr1-cell biomarkers, and the different methods to induce Tr1 cells in vitro and in vivo. We also address the role of Tr1 cells in infectious diseases, autoimmunity, and transplant rejection in different

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pre-clinical disease models and in patients. Finally, we highlight the pathological settings in which Tr1 cells can be beneficial to prevent or to cure the disease.

Contents

1 Introduction

The definite identification, more than two decades ago, of T regulatory (Treg) cells specifically devoted to suppress T effector cells and to inhibit undesired immune responses, has significantly broaden the concept of immune function and has open to new challenges for immunomodulation in health and diseases.

Over the years several types of Treg cells have been identified. The best characterized among the $CD4^+$ T cells, are the Treg cells expressing the forkhead box P3 ($FOXP3^+$ Tregs) and the T regulatory type 1 (Tr1) cells. $FOXP3^+$ Tregs can be either thymus-derived (tTregs), or induced in the periphery (pTregs). Regardless of their origin, both subsets are characterized by constitutive expression of cell surface IL-2Rα chain (CD25), and of FOXP3, which is widely recognized as the master transcription factor for their function. Tr1 cells are induced in the periphery independently from FOXP3. They are distinct from other $CD4^+$ T cell subsets because of their unique cytokine production profile. Their differentiation and function rely on the presence of IL-10, a potent immunosuppressive cytokines. However, a Tr1-cell specific transcription factor related to the IL-10 pathway has not been yet identified.

Studies in murine models and in primary immunodeficiency patients have contributed to elucidate the biological properties and the function of these two subsets of Treg cells and to understand the pathogenesis of the diseases caused by altered generation and function of tTregs and Tr1 cells. $CD4^+CD25^+FOXP3^+$ tTregs are strictly FOXP3-dependent and are subject to a unique pattern of DNA demethylation of the FOXP3 gene promoter, which guarantees their stability. FOXP3 natural mice mutants, the *scurfy* mice, in which the FOXP3 gene is partially deleted, its expression is abrogated, and tTregs are absent, have demonstrated the essential role of FOXP3 as cytokine suppressor gene and of tTregs as key suppressor of lymphoproliferation (Brunkow et al. 2001). Conversely, FOXP3 transgenic mice showed the power of the suppressor function

transferred by FOXP3 overexpression to different cell types (Khattri et al. 2003). Finally, FOXP3 knock-in and reporter mice have elucidated the lineage specifications and timing of gene expression during development (Wan and Flavell 2005). tTregs are important early in life, and loss of their function is life-threatening at very young age, as it is demonstrated in patients with Immune dysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) syndrome, bearing FOXP3 mutations. tTregs expansion and function is IL-2 dependent, as shown by both IL-2 or CD25 knock-out (KO) mice and CD25-deficient patients, whose phenotype resembles FOXP3 deficiency (Goudy et al. 2013). On the other hand, IL-10 KO mice have originally revealed the importance of this cytokine in the gut homeostasis and in preventing overwhelming inflammation (Kuhn et al. 1993). These mice spontaneously develop intestinal inflammation characterized by transmural lesions affecting the small and large intestine and uncontrolled generation of IFN-γ producing T cells. Similarly, the genetic loss of IL-10R or IL-10 in humans (Glocker et al. 2009, 2010) leads to a devastating inflammatory disease with predominant pathology in the gut where it is now well known that Tr1 cells are required to maintain tolerance to commensal antigens and bacteria.

tTregs, pTregs and Tr1 cells share common properties, including the expression of several inhibitory receptors, the poor proliferative capacity in vitro, the requirement for Ag-specific recognition and the ability to suppress proliferation and cytokine production by effector Tcells. The different Treg cell subsets are physiologically present in peripheral sites of close contact with the environment, such as the skin and the gut, in which they actively preserve the immune homeostasis in healthy subjects. Dissecting the biology and the role of the different Treg cell subsets is an essential step to define the best experimental conditions and the most appropriate disease indication for cell-based clinical trials.

In the present chapter we focus on Tr1 cells, describing their unique and distinctive biological properties, biomarkers, their specific mode of action, and different methods for their induction. We highlight the pathological settings in which their healing role has been suggested and the clinical indications for possible therapeutic use of Tr1 cells.

2 Biology of Tr1 Cells

Tr1 cells are memory T lymphocytes identified by cell surface expression of the lymphocyte-activation gene 3 (LAG-3) and the integrin alpha2 subunit (CD49b) (Gagliani et al. 2013b). Upon activation, Tr1 cells secrete high levels of IL-10, and minimal amounts of IL-4 and IL-17 (Bacchetta et al. 1990, 1994; Gagliani et al. 2013b; Groux et al. 1997). Tr1 cells secrete also TGF-β, variable amounts of IL-5, GM-CSF and IFN-γ, but low levels of IL-2 ((Bacchetta et al. 1990; Groux et al. 1997). In contrast to other T-cell subsets that secrete IL-10, including T helper (Th)1, Th2 (Yssel et al. 1992; Chang et al. 2007; Saraiva et al. 2009), Th9 (Veldhoen et al. 2008b), Th17 cells (McGeachy et al. 2007), and FOXP3$^+$ Tregs (Ito et al. 2008), Tr1 cells show a distinct kinetic of IL-10 production. Tr1 cells

secrete IL-10 as early as 4 hours post-activation. The levels of IL-10 increase rapidly in the supernatants of activated Tr1 cells and the highest concentration is reached 12–24 hours after activation (Bacchetta et al. 1994).

Tr1 cells express Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) (Bacchetta et al. 2002; Akdis 2008; Desreumaux et al. 2012), Programmed cell Death protein 1 (PD-1) (Akdis 2008), and inducible co-stimulatory molecule (ICOS) (Haringer et al. 2009), which are inhibitory receptors known to modulate T-cell functions. Tr1 cells express also the ectoenzymes CD39 and CD73 that generate adenosine via the enzymatic hydrolysis of extracellular ATP and disrupt the metabolic state of effector T cells (Bergmann et al. 2007; Mandapathil et al. 2010). Tr1 cells do not constitutively express FOXP3 (Vieira et al. 2004); however, upon activation, they can transiently up-regulate FOXP3 (Levings et al. 2005; Brun et al. 2009, 2011). However, FOXP3 expression in Tr1 cells never reaches that detected in tTregs or pTregs.

Tr1 cells proliferate poorly upon polyclonal T Cell Receptor (TCR)-mediated or Ag-specific activation in vitro (Bacchetta et al. 1994; Groux et al. 1997; Bacchetta et al. 2002). The autocrine production of IL-10 by Tr1 cells contributes to their low proliferative capacity, since addition of neutralizing anti-IL-10 antibody partially restores their proliferation (Bejarano et al. 1992; Bacchetta et al. 1994; Groux et al. 1997). IL-15 supports Tr1 cell proliferation, and, in combination with IL-2, promotes a significant in vitro expansion of Tr1 cell clones (Bacchetta et al. 2002) and cell lines (Magnani et al. 2011). It has also been shown that IL-21 acts as an autocrine growth factor for the expansion and/or maintenance of IL-27-induced (Pot et al. 2009) or IL-6-induced (Jin et al. 2013) murine Tr1 cells. Human Tr1 cell clones express significantly higher levels of mRNA encoding for IL-21 as compared to Th0 cell clones, but it remains to be determined whether this cytokine is necessary for their expansion (Gregori et al. unpublished data).

Tr1 cells modulate immune responses mainly through the secretion of IL-10 and TGF-β (Bacchetta et al. 1994; Groux et al. 1997; Barrat et al. 2002; Veldman et al. 2004; Brun et al. 2009, 2011). To exert their suppressive function Tr1 cells need to be activated via their TCR, but, once activated, Tr1 cells can mediate bystander suppressive activity against other Ags. IL-10 and TGF-β directly inhibits T-cell responses by suppressing IL-2, and IFN-γ (Vieira et al. 2004) and by preventing T-cell proliferation (Taga and Tosato 1992; Gorelik et al. 2002; Gorelik and Flavell 2002). IL-10, locally released by activated Tr1 cells, acts also on APCs, by down modulating co-stimulatory molecules and production of pro-inflammatory cytokines (de Waal Malefyt et al. 1991; Gregori et al. 2012), and on B cells by promoting isotype switching (Satoguina et al. 2005; Meiler et al. 2008). Overall, Tr1 cells control immune responses by modulating directly and indirectly T-, APC- and B-cell functions.

In addition to the cytokine-mediated suppression, Tr1 cells inhibit T cell responses by additional mechanisms, such as cytolysis (Grossman et al. 2004; Magnani et al. 2011), up-regulation of inhibitory receptors (Akdis 2008), and expression of ectoenzymes (Mandapathil et al. 2010). Human Tr1 cells express and release high levels of Granzyme B (GzB), and specifically lyse cells of

myeloid origin, but not other APCs or T and B lymphocytes (Magnani et al. 2011). Tr1-cell–mediated cytotoxicity of myeloid APCs is Ag-independent and requires recognition and activation via HLA class I molecules expressed on target cells. Specific killing of myeloid APCs by Tr1 cells depends also on high expression levels of CD54, CD58, CD155, and CD112 on target myeloid cells, which, upon interaction with their ligands (LFA-1, CD2, and CD226, respectively) on Tr1 cells, mediate stable Tr1-cell/myeloid target adhesion and Tr1-cell activation (Magnani et al. 2011). Killing of myeloid cells by Tr1 cells represents an additional indirect mechanism of suppression, which is not Ag specific but may contribute to bystander suppression mediated by Tr1 cells.

In conclusion, several mechanisms of Tr1-cell–mediated suppression have been identified in vitro. It remains to define how each of this mechanism contributes to the regulatory function of Tr1 cells in vivo.

3 Biomarkers of Tr1 Cells

A number of candidate molecules, transcription factors or surface molecules, have been proposed as biomarkers for Tr1 cells but most showed to be not specific. Cobbold et al. suggested that the repressor of GATA-3 (ROG) is specifically expressed in Tr1 cells (Cobbold et al. 2003). However, ROG is not specific for Tr1 cells since it is rapidly induced in other Th cells upon activation and it controls Th cell differentiation and cytokine production (Miaw et al. 2000). The early response gene 2 (Erg-2) was also proposed to be specifically expressed in IL-10-producing $CD4^+$ T cells and its enforced expression converts murine naïve $CD4^+$ T cells into IL-10-producing suppressor T cells (Okamura et al. 2009). Recently, it has been demonstrated that Erg-2 plays a dominant role in inducing IL-10 production in IL-27-stimulated murine $CD4^+$ T cells via the direct binding to the B lymphocyte induced maturation protein-1 (Blimp-1) promoter (Iwasaki et al. 2013). Moreover, Erg-2 expression strongly correlates with LAG-3 expression on $CD4^+$ IL-10-producing suppressor T cells (Okamura et al. 2012). Based on these findings Erg-2 has been proposed to be a regulator of IL-10 production in Tr1 cells (Okamura et al. 2012). However, we found that Tr1 cells ex vivo isolated from the small intestine of anti-CD3 mAb treated tolerant mice or induced in vitro with IL-27 and TGF-β express Erg-2 at levels comparable to that observed in other effector T cells (Gagliani et al. 2013b). Thus, it remains controversial whether Erg-2 expression is specific for Tr1 cells.

c-Maf, originally considered a Th2-cell specific transcription factor, has been shown to activate the *il10* promoter in Tr1 cells (Pot et al. 2009; Apetoh et al. 2010). Murine IL-27-induced Tr1 cells express both c-Maf and aryl hydrocarbon receptor (AhR) (Pot et al. 2009; Apetoh et al. 2010), but the expression of these transcription factors is not specific for Tr1 cells since both human and murine Th17 cells also express cMaf and AhR at levels comparable, or even higher, than those observed in IL-27-induced Tr1 cells (Veldhoen et al. 2008a;

Bauquet et al. 2009). Overall, it is still unclear if these transcription factors can be considered *bona fide* markers for Tr1 cells and master regulators for Tr1-cell function.

In addition to transcription factors, several surface molecules have been proposed to discriminate Tr1 cells from other $CD4^+$ T cell subsets. ICOS (Haringer et al. 2009) or PD-1 (Akdis 2008) have been associated with human IL-10-producing T cells, but they are also expressed on other T-cell subsets. Our group showed that CD226 (DNAX accessory molecule-1; DNAM-1), an adhesion/signaling molecule that contributes to the NK-mediated lysis, is expressed at high levels on human Tr1 cells and it is critically involved in the specific killing of myeloid APCs by Tr1 cells (Magnani et al. 2011).

In mice, LAG-3 expression was shown to be mainly restricted to $CD4^+CD25^-CD45RB^{low}$ T cells, which include IL-10-producing $FOXP3^-$ T cells, resembling Tr1 cells (Okamura et al. 2009). More recently, it was shown that IL-27 induces LAG-3 as well as IL-10 expression in murine $CD4^+$ T cells (Iwasaki et al. 2013). Based on these observations, it has been proposed that LAG-3 is a phenotypic marker of IL-10-producing FOXP3-independent Treg cells (Okamura et al. 2012). However, LAG-3 is also expressed by $FOXP3^+$ Tregs (Huang et al. 2004; Camisaschi et al. 2010), and it is required for the optimal regulatory function of $FOXP3^+$ Tregs (Huang et al. 2004). Upon activation, human and mouse T cells express LAG-3 (Huard et al. 1997; Bruniquel et al. 1998; Workman and Vignali 2005; Bettini et al. 2011; Lee et al. 2012; Gagliani et al. 2013b), which controls their expansion and homeostasis (Workman and Vignali 2005). Thus, LAG-3 expression is associated with IL-10 production, but it is not specific for Tr1 cells since other Treg-cell subsets and activated effector T cells can be LAG-3 positive.

CD49b, the α2 integrin subunit of the very-late-activation antigen (VLA)-2, has been proposed as a marker for IL-10-producing T cells (Charbonnier et al. 2006). Repetitive injection of immature bone-marrow-derived dendritic cells (DC) triggers the induction/expansion of a population of $CD4^+$ T cells that express CD49b, and a significant proportion of the resulting $CD4^+CD49b^+$ T cells produces IL-10 once re-activated in vitro (Charbonnier et al. 2006). Transfer of these $CD4^+CD49b^+$ T cells in vivo dampens the immune response in a mouse model of contact hypersensitivity and reversed signs of established arthritis (Charbonnier et al. 2010). In humans, the expression of CD49b in combination with high levels of CD18 has been associated with suppressive IL-10-producing T cells in healthy, but not in allergic patients (Rahmoun et al. 2006). However, CD49b expression is not exclusive for Tr1 cells, since murine $CD4^+CD49b^+$ memory T cells can also produce TNF-α and are associated with immunopathology in acute viral infection (Kassiotis et al. 2006). Our studies showed that in vivo and in vitro induced murine effector Th1, Th2, and Th17 cells, or $FOXP3^+$ Tregs, and human IL-17-producing T cells express CD49b (Gagliani et al. 2013b). In addition, VLA-2 (CD49b/CD29) is the major integrin expressed by human Th17 cells (Boisvert et al. 2010). Thus, the single expression of CD49b is not specific for Tr1 cells.

We recently demonstrated that co-expression of CD49b and LAG-3 selectively identifies both human and murine Tr1 cells (Gagliani et al. 2013b). Memory $CD4^+CD49b^+LAG\text{-}3^+$ T cells secrete large amounts of IL-10 but low levels of IL-4 and IL-17, do not constitutively express FOXP3, and display regulatory activity both in vitro and in vivo. Both CD49b and LAG-3 are stably expressed on functional Tr1 cells. CD49b is expressed on Tr1 cells irrespectively of their activation, whereas LAG-3 is expressed on Tr1 cells upon activation when the cells produce IL-10 and display suppressor activity. Co-expression of CD49b and LAG-3 distinguishes Tr1 cells from Th1, Th2, Th17 cells during helminth infection and Inflammatory Bowel Disease (IBD) (Gagliani et al. 2013b). Moreover, CD49b and LAG-3 can be used to identify Tr1 cells in the peripheral blood of healthy donors and tolerant patients. We observed an increase in circulating $CD49b^+LAG\text{-}3^+$ T cells in the peripheral blood of tolerant β-thalassemic patients who developed persistent mixed chimerism of donor and host cells after allogeneic hematopoietic stem cell transplantation (HSCT) (Gagliani et al. 2013b). In these patients we previously demonstrated that high occurrence of circulating Tr1 cells is associated with long term tolerance (Serafini et al. 2009). In conclusion, the combined use of CD49b and LAG-3 as specific biomarkers of Tr1 cells will help us to further characterize these cells and will allow to study the role of Tr1 cells in healthy donors and patients.

4 In Vitro Induction of Tr1 Cells

Several methods have been identified to induce Tr1 cells in vitro but IL-10 remains the main driving force. Originally, we induced Ag-specific Tr1 cells by repeated TCR stimulation in presence of high doses of IL-10 (Groux et al. 1997). Human Tr1 cells were generated by addition of exogenous IL-10 in cultures of human peripheral blood mononuclear cells (PBMC) or purified $CD4^+$ T cells stimulated with allogeneic monocytes (mixed lymphocytes reactions- MLR/IL-10) (Groux et al. 1996). The resulting T cells were anergic and contained precursors of IL-10-producing Tr1 cells (Groux et al. 1997). Anergy induced by IL-10 was Ag-specific, since IL-10-anergized $CD4^+$ and $CD8^+$ T cells did not proliferate upon re-challenge with the same Ag used during cell priming. Notably, after IL-10 anergization, the bulk culture contained also non-anergic T cells that were non-alloAg-specific and maintained their ability to respond to other Ags, such as pathogens or third party allo Ags (Bejarano et al. 1992; Bacchetta et al. 2010). IL-10-anergized cultures had a peculiar molecular signature characterized by down-regulation of signal transduction, cell cycle, cell division, and cell activation pathways and up-regulation of wound repairing, inflammatory and defense responses, and cell migration pathways (Bacchetta et al. 2010).

Although the molecular mechanisms underlying Tr1 cell induction via IL-10 is still elusive, STAT3-dependent activation by the IL-10/IL-10R interaction is required for anergy induction and IL-10 secretion by $CD4^+$ T cells (Levings et al. 2001). The importance of the STAT3 pathway to induce Tr1 cells is underlined by

the observation that other STAT3 activating cytokines such as IFN-α (Levings et al. 2001), IL-27 (Awasthi et al. 2007; Fitzgerald et al. 2007; Stumhofer et al. 2007; Murugaiyan et al. 2009) or IL-6 (Jin et al. 2013) promote IL-10-producing Tr1 cells. Activation of STAT3 by IL-27 leads to the induction of cMaf (Yang et al. 2005; Pot et al. 2009; Apetoh et al. 2010) and of AhR (Apetoh et al. 2010). AhR/cMaf complex trans-activates the *il10* and *il21* promoters (Apetoh et al. 2010) and sustains induction of murine Tr1 cells in vitro (Apetoh et al. 2010; Pot et al. 2011). IL-27 has been shown to promote human IL-10-producing T cells (Murugaiyan et al. 2009). However, it is still unclear whether the molecular mechanism underlying the induction of these human IL-10-producing cells is comparable to that observed in murine T cells and whether the resulting T cells are *bona fide* Tr1 cells. Like in the mouse, AhR was shown to be involved in human Tr1-cell differentiation. Stimulation of human $CD4^+$ T cells in the presence of TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or FICZ (6-formylidolo[3,2-b]canbazole) selectively activates AhR that co-operate with cMaf to trans-activate the *il10* promoter and to enhance IL-10 production (Gandhi et al. 2010). More recently, IL-6 was also shown to promote murine IL-10-producing Tr1 cells (Jin et al. 2013). IL-6-mediated activation of STAT3 promotes IL-21 production, which in turn co-operates with IL-2 to induce the expression of cMaf and AhR, thereby leading to IL-10 production in $CD4^+$ T cells. This mechanism is independent of both IL-27 and TGF-β (Jin et al. 2013).

Additional stimuli have been involved in human Tr1-cell induction including signaling via CD2, the ligand for CD58 (Wakkach et al. 2001), co-stimulation via ICOS-L (Ito et al. 2008), and co-signaling via CD46 in the presence of IL-2 (Kemper et al. 2003; Astier et al. 2006). It is still unclear whether CD3/CD46-stimulated T cells are *bona fide* Tr1 cells or they represent distinct inducible Treg cells, since they are not anergic or Ag-specific. Repetitive stimulation of $CD4^+$ T cells in the presence of vitamin D3 and dexamethasone induces a population of Ag-specific Tr1 cells in an IL-10-dependent manner (Barrat et al. 2002).

Immature DC or specialized subsets of DC, termed tolerogenic DC, can drive Tr1-cell differentiation (Gregori 2011). Repetitive stimulation of human naïve $CD4^+$ T cells (Jonuleit et al. 2000; Levings et al. 2005) with allogeneic immature DC induces differentiation of human Tr1 cells in vitro. Several molecules such as IL-10 alone (Jonuleit et al. 2000) or in combination with IFN – α (Manavalan et al. 2003), G-CSF (Rutella et al. 2004; Rossetti et al. 2010), or vitamin D3 alone (Penna and Adorini 2000) or in combination with dexamethasone (Pedersen et al. 2004) can differentiate tolerogenic DC with the ability to generate Tr1 cells. We established a protocol for in vitro differentiation of human tolerogenic DC, termed DC-10, which secrete spontaneously large amounts of IL-10 (Gregori et al. 2010). DC-10 differentiated from peripheral blood monocytes in the presence of GM-CSF, IL-4 and IL-10, are $CD11c^+CD11b^+$, express CD14 and CD16 but not CD1a, and display a mature myeloid phenotype, being $CD83^+$, $CD86^+$ and $HLA\text{-}DR^+$. Importantly, DC-10 express high levels of the tolerogenic molecules Immunoglobulin-Like Transcript (ILT)2, ILT3, ILT4 and the non classical HLA class I molecule HLA-G. Functional assays showed that DC-10 induce the differentiation of anergic allo-specific IL-10-producing Tr1 cells (Bacchetta et al.

2010; Gregori et al. 2010). Moreover, when activated with LPS, DC-10 maintain their phenotype and their ability to induce Tr1 cells. The secretion of IL-10 and the high expression levels of ILT4 and HLA-G are necessary for the tolerogenic activity of DC-10, as addition of neutralizing antibodies against IL-10R, ILT4, or HLA-G during co-culture of DC-10 and naïve T cells completely prevents Tr1-cell induction (Gregori et al. 2010). This observation has been indirectly confirmed by studies in which we compared the ability of G-CSF and IL-10 to promote human tolerogenic DC. Addition of G-CSF and IL-4 during monocyte-derived DC differentiation gives rise to a population of cells (G-DC) that are phenotypically similar to DC-10, since that are $CD14^{+}CD16^{+}$, but $CD1a^{-}$, display a mature myeloid phenotype, and express the tolerogenic markers ILT4 and HLA-G (Rossetti et al. 2010). However, as compared to DC-10, G-DC produce lower levels of basal IL-10 and IL-6 and higher levels of IL-12 and TNF-α upon activation, and express significantly lower levels of HLA-G and ILT4 (Amodio and Gregori 2012b). Consistent with these findings, G-DC had low stimulatory capacity, but they did not induce T-cell anergy and differentiation of suppressive Tr1 cells (Rossetti et al. 2010). Notably, the use of DC-10 represents an advantage as compared to that of monocytes and recombinant human IL-10 in promoting allo-specific T-cell anergy in HLA matched-unrelated donor pairs in whom the HLA disparity is null or very low. Moreover, DC-10-anergized T cells contain not only precursors of allo-specific Tr1 cells but also up to 15 % of allo-specific IL-10-producing Tr1 cells (Bacchetta et al. 2010; Gregori et al. 2010) which are $CD49b^{+}$ $LAG\text{-}3^{+}$ (Gagliani et al. 2013b).

As an alternative approach to generate an homogeneous population of IL-10-producing Tr1 cells we transduced human $CD4^{+}$ T cells with a bidirectional lentiviral vector (LV) encoding for human IL-10 and the marker gene, GFP, which are independently co-expressed (Andolfi et al. 2012). The resulting GFP^{+} LV-IL-10-transduced human $CD4^{+}$ T ($CD4^{IL-10}$) cells secrete high levels of IL-10 and low levels of IL-4 upon activation, and express markers associated with IL-10. Moreover, $CD4^{IL-10}$ T cells are anergic, suppress T cell responses in an IL-10- and TGF-β- dependent manner and in a cell-to-cell contact independent manner in vitro, and kill myeloid APCs. $CD4^{IL-10}$ T cells control xeno Graft-versus-Host Disease, demonstrating their suppressive function in vivo (Andolfi et al. 2012). Thus, constitutive over-expression of IL-10 in human $CD4^{+}$ T cells leads to a stable cell population that recapitulates the phenotype and function of Tr1 cells.

The possibility to generate Ag-specific Tr1 cells in vitro with minimal cell manipulation represents an important and critical step for the use of these cells as cell therapy to promote or restore Ag-specific tolerance in vivo. Moreover, the recent discovery of CD49b and LAG-3 as specific biomarkers of Tr1 cells that allow the isolation of Tr1 cells from in vitro Tr1-cell polarized populations and from Ag-specific IL-10-anergized T cells (Gagliani et al. 2013b), will open the possibility to select highly suppressive Tr1 cells from Ag-specific IL-10-anergized T cell cultures induced with either allogeneic DC-10 or autologous DC-10 pulsed with a given Ag. These recent advances will help in developing effective protocols for the isolation of pure clinical grade Tr1 cells.

5 In Vivo Induction of Tr1 Cells

Tr1 cells are physiologically generated in vivo through yet undefined mechanisms and are key in maintaining immunological homeostasis and preventing undesired immune responses. Here we describe the means reported to lead to the in vivo generation of Tr1 cells upon external intervention in mice and humans.

IL-10 is key for Tr1-cell induction, in accordance, any compound that triggers IL-10 production with simultaneous inhibition of pro-inflammatory signals is a possible good candidate for Tr1-cell generation in vivo. Over the years we demonstrated the ability of IL-10 or IL-10-inducing agents in combination with standard immunosuppressive treatments to generate Tr1 cells in vivo in models of autoimmunity or allogeneic transplantation (Battaglia et al. 2006a, b; Gagliani et al. 2010, 2011). Rapamycin + IL-10 showed to be an excellent combination therapy in both protecting from autoimmunity (Battaglia et al. 2006b) and inducing tolerance to allogeneic islets (Battaglia et al. 2006a). The addition of depleting agents such as the anti-CD45RB mAb or replacement of IL-10 with G-CSF, a molecule with several biological activities similar to IL-10 (Morris et al. 2004), also led to the in vivo generation of Ag-specific Tr1 cells in more stringent allograft models (Gagliani et al. 2011). In all our in vivo studies, Tr1 cells always localize in the spleen of tolerant mice (Gagliani et al. 2013a). It is still a matter of debate whether Tr1 cells are also generated in the spleen or the spleen represents their in vivo natural "reservoir", and whether in humans this organ is also the privileged site for Tr1 cell accumulation.

Another interesting compound boosting a tolerogenic IL-10-enriched environment is the anti-CD3 mAb. A single course of treatment with a non-FcR binding anti-CD3 monoclonal antibody, the hOKT3g1 (Ala-Ala), leads to preservation of insulin production in patients with new-onset type 1 diabetes (T1D). The sustained insulin production correlates with improved glucose control and reduced use of insulin (reviewed in (Daifotis et al. 2013)). This monoclonal antibody appears to deliver an activation signal to T cells, resulting in disproportionate production of IL-10 relative to IFN-γ in vitro and detectable levels of IL-10, IL-5, but rarely IFN-γ in the sera of treated patients. In addition, this treatment induces a population of IL-10^{+}CCR4^{+}CD4^{+} T cells in vivo (Herold et al. 2003). Anti-CD3 treatment leads to powerful generation of Tr1 cells also in various murine models. Repetitive intraperitoneal anti-CD3 mAb treatment indeed induces Tr1 cells in intraepithelial lymphocytes of the small intestine, whereas the same treatment induces mostly FOXP3^{+} Treg cells in lamina propria lymphocytes of the colon (Kamanaka et al. 2006). Similarly, in humanized mice treated with anti-CD3 mAb, intestinal migration was also seen via a CCR6/CCL20 dependent gradient and induction of IL-10 (Waldron-Lynch et al. 2012). Intranasally-administered anti-CD3 mAb also proved to be a new approach for the therapeutic induction of Tr1 cells in vivo (Weiner et al. 2011). The group of H. Weiner showed that intranasal anti-CD3 mAb delivery triggers the differentiation of suppressive Tr1 cells by a mechanism dependent on the production of IL-27 by upper airway-resident DC.

These data suggest that the same compound given via different route and in different settings (autoimmune vs. non pathogenic) generates Tr1 cells in different tissues and likely through different mechanisms.

We clearly showed that human DC-10 are one of the best Tr1-cell inducer in vitro. Similar data were generated in vivo in mice in a model of tolerance to transgene delivered by Adenoviral (Ad) vectors. Ad vectors have been extensively used for pulmonary gene therapy due to their ability to efficiently transduce a wide variety of proliferating and non-proliferating cells. Helper-dependent adenoviral (HD-Ad) vectors do not encode any viral genes and their use have led to significant improvement in the safety and efficacy of Ad-based vectors (Kushwah et al. 2007). Although with HD-Ad vectors the immune response to vector-encoded transgene expression is reduced, subsequent vector re-administration can increase the immune response thereby limiting transgene expression. Kushwah et al. delivered HD-Ad-pulsed IL-10-modified murine DC (generated in vitro in a very similar way to the human DC-10, Gregori et al. 2010) and subsequently assessed pulmonary DC maturation and migration following intranasal challenge with HD-Ad particles. Delivery of HD-Ad-pulsed IL-10-modified DC to mice led to induction of tolerance to HD-Ad vectors. In tolerant mice, maturation of pulmonary DC in response to intranasal HD-Ad delivery was impaired. Moreover, upon several rounds of HD-Ad challenge, in tolerant mice the T cell response towards HD-Ad vectors was significantly diminished along with a significant reduction in anti-Ad antibody titers, confirming the ability of HD-Ad-pulsed IL-10-modified DC in inducing long-term tolerance toward HD-Ad vectors in vivo. These data support the use of IL-10-modified DC as a stable means for the in vivo generation of Tr1 cells also in humans.

In mice, DC subsets other than IL-10-modified DC have been shown to generate Tr1 cells in vivo. $CD11c^{low}CD45RB^{high}$ DC are present in the spleen and lymph nodes of normal mice and are significantly enriched in the spleen of IL-10 transgenic mice (Groux et al. 1999; Wakkach et al. 2003). This DC subset can also be generated in vitro from bone marrow cells in the presence of exogenous IL-10. These natural or in vitro-derived DC display plasmacytoid morphology and an immature-like phenotype even after in vitro activation with LPS or CpG oligodeoxynucleotides, and secrete high levels of IL-10 after activation. In addition, $CD11c^{low}CD45RB^{high}$ DC induce the differentiation of Tr1 cells and adoptive transfer of OVA-pulsed $CD11c^{low}CD45RB^{high}$ DC induces OVA-specific unresponsiveness in recipient mice (Wakkach et al. 2003).

Intestinal $CD103^+$ DC mediate *Bifidobacterium breve (B. breve)*-induced development of Tr1 cells that express cMaf, IL-21, and AhR in the large intestine (Jeon et al. 2012). *B. breve* activates intestinal $CD103^+$ DC to produce IL-10 and IL-27 via the TLR2/MyD88 pathway, thereby inducing Tr1 cells in the large intestine. Oral *B. breve* administration ameliorated colitis in immune compromised mice given naive $CD4^+$ T cells from wild-type mice but not from *Il10*$^{-/-}$ mice. These findings demonstrate that *B. breve* prevents intestinal inflammation through the induction of intestinal IL-10-producing Tr1 cells via local $CD103^+$ DC (Jeon et al. 2012).

Overall, different DC subsets naturally present or generated in vivo localize in different tissues and drive the differentiation of Tr1 cells that control the specific tissue-immune response. One can envisage to boost the generation of specific DC subset that would lead to tissue-local specific Tr1-cell mediated tolerance.

An interesting alternative way to generate Tr1 cells in vivo is via the administration of soluble Ag. The group of L. Steinman demonstrated, back in 1994, that administration of the soluble form of the encephalitogenic determinant reverses ongoing experimental autoimmune encephalomyelitis (EAE) induced by T cells recognizing the same determinant (Karin et al. 1994). It was later proved that the rapid effect of soluble peptide therapy is due to in vivo repolarization of autoimmune T cells undergoing activation into Tr1 cells. These cells produce high levels of IL-10 in response to the determinant to which the immune response spreads, but not in response to the determinant with which the disease was induced (Gizi et al. 2002). The ability of such cells to suppress EAE and the competence of anti-IL-10 mAb to reverse tolerance induced by soluble peptide therapy with this determinant further support their regulatory role. Thus, soluble peptide therapy with the determinant to which the autoimmune response spreads amplifies a de novo regulatory mechanism aimed to reduce the pathological consequences of determinant spreading. Importantly, Tr1 cells play a pivotal role in the regulation of T-cell tolerance during determinant spreading.

6 Tr1 Cells in Transplantation

Tr1 cells were first identified and characterized in Severe Combined Immune-Deficient (SCID) patients who were immune-reconstituted after hematopoietic stem cell transplantation (HSCT) from HLA-mismatched donors. These patients developed spontaneous split chimerism, with T and natural killer (NK) cells donor-derived and B cells and APCs of host origin (Roncarolo et al. 1986, 1988a, b; Bacchetta et al. 1990, 1994), in the absence of graft versus host disease (GvHD) (Bacchetta et al. 1994). Cultures of the PBMC of these chimeras led to the isolation of donor-derived $CD4^+$ and $CD8^+$ T cell clones that produced IFN-γ and were specific for HLA-Ags of the host. A high proportion of these T cell clones had the Tr1-cell distinctive pattern of cytokine production (Bacchetta et al. 1990, 1994). Similar data were also obtained in transplanted β thalassemic patients who developed persistent mixed chimerism (Serafini et al. 2009). In these patients, allo-reactive T cells were not deleted and a high proportion of IL-10-producing Tr1 cell clones were identified in vitro. Interestingly, Tr1 cell clones isolated from this mixed-chimeric environment were both donor and host derived, and their regulatory function was exerted in both directions (i.e. G*v*HD and rejection). Notably, Tr1 cells were not detected at high frequency in patients with complete donor chimerism, indicating that their presence correlate with a state of active tolerance in mixed chimeric patients in the absence of GvHD or rejection, despite the chronic donor-host allo-Ag stimulation in vivo (Serafini et al. 2009).

The increased proportion of Tr1 cells in patients with mixed chimerism was recently confirmed using CD49b and LAG-3 as specific surface biomarkers of Tr1 cells (Gagliani et al. 2013b). Increased percentages of $CD49b^+$ and $LAG\text{-}3^+$

T cells were detected in PBMC from chimeric patients. Studies at clonal level in hematopoietic stem cell transplanted patients are laborious but the identification of CD49b and LAG-3 as specific surface biomarkers of Tr1 cells will allow an easier assessment of these cells in clinical samples.

Studies on establishment of tolerance after organ transplantation have been hampered by the fact that these patients undergo immunosuppressive treatments, given the high HLA disparity and the high risk of rejection in this context. Ongoing pharmacological immunosuppression interferes with detection of functional T cell responses in vitro and, possibly, with the induction of active tolerance in vivo. However, some patients do not reject the transplants after immunosuppression withdraw or require less immunosuppression than others, suggesting that their immune system is more prone to tolerance. Several studies have indicated IL-10 as part of the cytokine profile associated to an individual trend to better tolerate the transplant. This has been observed after different type of organ transplants, including lung, heart, liver, and kidney, suggesting that mechanisms of tolerance once established are similar in different settings. Increased IL-10-producing T cells, or increased IL-10 mRNA gene expression, have been commonly observed after different kind of organ transplants, even several years after transplantation (Bharat et al. 2006a, b; Dijke et al. 2006). In addition, specific IL-10 gene polymorphisms have been associated to better engraftment, although data are still controversial (Roncarolo et al. 2011). Interestingly, not only T cells have been indicated as source of IL-10 to promote tolerance. For example, cells of the innate immune system, such as mast cells, may contribute to create an IL-10 enriched milleu, and this may favor differentiation of Tr1 cells (Leveson-Gower et al. 2013). Similarly, stromal cells and B cells can secrete IL-10 and influence adaptive immunity, namely influence adaptive immunity towards tolerance.

Importantly, several cell transfer studies in murine disease models enforce the role of Tr1/IL-10 producing T cells in inducing Ag-specific transplantation tolerance. Transfer of Tr1 cells generated in vitro in the presence of TGF-β and IL-10 has been shown to protect from GvHD in murine models of mismatched hematopoietic stem cell transplantation (Zeller et al. 1999). In addition, cell-transfer of Ag-specific Tr1 cells was superior to that of polyclonal Tr1 cells in successfully prevent allograft rejection in two different models of islet transplantation (Gagliani et al. 2010).

Overall, these results indicate that IL-10 and Tr1 cells are associated with long-term transplantation tolerance either induced or established spontaneously in both mice and humans. These data built the rationale for medical intervention with Tr1-cell based therapy to promote transplantation tolerance not only in HSCT but also in organ transplantation.

7 Tr1 Cells in Autoimmune Diseases

Tr1 cells are fundamental for suppressing self-reactivity and maintaining self-tolerance, not only in experimental autoimmune disease models but also in many human autoimmune diseases.

The original finding that Tr1 cells efficiently prevent auto-inflammation in mice was reported by our group in a model of colitis. The pathogenicity of transferred $CD45RB^{high}CD4^+$ T cells into SCID mice was indeed inhibited by co-transfer of murine Tr1 cell clones derived from $CD4^+$ T cells expressing a transgenic T cell receptor specific for an ovalbumin (OVA) peptide. These Tr1 cell clones inhibited colitis only in recipients receiving OVA in their drinking water, demonstrating that the immune suppression relies on the Ag-specific activation of Tr1 cells in vivo (Groux et al. 1997). Maynard et al. have also suggested that Tr1 cells play a major role in maintaining immune homeostasis to the intestinal microbiota (Maynard et al. 2007). This hypothesis is consistent with the finding that loss of IL-10 results in the development of spontaneous enterocolitis in IL-10 deficient mice (Kuhn et al. 1993) and that patients with mutation in either IL-10 or in IL-10R suffer from severe colitis (Glocker et al. 2009, 2010; Gorelik et al. 2002; Gorelik and Flavell 2002). Similarly to the colitis model, transfer of in vitro generated OVA-specific Tr1 cells in mice suffering from EAE prevented development of neurological symptoms when OVA peptide was injected intracranially (Barrat et al. 2002). In addition, Meiron et al. reported that stromal cell-derived factor 1a (CXCL12), redirects the polarization of effector Th1 cells into $CD4^+CD25^-Foxp3^-IL\text{-}10^{high}$ Ag-specific Tr1 cells that suppress ongoing EAE (Meiron et al. 2008).

Of particular interest is the wide variety of human autoimmune diseases in which a defect in Tr1-cell frequency/function has been demonstrated, raising the attractive possibility that this may be a common denominator in uncontrolled immune responses to self-Ag. A defect in Tr1 cells has been proved in patients with T1D, multiple sclerosis (MS), pemphigus volgaris, myasthenia gravis (MG), and celiac disease (CD). In patients with T1D, higher numbers of insulin-specific IL-10 producing cells in early onset T1D subjects are associated with better future glucose control (Sanda et al. 2008), which in turn suggests that the presence of Ag-specific Tr1 cells enables better disease control. In addition, T cells from non-diabetic individuals carrying HLA class II molecules associated with the disease show an IL-10 response to islet peptides, while T cells from diabetic subjects predominantly produce IFN-γ in response to the same Ag (Arif et al. 2004). Therefore, islet destruction is characterized by pro-inflammatory autoreactive T cells while the tolerant non-diabetic state is characterized by autoreactive IL-10-producing Tr1 cells. Interestingly, the frequencies of IFN-γ^+ and IL-10^+ cells are similar between autoantibody negative first degree relatives (FDR) of T1D patients and T1D patients. However, cells from autoantibody negative FDR produce higher amounts of IL-10 compared to IFN-γ than those from T1D patients. Thus, despite the observation that FDR of T1D have cells that are capable of responding to T1D-autoAgs, they do not have the disease. This is most probably because the balance of auto Ag reactive T cells is favored towards IL-10 production (regulatory response) as compared to IFN-γ production (inflammatory response) (Petrich de Marquesini et al. 2010). This data leads to the hypothesis that even a moderate IL-10-regulatory response may be sufficient to prevent the development of T1D in genetically predisposed individuals.

MS is a complex genetic disease characterized by inflammation in the central nervous system white matter mediated by activated autoreactive lymphocytes.

CD46-activated T cells acquire a Tr1-cell phenotype (Kemper et al. 2003), and the group of D. Hafler observed that CD46-activated T cells from patients with MS secrete lower levels of IL-10 as compared to those from healthy donors (Astier et al. 2006). This defect was specific to CD46-mediated activation, as IL-10 secretion upon CD28 stimulation was not affected. Furthermore, levels of IFN-γ secreted by CD46-activated T cells were not influenced and reflected cell proliferation. While no difference in expression of CD46 cytoplasmic isoforms was detected in freshly isolated T cells, an increase in Cyt2 (one of the two possible splicing forms of the intracytoplasmic tails of CD46) expression was observed in T cells from patients with MS upon CD46 activation, indicating that CD46 is dysregulated in patients with impaired IL-10 production (Astier et al. 2006). Similarly, Martinez-Forero and colleagues confirmed that $CD4^+$ T cells from MS patients were less prone to differentiate to functional Tr1 cells after induction with anti-CD46 mAb (Martinez-Forero et al. 2008). In addition, IL-10 obtained from Tr1-cell culture supernatants was unable to suppress $CD4^+$ T cell proliferation in MS patients and the IL-10 signaling pathway was altered. A down-regulation of STAT3 phosphorylation was reported and this was ascribed to SOCS3, an inhibitor of cytokine signaling, in MS patients (Martinez-Forero et al. 2008). In summary, all these studies indicate that the function and IL-10 signaling pathway of CD46-induced Tr1 cells is altered in MS patients. It remains to be defined whether this is a feature of only CD46-induced Tr1 cells or it is a general characteristic of Tr1 cells in this disease.

Pemphigus vulgaris is primarily associated with circulating autoantibodies against the desmosome component desmoglein 3 (Dsg3). Dsg3-specific Tr1 cells that may maintain and restore natural tolerance against Dsg3 were identified (Veldman et al. 2004). Dsg3-responsive IL-10-secreting Tr1 cells were isolated from healthy carriers of the pemphigus-associated HLA class II alleles DRB1*0402 and DQB1*0503, but were only rarely detected in pemphigus patients. In addition, it was shown that the growth of Dsg3-responsive Tr1 cells requires the presence of IL-2, and that they exert their Dsg3-dependent inhibitory function by secreting IL-10 and TGF-β. Thus, these Tr1 cells may be critically involved in the maintenance and restoration of tolerance against the autoAg Dsg3.

MG is an autoimmune disease characterized by fluctuating pathological weakness involving skeletal muscle. It is primarily caused by autoantibodies to the nicotinic acetylcholine receptor (AChR) at the postsynaptic site of the neuromuscular junction. Decreased in vitro IL-10 mRNA expression from non-stimulated PBMC in non-thymectomized MG patients was reported (Huang et al. 2000). When PBMC from healthy individuals are stimulated by Con A, the expression level of IL-10 is related to three polymorphisms in the promoter region of the IL-10 gene (Turner et al. 1997); G/A at position −1082, T/C at position −819 and A/C at position −592. They constitute three haplotypes (GCC, ATA and ACC), which in combination are associated with high (GCC/GCC), medium (GCC/ATA, GCC/ACC) or low (ATA/ATA, ATA/ACC and ACC/ACC) expression of IL-10. The distribution of IL-10 polymorphisms in MG patients was analyzed to determine whether these influenced disease susceptibility and interestingly low producer IL-10 haplotypes were highly associated with MG (Alseth et al. 2009).

Celiac disease (CD) is a common disorder of small intestine due to permanent intolerance to dietary wheat gluten. An abnormal T-cell mediated immune response to gliadin, together with the absence of specific immune regulation, plays a crucial role in inducing the celiac enteropathy (Troncone et al. 1998). We found that gliadin-specific T-cell activation was suppressed in celiac intestinal mucosa cultured 24 hours ex vivo with gliadin and IL-10. Interestingly, these gliadin-specific T-cell lines generated in the presence of IL-10 were anergic in response to gliadin (Salvati et al. 2005). Moreover, we isolated gliadin-specific Tr1 cell clones from intestinal mucosa of celiac patient in gluten free diet. These Tr1 cell clones were anergic, produced IL-10 and TGF-β, and had a strong inhibitory capacity of gliadin-specific T cell response (Gianfrani et al. 2006).

All these data show that IL-10 and, specifically Tr1 cells, are relevant for autoimmune disease protection. Strategies for in vivo boosting of Tr1 cells or cell therapy with ex vivo generated Ag-specific Tr1 cells might be envisaged for prevention/cure of auto-immune diseases.

8 Tr1 Cells in Infectious Diseases

Induction of Treg cells during infectious diseases may have both beneficial and harmful effects for the host. The host can benefit from pathogen-mediated Treg cell induction particularly during chronic infections, as Treg cells limit inflammatory responses and thereby reduce host tissue damage (Belkaid 2008). However the downside may be that the pathogen may not be fully eradicated, allowing the establishment of persistent infection. This enhances the risk of disease reactivation later in life, when facing (temporal) immune dysfunction, for example, during co-infections or co-morbidity (Joosten and Ottenhoff 2008). One interpretation of the interplay between pathogenic organisms and Treg cells is that pathogens have evolved to take advantage of the inherent regulatory mechanisms in the body, hijacking an existing system to evade host immune responses. Whether this regulatory response is ultimately beneficial or detrimental to the host strictly depends on the type of infection.

The intestine contains a large quantity of resident bacteria that are a significant source of both Ags and pro-inflammatory molecules. However, despite these immune responses the intestine remains in a state of controlled inflammation, suggesting that mucosal immune responses to enteric bacteria are tightly regulated. One potentially important immune regulatory function of the intestinal microflora is their involvement in the generation of distinct T-cell subtypes and maintenance of homeostasis. In the study by Christensen et al., various lactobacilli species have been demonstrated to differentially activate DC. DC stimulated with *Lactobacillus reuteri* (*L. reuteri*) were found to secrete high levels of IL-10, in contrast to *L. casei* treated DC, which preferentially induced IL-12 production (Christensen et al. 2002). The results of this study suggest that species of lactobacilli exert very different and opposing effects on DC activation. It is possible

that *L. casei* as a strong IL-12 inducer could be a contributing type 1 DC polarizing factor, whereas *L. reuteri* which induces IL-10 may promote the differentiation of Tr1 cells. However, the precise mechanism by which probiotics induce IL-10-producing T cells in the intestinal lamina propria is unclear. Jeon and colleagues recently demonstrated that the probiotic strains of bacteria *B. breve*, but not *L. casei* as previously shown (Christensen et al. 2002), induced development of Tr1 cells that express cMaf, IL-21, and AhR in the large intestine (Jeon et al. 2012). Intestinal $CD103^+$ DC mediated *B. breve*-induced development of Tr1 cells. $CD103^+$ DC from Il10(-/-), Tlr2(-/-), and Myd88(-/-) mice showed defective *B. breve*-induced Tr1 cell development. *B. breve*-treated $CD103^+$ DC failed to induce IL-10 production from co-cultured Il27ra(-/-) T cells. Finally, *B. breve* treatment of Tlr2(-/-) mice did not increase IL-10-producing T cells in the colonic lamina propria. Thus, *B. breve* activates intestinal $CD103^+$ DC to produce IL-10 and IL-27 via the TLR2/MyD88 pathway thereby inducing IL-10-producing Tr1 cells in the large intestine (Jeon et al. 2012). Oral *B. breve* administration ameliorated colitis in immune compromised mice infused with naïve $CD4^+$ T cells from wild-type mice, but not from Il10(-/-) mice. These findings demonstrate that *B. breve* prevents intestinal inflammation through the induction of intestinal IL-10-producing Tr1 cells.

Another example of beneficial Treg-cell activity after infection comes from studies with *Bordetella pertussis* (*B. pertussis*), the etiologic agent of whooping cough. *B. pertussis* causes a severe and protracted respiratory disease, often complicated by secondary infections that can have a lethal outcome in young children. Recovery from infection in humans and mice is associated with the development of *B. pertussis*-specific Th1 cells (Barnard et al. 1996; Ryan et al. 1997). However, Ag-specific Th1-cell immune responses in the lungs of infected mice are severely suppressed during the acute phase of infection (McGuirk et al. 1998). Two virulence factors, filamentous hemagglutinin (FHA) and adenylate cyclase toxin (CyaA), have been shown to induce IL-10 production from macrophages and DC, either alone or in synergy with TLR ligands, and thereby selectively stimulate the induction of Tr1 cells (McGuirk et al. 2002; Ross et al. 2004). Furthermore, Tr1-cell clones specific for FHA and pertactin have been generated from the lungs of acutely infected mice (McGuirk et al. 2002). Thus, simultaneous induction of Tr1 cells helps to dampen Th1 cells and inflammatory responses and, although exerting temporary suppression of the protective Th1 cells, eventually allow resolution of the infection with minimal collateral damage to host tissues.

During chronic helminth infection onchocerciasis (river blindness), where patients have relatively little signs of dermatitis despite the presence of millions of small worms in the skin, T cells that bear characteristics of Tr1 cells can be obtained (Satoguina et al. 2005). A significant part of T cells present in the onchocercomas are Ag driven and may account for the finding that down-regulation is Ag specific and does not extend to other closely related nematode Ag such as *Ascaris* (Doetze et al. 2000). However, also non-Ag-specific T cell clones derived from onchocercomas bear the Tr1-cell phenotype. It is conceivable that these cells have been educated on-site to adopt the suppressor phenotype by infectious

tolerance. Thus, Tr1 cells could have a role in counteracting Th2-cell-driven effector/inflammatory responses, enabling the host to coexist with a chronic parasite without destruction of self-tissues.

A detrimental effect of Tr1 cells in infections has also been reported. In tuberculosis, 15 % of infected patients fail to respond to intradermal injection with purified protein derivative (PPD) (Boussiotis et al. 2000). T cells from patients with normal DTH responses proliferate and secrete IFN-γ. In contrast, T cells from unresponsive patients proliferate poorly and secrete high levels of IL-10 (Boussiotis et al. 2000; Delgado et al. 2002). Furthermore, studies in IL-10 transgenic mice demonstrate that increased susceptibility to reactivation tuberculosis and suppression of protective Th1-cell responses is strongly influenced by the expression of IL-10 during the latent phase of infection (Turner et al. 2002). In malaria, certain circumsporozoite variants have been shown to induce IL-10, rather than IFN-γ, production by $CD4^+$ T cells, suggesting a mechanism whereby malaria can persist in an infected individual (Plebanski et al. 1999). Similarly, individuals who are infected with *Wuchereria bancrofi* (filariasis) and are microfilaremic lack T-cell proliferation and IFN-γ responses to parasite Ags. These individuals, however, do produce high levels of IL-10 in response to parastic Ags (Montenegro et al. 1999).

Finally, Human Immunodeficiency Virus (HIV)-1–infected individuals with active viral replication or progression have increased frequencies of circulating IL-10–producing $CD4^+$ T cells, in comparison to non-progressors or non-infected-individuals. In addition, constitutive IL-10 production by $CD4^+$ T cells was significantly associated with the inability to mount an IFN-γ producing $CD4^+$ T-cell immune response to HIV-1 *gag* (Ostrowski et al. 2001). The inverse nature of IFN-γ and IL-10 production by $CD4^+$ T cells is further illustrated by HIV infected individuals who were treated with Highly Active Anti-Retroviral Therapy (HAART). In 2 of the 3 individuals studied, Ostrowski and colleagues observed a dramatic decline in levels of circulating IL-10–producing $CD4^+$ T cells associated with the development of IFN-γ producing $CD4^+$ T-cells response to HIV-1 (Ostrowski et al. 2001). It remains unclear whether these circulating IL-10–producing $CD4^+$ T cells are HIV-1 specific or simply reflect a down-regulation to generalized immune activation. An enhancement in IL-10 produced by $CD4^+$ T cells was observed after incubation with HIV-1 *gag* in around 50 % of HIV-1–infected individuals, which suggests that HIV-1 Ags themselves are responsible for IL-10 induction. Thus, in certain HIV-1–infected individuals, HIV-1 may subvert immune responses by inducing Tr1 cells, which may prevent the development of powerful anti-HIV-1 specific immune responses (Ostrowski et al. 2001).

9 Tr1-Cell Clinical Application

The use of Treg cells as a therapeutic modality to modulate immune responses in human has become a major goal for several investigators. Treg cell therapy may represent an innovative treatment for patients with autoimmunity, chronic

inflammatory diseases, allergies and other immune mediated pathologies. While Treg cell therapy, as compared to drugs, could provide obvious advantages, including an Ag-specific immunomodulation in the absence of general immunosuppression, several hurdles need to be overcome before this approach becomes widely accepted and feasible. One of the major challenges will be the identification of the optimal Treg cell dose to be infused in order to counteract the potency of inflammatory tissue damage that differ in different pathologies. An additional issue regards the in vivo cell survival and persistence of Treg cells. Indeed, it is still undefined whether a single cell infusion is sufficient or multiple administrations are required to obtain a persistent tolerogenic effect. Furthermore, it is still unclear whether infused Treg cells migrate to tissues or, after ex vivo expansion they have limited recirculation capacity. Increasing evidences suggest that Treg cells, at least the FOXP3-expressing subset, are intrinsically plastic, especially when exposed to an inflammatory environment. Tr1 cells expanded/generated in vitro for immunotherapy may also have the potential risk of converting into effector T cells in vivo under inflammatory conditions, with consequent loss of their suppressive ability.

At present, results from two initial proof-of-concept trials are in favor of the safety of Tr1-cell therapy. We performed a clinical trial in which adoptive cell therapy with Tr1 cells was administered into patients affected with advanced hematological malignancies undergoing haploidentical HSCT therapy with the aim to provide fast immune-reconstitution and prevent GvHD (Bacchetta et al. 2014). Accordingly to the need of immune reconstitution within a high degree of HLA disparity, we provided these patients with donor T cells which have been primed for a short time (10-days) in the presence of the host APC and IL-10 to "instruct" the Ag-specific T cells to differentiate into Tr1 cells. The method to obtain the infused cells was validated in good-manufacturing-practice (GMP), to generate allo-specific IL-10-anergized T cells by using host monocytes in the presence of IL-10 (IL-10 DLI) (Bacchetta et al. 2010; Gregori et al. 2011; Roncarolo et al. 2011). These cells were infused in overall 12 patients 1-month after treatment with a mega-dose of $CD34^+$ cells and when the patients showed myeloid engraftment. The dose that showed to be safe was 10^5 $CD3^+$ T cells/kg. However, for some patients this cell dose was not sufficient to protect from infections or to prevent disease relapse. Overall, four patients could benefit of this adoptive immunotherapy with IL-10 DLI and fully recovered from the diseases with an uneventful long-term follow-up. There are evidences that in these long-term survivors (mean follow-up 7.5 years) tolerance has been established and circulating Tr1 cells are present at high frequency (Bacchetta et al. 2014). A similar cell-therapy protocol is now planned using DC-10 as APC to produce IL-10-anergized T cells in vitro. In this case, DC-10 in vitro differentiated from donor monocytes will be used to anergize recipient T cells. This Tr1 containing T cells will be injected into patients transplanted with kidney from living donors in combination with standard immunosuppressive drugs. This study will evaluate the benefit of the cell therapy in terms of preventing graft rejection even long after the transplant, for which standard immunosuppression has shown not to be efficient. This clinical trial is part of "The ONE Study", an integrated European Union-funded project, that aims

at developing and testing different subsets of regulatory cell products in kidney transplanted recipients allowing a direct comparison of the safety, clinical practicality and therapeutic efficacy of different cell types (http://www.onestudy.org/).

A second proof-of-concept trial of Tr1 cell therapy consisted of OVA-specific Tr1 cells clones in single injection to patients affected with refractory Chron's disease (CD) (Desreumaux et al. 2012). OVA-specific Tr1 cells were generated from culturing PBMC with native OVA, IL-2, and IL-4 to enrich and expand OVA-specific T cells, followed by cloning and expansion on a layer of feeder cells (i.e., Schneider cells) transfected to express a membrane-bound anti-CD3 antibody, as well as human CD80, CD58, IL-2, and IL-4. OVA-Tr1 cell clones were selected based on an OVA-specific IL-10 production. To ensure activation of OVA-specific Tr1 cells migrating to the gut, patients ingested an OVA-enriched diet. Twenty patients received a single injection. Different doses were tested in different patients groups. The injections were well tolerated. This trial resulted in a 40 % response rate in the intention-to-treat population. CD flares were documented in 7 patients. Six out of the 8 CD patients in the lowest dose group (10^6 Treg cells) had a measurable response, with 3 patients reaching remission, although transient. Therefore, the study was safe and also showed some benefit, however the clinical effect was time limited, reaching the maximum 5-weeks after treatment and declining thereafter. This observation suggests that the infusion of multiple doses of Tr1 cells may be required for long-term control of the disease.

Overall these first trials are promising. Tr1 cells have the advantage of being quickly generated in vitro in an Ag-specific manner, therefore their potential application is very broad.

As an alternative to generate homogeneous population of Tr1 cells LV-mediated IL-10 gene transfer can be utilized, as described above (Andolfi et al. 2012). LV-mediated gene transfer represents an innovative tool for generating cells suitable for therapy. These cells have overlapping characteristics with the IL-10 in vitro generated Tr1 cells, but they can be more easily expanded in vitro, attribute that could be very important in order to generate more cells for multiple infusions for example. In addition, since they constitutively overexpress IL-10 they have a very stable phenotype and therefore they could be more powerful suppressors. Finally, this approach can be applied to generate Ag-specific Tr1 cells by transducing Ag-experienced T cells.

Future clinical application of in vitro generated Tr1 cells envisage the selection of highly suppressive cells from IL-10-anergized cultures thanks to the recent discovery of CD49b and LAG-3 as specific biomarkers of Tr1 cells (Gagliani et al. 2013b). These biomarkers will allow the isolation of Tr1 cells from in vitro Tr1-polarized populations and from Ag-specific IL-10-anergized T cells.

Optimization of the best modality to obtain Tr1 cells suitable for cell therapy will contribute to facilitate their use in diseases different from those selected for the initial proof of concept trials. In allogeneic HSCT, Tr1 cells could be used in transplant from unrelated matched donors to prevent chronic GvHD; in organ transplantation, their use could be extended to liver and pancreatic islet transplant possibly to prevent or limit the need of long-term immunosuppressive therapy. Furthermore, autologous Tr1 cell therapy can be indicated in food allergy and in celiac disease, in which the patients could be fed with low doses of the specific

antigens to maintain the peripheral Tr1 cell pool operational and to favour the gut homing. Ultimately, a variety of autoimmune diseases, especially those with recurrent course and high inflammatory component such as rheumatoid arthritis, could also be a good indication for Tr1 cell therapy, which could be performed by local administration to further avoid systemic side effect of anti-inflammatory drugs.

10 Conclusions

Since the discovery of Tr1 cells nearly two decades ago, research has firmly established their role in controlling immune homeostasis and preventing a wide variety of diseases. A great deal of progress has been made in understanding the mechanisms of induction and suppression by Tr1 cells. However a number of important questions still remain: the intracellular mechanisms which lead to the induction of Tr1 cells, their in vivo localization in healthy and pathological conditions, their ability to recirculate and to home to the target tissue, their in vivo survival and plasticity etc. Moreover, although it is well established that $FOXP3^+$ Treg and Tr1 cells are distinct populations sharing similar mechanisms of suppression, additional research is needed to define the precise relationship between these Treg cell subsets. Addressing these questions will bring us closer to unravel Tr1-cell biology, to effectively use them for cellular therapy in a wide variety of diseases and possibly to identify specific compounds able to switch on and off these cells in vivo.

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References

Akdis M (2008) T-cell tolerance to inhaled allergens: mechanisms and therapeutic approaches. Expert Opin Biol Ther 8:769–777. doi:10.1517/14712598.8.6.769

Alseth EH, Nakkestad HL, Aarseth J, Gilhus NE, Skeie GO (2009) Interleukin-10 promoter polymorphisms in myasthenia gravis. J Neuroimmunol 210:63–66. doi:10.1016/S0165-5728(09)00066-6

Amodio G, Gregori S (2012a) Dendritic cells a double-edge sword in autoimmune responses. Front Immunol 3:233. doi:10.3389/fimmu.2012.00233

Amodio G, Gregori S (2012b) Human tolerogenic DC-10: perspectives for clinical applications. Transplant Res 1:14. doi:10.1186/2047-1440-1-14

Andolfi G, Fousteri G, Rossetti M, Magnani CF, Jofra T, Locafaro G, Bondanza A, Gregori S, Roncarolo MG (2012) Enforced IL-10 expression confers type 1 regulatory T cell (Tr1) phenotype and function to human CD4(+) T cells. Mol Ther J Am Soc Gene Ther 20:1778–1790. doi:10.1038/mt.2012.71

Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL, Kuchroo VK (2010) The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol 11:854–861. doi:10.1038/ni.1912

Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, Peakman M (2004) Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest 113:451–463

Astier A, Meiffren G, Freeman S, Hafler D (2006) Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. J Clin Investig 116:3252–3257. doi:10.1172/jci29251

Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 8:1380–1389. doi:10.1038/ni1541

Bacchetta R, Bigler M, Touraine JL, Parkman R, Tovo PA, Abrams J, De Waal Malefyt R, De Vries JE, Roncarolo MG (1994) High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. J Exp Med 179:493–502

Bacchetta R, De Waal Malefijt R, Yssel H, Abrams J, De Vries JE, Spits H, Roncarolo MG (1990) Host-reactive CD4 + and CD8 + T cell clones isolated from a human chimera produce IL-5, IL-2, IFN-gamma and granulocyte/macrophage-colony-stimulating factor but not IL-4. J Immunol 144:902–908

Bacchetta R, Gregori S, Serafini G, Sartirana C, Schulz U, Zino E, Tomiuk S, Jansen U, Ponzoni M, Paties CT, Fleischhauer K, Roncarolo MG (2010) Molecular and functional characterization of alloantigen-specific anergic T-cells suitable for cell therapy. Haematologica. doi:10.3324/haematol.2010.025825

Bacchetta R, Sartirana C, Levings MK, Bordignon C, Narula S, Roncarolo MG (2002) Growth and expansion of human T regulatory type 1 cells are independent from TCR activation but require exogenous cytokines. Eur J Immunol 32:2237–2245. doi:10.1002/1521-4141(200208)32:8<2237::AID-IMMU2237>3.0.CO;2-2

Bacchetta R., Lucarelli B, Sartirana C, Gregori S, Lupo Stanghellini MT, Miqueu P, Tomiuk S, Hernandez-Fuentes M, Gianolini ME, Greco R, Bernardi M, Zappone E, Rossini S, Janssen U, Ambrosi A, Salomoni M, Peccatori J, Ciceri F, Roncarolo MG (2014) Immunological outcome in haploidentical-HSC transplanted patients treated with IL-10-anergized donor T cells. Front Immunol 5:16. doi:10.3389/fimmu.2014.00016

Barnard A, Mahon BP, Watkins J, Redhead K, Mills KH (1996) Th1/Th2 cell dichotomy in acquired immunity to Bordetella pertussis: variables in the in vivo priming and in vitro cytokine detection techniques affect the classification of T-cell subsets as Th1, Th2 or Th0. Immunology 87:372–380

Barrat F, Cua D, Boonstra A, Richards D, Crain C, Savelkoul H, De Waal-Malefyt R, Coffman R, Hawrylowicz C, O'garra A (2002) In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. J Exp Med 195:603–616. doi:10.1084/jem.20011629

Battaglia M, Stabilini A, Draghici E, Gregori S, Mocchetti C, Bonifacio E, Roncarolo MG (2006a) Rapamycin and interleukin-10 treatment induces T regulatory type 1 cells that mediate antigen-specific transplantation tolerance. Diabetes 55:40–49. doi:10.2337/diabetes.55.01.06.db05-0613

Battaglia M, Stabilini A, Draghici E, Migliavacca B, Gregori S, Bonifacio E, Roncarolo MG (2006b) Induction of tolerance in type 1 diabetes via both CD4 + CD25 + T regulatory cells and T regulatory type 1 cells. Diabetes 55:1571–1580. doi:10.2337/db05-1576

Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH, Kuchroo VK (2009) The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat Immunol 10:167–175. doi:10.1038/ni.1690

Bejarano MT, De Waal Malefyt R, Abrams JS, Bigler M, Bacchetta R, De Vries JE, Roncarolo MG (1992) Interleukin 10 inhibits allogeneic proliferative and cytotoxic T cell responses generated in primary mixed lymphocyte cultures. Int Immunol 4:1389–1397

Belkaid Y (2008) Role of Foxp3-positive regulatory T cells during infection. Eur J Immunol 38:918–921. doi:10.1002/eji.200738120

Bergmann C, Strauss L, Zeidler R, Lang S, Whiteside TL (2007) Expansion and characteristics of human T regulatory type 1 cells in co-cultures simulating tumor microenvironment. Cancer Immunol Immunother 56:1429–1442. doi:10.1007/s00262-007-0280-9

Bettini M, Szymczak-Workman AL, Forbes K, Castellaw AH, Selby M, Pan X, Drake CG, Korman AJ, Vignali DA (2011) Cutting edge: accelerated autoimmune diabetes in the absence of LAG-3. J Immunol 187:3493–3498. doi:10.4049/jimmunol.1100714

Bharat A, Fields RC, Steward N, Trulock EP, Patterson GA, Mohanakumar T (2006a) CD4 + 25 + regulatory T cells limit Th1-autoimmunity by inducing IL-10 producing T cells following human lung transplantation. Am J Transplant Official J Am Soc Transplant Am Soc Transplant Surg 6:1799–1808. doi:10.1111/j.1600-6143.2006.01383.x

Bharat A, Fields RC, Trulock EP, Patterson GA, Mohanakumar T (2006b) Induction of IL-10 suppressors in lung transplant patients by CD4 + 25 + regulatory T cells through CTLA-4 signaling. J Immunol 177:5631–5638

Boisvert M, Chetoui N, Gendron S, Aoudjit F (2010) Alpha2beta1 integrin is the major collagen-binding integrin expressed on human Th17 cells. Eur J Immunol 40:2710–2719. doi:10.1002/eji.201040307

Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, Dascher CC, Berezovskaya A, Rousset D, Reynes JM, Goldfeld AE (2000) IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. J Clin Invest 105:1317–1325. doi:10.1172/JCI9918

Brun V, Bastian H, Neveu V, Foussat A (2009) Clinical grade production of IL-10 producing regulatory Tr1 lymphocytes for cell therapy of chronic inflammatory diseases. Int Immunopharmacol 9:609–613. doi:10.1016/j.intimp.2009.01.032

Brun V, Neveu V, Pers YM, Fabre S, Quatannens B, Bastian H, Clerget-Chossat N, Jorgensen C, Foussat A (2011) Isolation of functional autologous collagen-II specific IL-10 producing Tr1 cell clones from rheumatoid arthritis blood. Int Immunopharmacol 11:1074–1078. doi:10.1016/j.intimp.2011.03.001

Bruniquel D, Borie N, Hannier S, Triebel F (1998) Regulation of expression of the human lymphocyte activation gene-3 (LAG-3) molecule, a ligand for MHC class II. Immunogenetics 48:116–124

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27:68–73. doi:10.1038/83784

Camisaschi C, Casati C, Rini F, Perego M, De Filippo A, Triebel F, Parmiani G, Belli F, Rivoltini L, Castelli C (2010) LAG-3 expression defines a subset of CD4(+)CD25(high)Foxp3(+) regulatory T cells that are expanded at tumor sites. J Immunol 184:6545–6551. doi:10.4049/jimmunol.0903879

Chang HD, Helbig C, Tykocinski L, Kreher S, Koeck J, Niesner U, Radbruch A (2007) Expression of IL-10 in Th memory lymphocytes is conditional on IL-12 or IL-4, unless the IL-10 gene is imprinted by GATA-3. Eur J Immunol 37:807–817. doi:10.1002/eji.200636385

Charbonnier LM, Han WG, Quentin J, Huizinga TW, Zwerina J, Toes RE, Jorgensen C, Louis-Plence P (2010) Adoptive transfer of IL-10-secreting CD4 + CD49b + regulatory T cells suppresses ongoing arthritis. J Autoimmun 34:390–399. doi:10.1016/j.jaut.2009.10.003

Charbonnier LM, Van Duivenvoorde LM, Apparailly F, Cantos C, Han WG, Noel D, Duperray C, Huizinga TW, Toes RE, Jorgensen C, Louis-Plence P (2006) Immature dendritic cells suppress collagen-induced arthritis by in vivo expansion of CD49b + regulatory T cells. J Immunol 177:3806–3813. doi:10.4049/jimmunol.177.6.3806

Christensen HR, Frokiaer H, Pestka JJ (2002) Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J Immunol 168:171–178

Cobbold SP, Nolan KF, Graca L, Castejon R, Le Moine A, Frewin M, Humm S, Adams E, Thompson S, Zelenika D, Paterson A, Yates S, Fairchild PJ, Waldmann H (2003) Regulatory T cells and dendritic cells in transplantation tolerance: molecular markers and mechanisms. Immunol Rev 196:109–124

Daifotis, A.G., Koenig, S., Chatenoud, L., and Herold, K.C. (2013). Anti-CD3 clinical trials in type 1 diabetes mellitus. Clin Immunol. doi:10.1016/j.clim.2013.05.001

De Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, Te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, De Vries JE (1991) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 174:915–924

Delgado JC, Tsai EY, Thim S, Baena A, Boussiotis VA, Reynes JM, Sath S, Grosjean P, Yunis EJ, Goldfeld AE (2002) Antigen-specific and persistent tuberculin anergy in a cohort of pulmonary tuberculosis patients from rural Cambodia. Proc Natl Acad Sci USA 99:7576–7581. doi:10.1073/pnas.062056099

Desreumaux P, Foussat A, Allez M, Beaugerie L, Hebuterne X, Bouhnik Y, Nachury M, Brun V, Bastian H, Belmonte N, Ticchioni M, Duchange A, Morel-Mandrino P, Neveu V, Clerget-Chossat N, Forte M, Colombel JF (2012) Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease. Gastroenterology 143(1207–1217):e1201–e1202. doi:10.1053/j.gastro.2012.07.116

Dijke IE, Velthuis JH, Balk AH, Korevaar SS, Maat AP, Weimar W, Baan CC (2006) Donor-specific cytotoxic hyporesponsiveness associated with high interleukin-10 messenger RNA expression in cardiac allograft patients. J Heart Lung Transplant Official Publ Int Soc Heart Transplant 25:955–964. doi:10.1016/j.healun.2006.03.021

Doetze A, Satoguina J, Burchard G, Rau T, Loliger C, Fleischer B, Hoerauf A (2000) Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h)3/T(r)1-type cytokines IL-10 and transforming growth factor-beta but not by a T(h)1 to T(h)2 shift. Int Immunol 12:623–630

Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, Saris CJ, Gran B, Ciric B, Rostami A (2007) Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat Immunol 8:1372–1379. doi:10.1038/ni1540

Gagliani N, Gregori S, Jofra T, Valle A, Stabilini A, Rothstein DM, Atkinson M, Roncarolo MG, Battaglia M (2011) Rapamycin combined with anti-CD45RB mAb and IL-10 or with G-CSF induces tolerance in a stringent mouse model of islet transplantation. PLoS ONE 6:e28434. doi:10.1371/journal.pone.0028434

Gagliani N, Jofra T, Stabilini A, Valle A, Atkinson M, Roncarolo MG, Battaglia M (2010) Antigen-specific dependence of Tr1-cell therapy in preclinical models of islet transplant. Diabetes 59:433–439. doi:10.2337/db09-1168

Gagliani N, Jofra T, Valle A, Stabilini A, Morsiani C, Gregori S, Deng S, Rothstein DM, Atkinson M, Kamanaka M, Flavell RA, Roncarolo MG, Battaglia M (2013a) Transplant tolerance to pancreatic islets is initiated in the graft and sustained in the spleen. Am J Transplant 13:1963–1975. doi:10.1111/ajt.12333

Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, Guo B, Herbert DR, Bulfone A, Trentini F, Di Serio C, Bacchetta R, Andreani M, Brockmann L, Gregori S, Flavell RA, Roncarolo MG (2013b) Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nat Med. doi:10.1038/nm.3179

Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, Kozoriz D, Weiner HL, Quintana FJ (2010) Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. Nat Immunol 11:846–853. doi:10.1038/ni.1915

Gianfrani C, Levings MK, Sartirana C, Mazzarella G, Barba G, Zanzi D, Camarca A, Iaquinto G, Giardullo N, Auricchio S, Troncone R, Roncarolo MG (2006) Gliadin-specific type 1 regulatory T cells from the intestinal mucosa of treated celiac patients inhibit pathogenic T cells. J Immunol 177:4178–4186

Gizi W, Nir N, Nathan K (2002) Tr1 cell ìdependent active tolerance blunts the pathogenic effects of determinant spreading. J Clin Invest 110. doi:10.1172/jci200215176

Glocker EO, Frede N, Perro M, Sebire N, Elawad M, Shah N, Grimbacher B (2010) Infant colitis–it's in the genes. Lancet 376:1272. doi:10.1016/S0140-6736(10)61008-2

Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, Perro M, Diestelhorst J, Allroth A, Murugan D, Hatscher N, Pfeifer D, Sykora KW, Sauer M, Kreipe H, Lacher M, Nustede R, Woellner C, Baumann U, Salzer U, Koletzko S, Shah N, Segal AW, Sauerbrey A, Buderus S, Snapper SB, Grimbacher B, Klein C (2009) Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. New Engl J Med 361:2033–2045. doi:10.1056/NEJMoa0907206

Gorelik L, Constant S, Flavell RA (2002) Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. J Exp Med 195:1499–1505

Gorelik L, Flavell RA (2002) Transforming growth factor-beta in T-cell biology. Nat Rev Immunol 2:46–53. doi:10.1038/nri704

Goudy K, Aydin D, Barzaghi F, Gambineri E, Vignoli M, Ciullini Mannurita S, Doglioni C, Ponzoni M, Cicalese MP, Assanelli A, Tommasini A, Brigida I, Dellepiane RM, Martino S, Olek S, Aiuti A, Ciceri F, Roncarolo MG, Bacchetta R (2013) Human IL2RA null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity. Clin Immunol 146:248–261. doi:10.1016/j.clim.2013.01.004

Gregori S (2011) Dendritic cells in networks of immunological tolerance. Tissue Antigens 77:89–99. doi:10.1111/j.1399-0039.2010.01615.x

Gregori S, Goudy KS, Roncarolo MG (2012) The cellular and molecular mechanisms of immuno-suppression by human type 1 regulatory T cells. Front Immunol 3:30. doi:10.3389/fimmu.2012.00030

Gregori S, Roncarolo MG, Bacchetta R (2011) Methods for in vitro generation of human type 1 regulatory T cells. Methods Mol Biol 677:31–46. doi:10.1007/978-1-60761-869-0_3

Gregori S, Tomasoni D, Pacciani V, Scirpoli M, Battaglia M, Magnani CF, Hauben E, Roncarolo MG (2010) Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. Blood 116:935–944. doi:10.1182/blood-2009-07-234872

Grossman WJ, Verbsky JW, Tollefsen BL, Kemper C, Atkinson JP, Ley TJ (2004) Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. Blood 104:2840–2848

Groux H, Bigler M, de Vries JE, Roncarolo MG (1996) Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T cells. J Exp Med 184:19–29

Groux H, Cottrez F, Rouleau M, Mauze S, Antonenko S, Hurst S, Mcneil T, Bigler M, Roncarolo MG, Coffman RL (1999) A transgenic model to analyze the immunoregulatory role of IL-10 secreted by antigen-presenting cells. J Immunol 162:1723–1729

Groux H, O'garra A, Bigler M, Rouleau M, Antonenko S, De Vries JE, Roncarolo MG (1997) A CD4 + T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389:737–742. doi:10.1038/39614

Haringer B, Lozza L, Steckel B, Geginat J (2009) Identification and characterization of IL-10/IFN-gamma-producing effector-like T cells with regulatory function in human blood. J Exp Med 206:1009–1017. doi:10.1084/jem.20082238

Herold KC, Burton JB, Francois F, Poumian-Ruiz E, Glandt M, Bluestone JA (2003) Activation of human T cells by FcR nonbinding anti-CD3 mAb, hOKT3gamma1(Ala-Ala). J Clin Invest 111:409–418. doi:10.1172/JCI16090

Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HI, Powell JD, Pardoll DM, Drake CG, Vignali DA (2004) Role of LAG-3 in regulatory T cells. Immunity 21:503–513. doi:10.1016/j.immuni.2004.08.010

Huang WX, Huang P, Fredrikson S, Pirskanen R, Hillert J (2000) Decreased mRNA expression of TNF-alpha and IL-10 in non-stimulated peripheral blood mononuclear cells in myasthenia gravis. Eur J Neurol 7:195–202

Huard B, Mastrangeli R, Prigent P, Bruniquel D, Donini S, El-Tayar N, Maigret B, Dreano M, Triebel F (1997) Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. Proc Natl Acad Sci USA 94:5744–5749

Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, Qin FX, Gilliet M, Liu YJ (2008) Two functional subsets of FOXP3 + regulatory T cells in human thymus and periphery. Immunity 28:870–880. doi:10.1016/j.immuni.2008.03.018

Iwasaki Y, Fujio K, Okamura T, Yanai A, Sumitomo S, Shoda H, Tamura T, Yoshida H, Charnay P, Yamamoto K (2013) Egr-2 transcription factor is required for Blimp-1-mediated IL-10 production in IL-27-stimulated CD4(+) T cells. Eur J Immunol 43:1063–1073. doi:10.1002/eji.201242942

Jeon S, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, Tsuji N, Kiyono H, Ma J, Kusu T, Okumura R, Hara H, Yoshida H, Yamamoto M, Nomoto K, Takeda K (2012) Probiotic Bifidobacterium breve induces IL-10-producing Tr1 cells in the colon. PLoS Pathogens 8. doi:10.1371/journal.ppat.1002714

Jin JO, Han X, Yu Q (2013) Interleukin-6 induces the generation of IL-10-producing Tr1 cells and suppresses autoimmune tissue inflammation. J Autoimmun 40:28–44. doi:10.1016/j.jaut.2012.07.009

Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH (2000) Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J Exp Med 192:1213–1222

Joosten SA, Ottenhoff TH (2008) Human CD4 and CD8 regulatory T cells in infectious diseases and vaccination. Hum Immunol 69:760–770. doi:10.1016/j.humimm.2008.07.017

Kamanaka M, Kim S, Wan Y, Sutterwala F, Lara-Tejero M, Gal√°N J, Harhaj E, Flavell R (2006) Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. Immunity 25:941–952. doi:10.1016/j.immuni.2006.09.013

Karin N, Mitchell DJ, Brocke S, Ling N, Steinman L (1994) Reversal of experimental autoimmune encephalomyelitis by a soluble peptide variant of a myelin basic protein epitope: T cell receptor antagonism and reduction of interferon gamma and tumor necrosis factor alpha production. J Exp Med 180:2227–2237

Kassiotis G, Gray D, Kiafard Z, Zwirner J, Stockinger B (2006) Functional specialization of memory Th cells revealed by expression of integrin CD49b. J Immunol 177:968–975

Kemper C, Chan AC, Green JM, Brett KA, Murphy KM, Atkinson JP (2003) Activation of human CD4 + cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature 421:388–392. doi:10.1038/nature01315

Khattri R, Cox T, Yasayko SA, Ramsdell F (2003) An essential role for Scurfin in CD4 + CD25 + T regulatory cells. Nat Immunol 4:337–342. doi:10.1038/ni909

Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274. doi:10.1016/0092-8674(93)80068-p

Kushwah R, Cao H, Hu J (2007) Potential of helper-dependent adenoviral vectors in modulating airway innate immunity. Cell Mol Immunol 4:81–89

Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, Wu C, Kleinewietfeld M, Kunder S, Hafler DA, Sobel RA, Regev A, Kuchroo VK (2012) Induction and molecular signature of pathogenic T(H)17 cells. Nat Immunol 13:991–999. doi:10.1038/ni.2416

Leveson-Gower DB, Sega EI, Kalesnikoff J, Florek M, Pan Y, Pierini A, Galli SJ, Negrin RS (2013) Mast cells suppress murine GVHD in a mechanism independent of CD4 + CD25 + regulatory T cells. Blood 122:3659–3665. doi:10.1182/blood-2013-08-519157

Levings MK, Gregori S, Tresoldi E, Cazzaniga S, Bonini C, Roncarolo MG (2005) Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not CD25 + CD4 + Tr cells. Blood 105:1162–1169

Levings MK, Sangregorio R, Galbiati F, Squadrone S, De Waal Malefyt R, Roncarolo MG (2001) IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. J Immunol 166:5530–5539

Magnani CF, Alberigo G, Bacchetta R, Serafini G, Andreani M, Roncarolo MG, Gregori S (2011) Killing of myeloid APCs via HLA class I, CD2 and CD226 defines a novel mechanism of suppression by human Tr1 cells. Eur J Immunol 41:1652–1662. doi:10.1002/eji.201041120

Manavalan JS, Rossi PC, Vlad G, Piazza F, Yarilina A, Cortesini R, Mancini D, Suciu-Foca N (2003) High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. Transpl Immunol 11:245–258

Mandapathil M, Szczepanski MJ, Szajnik M, Ren J, Jackson EK, Johnson JT, Gorelik E, Lang S, Whiteside TL (2010) Adenosine and prostaglandin E2 cooperate in the suppression of immune responses mediated by adaptive regulatory T cells. J Biol Chem 285:27571–27580. doi:10.1074/jbc.M110.127100

Martinez-Forero I, Garcia-Munoz R, Martinez-Pasamar S, Inoges S, De Cerio Lopez-Diaz A, Palacios R, Sepulcre J, Moreno B, Gonzalez Z, Fernandez-Diez B, Melero I, Bendandi M, Villoslada P (2008) IL-10 suppressor activity and ex vivo Tr1 cell function are impaired in multiple sclerosis. Eur J Immunol 38:576–586. doi:10.1002/eji.200737271

Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, Weaver CT (2007) Regulatory T cells expressing interleukin 10 develop from Foxp3 + and Foxp3- precursor cells in the absence of interleukin 10. Nat Immunol 8:931–941. doi:10.1038/ni1504

Mcgeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, Mcclanahan T, Cua DJ (2007) TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol 8:1390–1397. doi:10.1038/ni1539

Mcguirk P, Mahon BP, Griffin F, Mills KH (1998) Compartmentalization of T cell responses following respiratory infection with Bordetella pertussis: hyporesponsiveness of lung T cells is associated with modulated expression of the co-stimulatory molecule CD28. Eur J Immunol 28:153–163. doi:10.1002/(SICI)1521-4141(199801)28:01<153::AID-IMMU153>3.0.CO;2-#

Mcguirk P, Mccann C, Mills KH (2002) Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. J Exp Med 195:221–231

Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M (2008) In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. J Exp Med 205:2887–2898. doi:10.1084/jem.20080193

Meiron M, Zohar Y, Anunu R, Wildbaum G, Karin N (2008) CXCL12 (SDF-1alpha) suppresses ongoing experimental autoimmune encephalomyelitis by selecting antigen-specific regulatory T cells. J Exp Med 205:2643–2655. doi:10.1084/jem.20080730

Miaw SC, Choi A, Yu E, Kishikawa H, Ho IC (2000) ROG, repressor of GATA, regulates the expression of cytokine genes. Immunity 12:323–333

Montenegro SM, Miranda P, Mahanty S, Abath FG, Teixeira KM, Coutinho EM, Brinkman J, Goncalves I, Domingues LA, Domingues AL, Sher A, Wynn TA (1999) Cytokine production in acute versus chronic human Schistosomiasis mansoni: the cross-regulatory role of interferon-gamma and interleukin-10 in the responses of peripheral blood mononuclear cells and splenocytes to parasite antigens. J Infect Dis 179:1502–1514. doi:10.1086/314748

Morris ES, Macdonald KP, Rowe V, Johnson DH, Banovic T, Clouston AD, Hill GR (2004) Donor treatment with pegylated G-CSF augments the generation of IL-10-producing regulatory T cells and promotes transplantation tolerance. Blood 103:3573–3581. doi:10.1182/blood-2003-08-2864

Murugaiyan G, Mittal A, Lopez-Diego R, Maier LM, Anderson DE, Weiner HL (2009) IL-27 is a key regulator of IL-10 and IL-17 production by human CD4 + T cells. J Immunol 183:2435–2443. doi:10.4049/jimmunol.0900568

Okamura T, Fujio K, Shibuya M, Sumitomo S, Shoda H, Sakaguchi S, Yamamoto K (2009) CD4 + CD25-LAG3 + regulatory T cells controlled by the transcription factor Egr-2. Proc Natl Acad Sci USA 106:13974–13979. doi:10.1073/pnas.0906872106

Okamura T, Fujio K, Sumitomo S, Yamamoto K (2012) Roles of LAG3 and EGR2 in regulatory T cells. Ann Rheum Dis 71(Suppl 2):i96–i100. doi:10.1136/annrheumdis-2011-200588

Ostrowski MA, Gu JX, Kovacs C, Freedman J, Luscher MA, Macdonald KS (2001) Quantitative and qualitative assessment of human immunodeficiency virus type 1 (HIV-1)-specific CD4 + T cell immunity to gag in HIV-1-infected individuals with differential disease progression: reciprocal interferon-gamma and interleukin-10 responses. J Infect Dis 184:1268–1278

Pedersen AE, Gad M, Walter MR, Claesson MH (2004) Induction of regulatory dendritic cells by dexamethasone and 1alpha,25-Dihydroxyvitamin D(3). Immunol Lett 91:63–69

Penna G, Adorini L (2000) 1 Alpha, 25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 164:2405–2411. doi:10.4049/jimmunol.164.5.2405

Petrich De Marquesini LG, Fu J, Connor KJ, Bishop AJ, Mclintock NE, Pope C, Wong FS, Dayan CM (2010) IFN-gamma and IL-10 islet-antigen-specific T cell responses in autoantibody-negative first-degree relatives of patients with type 1 diabetes. Diabetologia 53:1451–1460. doi:10.1007/s00125-010-1739-3

Plebanski M, Flanagan KL, Lee EA, Reece WH, Hart K, Gelder C, Gillespie G, Pinder M, Hill AV (1999) Interleukin 10-mediated immunosuppression by a variant CD4 T cell epitope of Plasmodium falciparum. Immunity 10:651–660. doi:10.1016/S1074-7613(00)80064-3

Pot C, Apetoh L, Kuchroo VK (2011) Type 1 regulatory T cells (Tr1) in autoimmunity. Semin Immunol 23:202–208. doi:10.1016/j.smim.2011.07.005

Pot C, Jin H, Awasthi A, Liu SM, Lai CY, Madan R, Sharpe AH, Karp CL, Miaw SC, Ho IC, Kuchroo VK (2009) Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. J Immunol 183:797–801. doi:10.4049/jimmunol.0901233

Rahmoun M, Foussat A, Groux H, Pene J, Yssel H, Chanez P (2006) Enhanced frequency of CD18- and CD49b-expressing T cells in peripheral blood of asthmatic patients correlates with disease severity. Int Arch Allergy Immunol 140:139–149. doi:10.1159/000092533

Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK (2006) Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunol Rev 212:28–50. doi:10.1111/j.0105-2896.2006.00420.x

Roncarolo MG, Gregori S, Lucarelli B, Ciceri F, Bacchetta R (2011) Clinical tolerance in allogeneic hematopoietic stem cell transplantation. Immunol Rev 241:145–163. doi:10.1111/j.1600-065X.2011.01010.x

Roncarolo MG, Touraine JL, Banchereau J (1986) Cooperation between major histocompatibility complex mismatched mononuclear cells from a human chimera in the production of antigen-specific antibody. J Clin Invest 77:673–680. doi:10.1172/JCI112361

Roncarolo MG, Yssel H, Touraine JL, Bacchetta R, Gebuhrer L, De Vries JE, Spits H (1988a) Antigen recognition by MHC-incompatible cells of a human mismatched chimera. J Exp Med 168:2139–2152

Roncarolo MG, Yssel H, Touraine JL, Betuel H, De Vries JE, Spits H (1988b) Autoreactive T cell clones specific for class I and class II HLA antigens isolated from a human chimera. J Exp Med 167:1523–1534

Ross PJ, Lavelle EC, Mills KH, Boyd AP (2004) Adenylate cyclase toxin from Bordetella pertussis synergizes with lipopolysaccharide to promote innate interleukin-10 production and enhances the induction of Th2 and regulatory T cells. Infect Immun 72:1568–1579

Rossetti M, Gregori S, Roncarolo MG (2010) Granulocyte-colony stimulating factor drives the in vitro differentiation of human dendritic cells that induce anergy in naive T cells. Eur J Immunol 40:3097–3106. doi:10.1002/eji.201040659

Rutella S, Bonanno G, Pierelli L, Mariotti A, Capoluongo E, Contemi AM, Ameglio F, Curti A, De Ritis DG, Voso MT, Perillo A, Mancuso S, Scambia G, Lemoli RM, Leone G (2004) Granulocyte colony-stimulating factor promotes the generation of regulatory DC through induction of IL-10 and IFN-alpha. Eur J Immunol 34:1291–1302. doi:10.1002/eji.200324651

Ryan M, Murphy G, Gothefors L, Nilsson L, Storsaeter J, Mills KH (1997) Bordetella pertussis respiratory infection in children is associated with preferential activation of type 1 T helper cells. J Infect Dis 175:1246–1250

Salvati VM, Mazzarella G, Gianfrani C, Levings MK, Stefanile R, De Giulio B, Iaquinto G, Giardullo N, Auricchio S, Roncarolo MG, Troncone R (2005) Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in ex vivo cultured coeliac intestinal mucosa. Gut 54:46–53. doi:10.1136/gut.2003.023150

Sanda S, Roep BO, Von Herrath M (2008) Islet antigen specific IL-10 + immune responses but not CD4 + CD25 + FoxP3 + cells at diagnosis predict glycemic control in type 1 diabetes. Clin Immunol 127:138–143

Saraiva M, Christensen JR, Veldhoen M, Murphy TL, Murphy KM, O'garra A (2009) Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. Immunity 31:209–219. doi:10.1016/j.immuni.2009.05.012

Satoguina JS, Weyand E, Larbi J, Hoerauf A (2005) T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. J Immunol 174:4718–4726. doi:10.4049/jimmunol.174.8.4718

Serafini G, Andreani M, Testi M, Battarra M, Bontadini A, Biral E, Fleischhauer K, Marktel S, Lucarelli G, Roncarolo MG, Bacchetta R (2009) Type 1 regulatory T cells are associated with persistent split erythroid/lymphoid chimerism after allogeneic hematopoietic stem cell transplantation for thalassemia. Haematologica 94:1415–1426. doi:10.3324/haematol.2008.003129

Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'shea JJ, Hunter CA (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8:1363–1371. doi:10.1038/ni1537

Taga K, Tosato G (1992) IL-10 inhibits human T cell proliferation and IL-2 production. J Immunol 148:1143–1148

Troncone R, Gianfrani C, Mazzarella G, Greco L, Guardiola J, Auricchio S, De Berardinis P (1998) Majority of gliadin-specific T-cell clones from celiac small intestinal mucosa produce interferon-gamma and interleukin-4. Dig Dis Sci 43:156–161

Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV (1997) An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 24:1–8

Turner J, Gonzalez-Juarrero M, Ellis DL, Basaraba RJ, Kipnis A, Orme IM, Cooper AM (2002) In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. J Immunol 169:6343–6351

Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B (2008a) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453:106–109. doi:10.1038/nature06881

Veldhoen M, Uyttenhove C, Van Snick J, Helmby H, Westendorf A, Buer J, Martin B, Wilhelm C, Stockinger B (2008b) Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 9:1341–1346. doi:10.1038/ni.1659

Veldman C, Hohne A, Dieckmann D, Schuler G, Hertl M (2004) Type I regulatory T cells specific for desmoglein 3 are more frequently detected in healthy individuals than in patients with pemphigus vulgaris. J Immunol 172:6468–6475

Vieira PL, Christensen JR, Minaee S, O'neill EJ, Barrat FJ, Boonstra A, Barthlott T, Stockinger B, Wraith DC, O'garra A (2004) IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4 + CD25 + regulatory T cells. J Immunol 172:5986–5993

Wakkach A, Cottrez F, Groux H (2001) Differentiation of regulatory T cells 1 is induced by CD2 costimulation. J Immunol 167:3107–3113

Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H (2003) Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity 18:605–617

Waldron-Lynch F, Henegariu O, Deng S, Preston-Hurlburt P, Tooley J, Flavell R, Herold KC (2012) Teplizumab induces human gut-tropic regulatory cells in humanized mice and patients. Sci Transl Med 4:118ra112. doi:10.1016/4/118/118ra12

Wan YY, Flavell RA (2005) Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. Proc Natl Acad Sci USA 102:5126–5131. doi:10.1073/pnas.0501701102

Weiner HL, Pires da Cunha A, Quintana F, Wu H (2011) Oral tolerance. Immunol Rev 24:241–259

Workman CJ, Vignali DA (2005) Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223). J Immunol 174:688–695

Yang Y, Ochando J, Yopp A, Bromberg JS, Ding Y (2005) IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. J Immunol 174:2720–2729

Yssel H, De Waal Malefyt R, Roncarolo MG, Abrams JS, Lahesmaa R, Spits H, De Vries JE (1992) IL-10 is produced by subsets of human CD4 + T cell clones and peripheral blood T cells. J Immunol 149:2378–2384

Zeller JC, Panoskaltsis-Mortari A, Murphy WJ, Ruscetti FW, Narula S, Roncarolo MG, Blazar BR (1999) Induction of CD4 + T cell alloantigen-specific hyporesponsiveness by IL-10 and TGF-beta. J Immunol 163:3684–3691

Interleukin-10-Producing B Cells and the Regulation of Immunity

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Abstract B cells are usually considered primarily for their unique capacity to produce antibodies after differentiation into plasma cells. In addition to their roles as antibody-producing cells, it has become apparent during the last 10 years that B cells also perform important functions in immunity through the production of cytokines. In particular, it was shown that B cells could negatively regulate immunity through provision of interleukin (IL)-10 during autoimmune and infectious diseases in mice. Here, we review data on the suppressive functions of B cells in mice with particular emphasis on the signals controlling the acquisition of such suppressive functions by B cells, the phenotype of the B cells involved in the negative regulation of immunity, and the processes targeted by this inhibitory circuit. Finally, we discuss the possibility that human B cells might also perform similar inhibitory functions through the provision of IL-10, and review data suggesting that such B cell-mediated regulatory activities might be impaired in patients with autoimmune diseases.

Contents

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1 Introduction

In addition to their roles as antibody-producing or antigen-presenting cells, B lymphocytes can markedly influence immunity through the secretion of cytokines. This notion gained momentum about 10 years ago, when it was observed that B cells could differentiate into distinct cytokine-producing subsets termed Be1 and Be2, which could subsequently instruct the differentiation of naïve $CD4^+$ T cells into T_H1 or T_H2 cells, respectively, in vitro (Harris et al. 2000). The fact that endogenous B cells could control immunity through the production of cytokine in vivo was then demonstrated in two models of inflammatory diseases, namely ulcerative colitis (UC) and experimental autoimmune encephalomyelitis (EAE) (Fillatreau et al. 2002; Mizoguchi et al. 2002). In both cases, B cells limited pathogenic immunity, and improved the disease course through the production of interleukin (IL)-10, a cytokine classically thought to be produced by regulatory T cells and some innate immune cells. These findings pioneered the notion that endogenous B cells could mediate regulatory functions limiting immunopathology during inflammatory or autoimmune diseases. It is now known that IL-10 production by B cells can also result in impairment of protective immunity during infection, emphasizing the general relevance of this regulatory pathway. Importantly, evidence is accumulating that B cells can exert similar suppressive functions in humans. For instance, B cells from patients with relapsing-remitting multiple sclerosis (MS) produce less IL-10 than B cells from healthy patients upon stimulation in vitro, suggesting that a defect in this suppressive mechanism facilitates onset or progression of MS (Duddy et al. 2007). Moreover, B cell depletion therapy resulted in an aggravation of autoimmune disease in a few patients treated with rituximab, and in appearance of new immune-driven diseases in some individuals. Indeed, a patient with UC suffered a severe exacerbation of disease after rituximab treatment, and the elimination of B cells temporally correlated with a loss of IL-10 expression in the intestinal mucosa (Goetz et al. 2007). Several patients with rheumatoid arthritis (RA) or B cell lymphoma developed psoriasis, a cutaneous disorder driven by aberrant inflammatory T cell responses, after rituximab treatment (Dass et al. 2007; Mielke et al. 2008). The development of psoriasis in B cell-depleted individuals could result from an elimination of IL-10-producing B cells because such cells provided protection from a psoriasis-like disease in mice (Yanaba et al. 2013). These findings collectively fuelled enormous interest in the possible implication of B cell-mediated immune regulation in human diseases. It is now reasonable to ask whether it might be possible to treat patients suffering from deleterious immune responses by promoting the IL-10-mediated regulatory functions of B cells, or whether antimicrobial immunity could be improved by tuning down these inhibitory processes. This chapter aims at providing a frame for the discussion of these questions by presenting a few examples illustrating the suppressive functions of IL-10-producing B cells in autoimmune and infectious diseases in mice as well as in humans, and by concluding with a few examples suggesting the possibility of using the IL-10-mediated suppressive functions of B cells for dampening unwanted immunity.

2 IL-10-Producing B Cells in Immune-Driven Pathologies

2.1 Experimental Autoimmune Encephalomyelitis

EAE is a T cell-mediated demyelinating autoimmune disease of the central nervous system (CNS), and is the primary animal model for the human disease MS. Most of the novel drugs nowadays used to treat patients with MS were initially developed in EAE. EAE can be induced in mice by immunization with myelin antigens, such as myelin oligodendrocyte glycoprotein (MOG). Following immunization, mice typically start displaying clinical signs of the disease within 10–14 days, reach a maximum of disability score around day 20, and then enter a phase of spontaneous remission from paralysis that can result in complete recovery of motor functions within about 30 days (Fillatreau and Anderton 2007). The regulatory functions of B cells in EAE were discovered by demonstrating that mice with a selective lack of IL-10 expression in B cells failed to recover from disease, and instead developed a severe chronic pathology after immunization with MOG (Fillatreau et al. 2002). Moreover, B cells isolated from wild-type mice after recovery from EAE markedly improved the disease course in recipient mice upon adoptive transfer (Fillatreau et al. 2002). The signals controlling the suppressive functions of B cells in this disease, the implicated B cell subset, and the cell types targeted by IL-10-producing B cells are discussed below.

Naïve B cells can promote immunological tolerance as a result of their low expression of major histocompatibility complex (MHC) and co-stimulatory molecules. However, naïve B cells do not constitutively produce IL-10, suggesting that they were not responsible for the suppression of disease in EAE. Indeed, activated B cells mediated this protective effect because mice lacking CD40 only in B cells, or mice expressing a single BCR of irrelevant antigen specificity developed a chronic disease, alike mice with IL-10-deficient B cells, whereas mice with wild-type B cells rapidly recovered from disease after a short episode of paralysis (Fillatreau et al. 2002). Thus, the B cell-mediated suppression involved antigen-specific B cells activated via the BCR and CD40. BCR and CD40 signaling might directly contribute to the production of IL-10 by B cells in vivo because B cells isolated from wild-type mice after recovery from EAE produced IL-10 upon co-stimulation with MOG and an agonistic antibody to CD40 in vitro. However, these signals were insufficient to stimulate the production of IL-10 by naïve B cells, indicating that an additional factor was required to initiate the production of this cytokine.

The signals capable of initiating suppressive functions in naïve B cells were sought after by investigating which factor could trigger IL-10 production by naïve B cells in vitro. Naïve B cells secreted large amounts of IL-10 upon stimulation with agonists of Toll-like receptors (TLR), such as lipopolysaccharides (LPS) for TLR-4 or CpG motif-containing oligonucleotides for TLR-9, indicating that TLR could provide the signal initiating suppressive functions in naïve B cells (Lampropoulou et al. 2008). In keeping with this, mice with a B cell-restricted deficiency in MyD88, an essential signaling adaptor for all TLR except TLR3,

or in both TLR-2 and TLR-4, developed a chronic form of EAE clinically indistinguishable from the one observed with mice lacking IL-10 production by B cells, indicating that intrinsic TLR signaling in B cells was indeed required for B cell-mediated inhibition of disease pathogenesis (Lampropoulou et al. 2008). The IL-10 production induced in vitro in naïve B cells via TLR triggering could be augmented upon secondary stimulation of the B cells via the BCR and CD40 (Lampropoulou et al. 2008; Matsumoto et al. 2011). This suggests that the suppressive functions of B cells are instructed via a two-step process initiated by TLR signaling, and subsequently amplified via BCR and CD40 (Lampropoulou et al. 2008, 2010). Recently, IL-21 was proposed as a distinct signal capable of inducing IL-10 production by naïve B cells, and B cells lacking IL-21 receptor were unable to suppress EAE in recipient mice upon adoptive transfer (Yoshizaki et al. 2012).

The initiation and subsequent amplification of IL-10 production by B cells involve distinct molecular mechanisms. The calcium sensor stromal interaction molecules (STIM1) and STIM2 contribute selectively to the amplification of IL-10 production by B cells upon BCR engagement, without impacting on the initial IL-10 secretion induced by TLR stimulation (Matsumoto et al. 2011). Accordingly, lack of STIM1 and STIM2 resulted in a profound reduction of IL-10 expression by MOG-reactive B cells during EAE, and mice with a B cell-restricted deficiency in both STIM1 and STIM2 displayed an exacerbated EAE course compared to control mice (Matsumoto et al. 2011). Other signaling adaptors might be involved in both the initiation and the amplification of IL-10 production by B cells. For instance, BLNK (also called SLP65) is implicated in signaling via TLR, BCR, and CD40 (Koretzky et al. 2006), and might have such a general function. BLNK-deficient B cells were unable to activate STAT3 and secrete IL-10 upon stimulation with LPS in vitro, and BLNK-deficient mice developed an exacerbated form of EAE upon immunization with MOG (Jin et al. 2013).

The implication of the BCR in the suppressive functions of B cells suggests that BCR co-receptors might modulate this activity. In keeping with this, CD19-deficient mice displayed a reduced production of IL-10 by B cells during EAE, and they developed an exacerbated disease course with enhanced demyelination and stronger accumulation of $CD8^+$ T cells in CNS compared to control mice (Matsushita et al. 2006; Yanaba et al. 2008). However, this phenotype involved more complex mechanisms because CD19-deficient mice reconstituted with wild-type B cells also suffered a more severe disease than control mice, indicating that the disease exacerbation was not solely due to a lack of B cell-mediated regulation in CD19-deficient mice. In fact, B cells displayed heightened pathogenic functions in these mice: CD19-deficient B cells produced increased amounts of IFN-γ during EAE, and exacerbated disease in recipient wild-type mice upon adoptive transfer (Matsushita et al. 2006). Some cell surface receptors can therefore act as switches between the protective and the pathogenic functions of B cells in autoimmune diseases. Targeting such BCR co-receptors, which are expressed solely on B cells, might be a fruitful approach to manipulate the function of B cells therapeutically. However, it is important to keep in mind that CD19 has key roles in humoral immunity, so that enhancing its function might have deleterious consequences in

the context of autoantibody-mediated pathology. Some of the effects observed as a result of CD19 deficiency might be due to the role of this molecule in regulating B cell development rather than to its function in the control of B cell activation. This would suggest that distinct B cell subsets might have different propensity to produce particular cytokines upon activation in naïve mice.

There is enormous interest in identifying the phenotype of the B cells expressing IL-10 in vivo, and suppressing pathology during autoimmune diseases. A first strategy to look for such cells is to assess whether a particular B cell subset in naïve mice is more prone to differentiate into IL-10-producing B cells upon activation than others. In the spleen of naïve mice, distinct B cell subsets had different tendencies to secrete IL-10 upon TLR stimulation in vitro. Marginal zone B cells, either defined as $CD1d^{hi}$ or $CD21^{hi}CD23^{lo}$ cells, displayed a superior capacity to secrete large amounts of IL-10 within 24 h after LPS stimulation in vitro, compared to follicular $CD1d^{lo}CD21^{lo}CD23^{hi}$ B cells. A suppressive role for these cells has been documented in various autoimmune disease models (Kalampokis et al. 2013). In EAE, adoptive transfer of $CD1d^{hi}CD5^{+}$ B cells isolated from CD20-deficient mice into wild-type mice previously depleted of endogenous B cell with an anti-CD20 antibody resulted in an IL-10-dependent reduction of the disease progression (Matsushita et al. 2008). In contrast, $CD1d^{hi}CD5^{+}$ B cells were not protective upon adoptive transfer in mice with B cells lacking STIM1 and STIM2, which developed an exacerbated EAE due to a defect in IL-10 production by B cells (Matsumoto et al. 2011). The reason for this discrepancy is not clear, but could be due to the fact that $CD1d^{hi}CD5^{+}$ B cells can acquire different functions depending on their microenvironment, and their mode of activation. Indeed, $CD1d^{hi}CD5^{+}$ B cells also displayed the strongest capacity to rapidly produce IL-6 upon stimulation via TLR4 and CD40 in vitro, and were, in comparison to other B cell subsets, the main source of B cell-derived IL-6 during EAE (Barr et al. 2012). Since IL-6 production is the major mechanism of B cell-mediated pathogenesis in EAE these data indicate that $CD1d^{hi}CD5^{+}$ B cells might have some deleterious effects during this disease. This shall be taken into account when considering the application of such cells in adoptive cell therapy. It might be possible to drive these cells preferentially towards an anti-inflammatory phenotype by using appropriate culture conditions before adoptive transfer. Such a culture system, which also allowed an expansion of these cells, was recently reported (Yoshizaki et al. 2012). This protocol involved first a culture of B cells in the presence of IL-4, BAFF, and CD40L for 4 days, and then in the presence of IL-21, BAFF, and CD40L for another 4–5 days. At the end of these 9 days, the culture contained two subsets of B cells, $CD19^{lo}CD5^{hi}$ and $CD19^{hi}CD5^{lo}$ B cells, and about 75 % of the $CD19^{lo}CD5^{hi}$ B cells expressed intracellular IL-10 (Yoshizaki et al. 2012). These cells could inhibit the development of EAE in recipient mice upon adoptive transfer, even when injected in mice with ongoing EAE indicating that they could control established disease (Yoshizaki et al. 2012). These results are remarkable because the B cells used in this study were initially isolated from naïve mice, implying that they were not enriched for any BCR specificity towards disease-relevant autoantigen(s). However, B cells required MHC-II expression to mediate

their protective effect. It will be important to clarify the role of bystander versus antigen-specific mechanisms in this protective effect. The phenotype of the B cells that actually expressed IL-10 in vivo, and suppressed disease in recipient mice after transfer also remains to be characterized.

The direct target(s) of IL-10-producing B cells in EAE have not been definitively identified. The lack of recovery from disease in mice with an IL-10-deficiency restricted to B cells correlated with heightened autoreactive T cell responses of T_H1 and T_H17 types, and with a higher accumulation of immune cells in the CNS during EAE, as observed also in mice lacking TLR2 and TLR4, or MyD88, or CD40, selectively in B cells (Fillatreau et al. 2002; Lampropoulou et al. 2008). The inhibitory effect of B cells on pathogenic T cells is unlikely to be mediated through $Foxp3^+$ regulatory $CD4^+$ T cells, because the latter were activated normally in B cell-deficient mice, as indicated by their efficient accumulation in inflamed CNS and local acquisition of enhanced suppressive functions (Hoehlig et al. 2012). An alternative possibility is that IL-10 from B cells targets dendritic cells (DC), which are instrumental for the induction of T cell responses. In support to this possibility, IL-10 from TLR-activated B cells suppressed the provision of multiple pro-inflammatory mediators by DC in vitro, and subsequently reduced their capacity to stimulate T cells (Lampropoulou et al. 2008). Furthermore, DC from B cell-deficient mice produced increased amounts of IL-12, and displayed a stronger capacity to induce IFN-γ production by $CD4^+$ T cells upon immunization compared to DC from wild-type mice (Moulin et al. 2000). A precise identification of the mechanisms targeted by IL-10 producing B cells might be valuable to optimally employ these cells therapeutically.

2.2 Inflammatory Bowel Disease

Inflammatory bowel diseases (IBD) are a heterogeneous group of intestinal disorders including ulcerative colitis (UC) and Crohn's disease (CD) (Kaser et al. 2010). UC and CD share in common an aberrant T cell response towards enteric bacteria, but involve different mechanisms of pathogenesis, and are associated preferentially with T_H2 or T_H1 cells, respectively. We review below the data documenting the protective roles of IL-10-producing B cells in these two types of inflammatory disorders of the gut.

Mice with a null mutation in *Tcrα* gene ($TCR\alpha^{-/-}$ mice), which harbor an unconventional population of $TCR\alpha^-\beta^+$ $CD4^+$ T cells (Takahashi et al. 1997), spontaneously develop an inflammatory disease of the gut that shares multiple features with UC, including a restriction of the pathology to the colon and a dominant role in pathogenesis for T cells producing T_H2-type cytokines, such as IL-4 (Mizoguchi et al. 1996a). The B cell compartment gets strongly activated during this disease, which leads to an augmentation of the number of B cells and plasma cells in mesenteric lymph nodes (MLN), as well as to an increase of serum levels of autoantibodies against neutrophil cytoplasmic antigens (ANCA), tropomyosin,

histone, and DNA (Mizoguchi et al. 1996a, b; 1997b; 2002; Wen et al. 1994). The functional relevance of B cells in UC was addressed using TCRα$^{-/-}$ mice on a B cell-deficient background. Unexpectedly, these mice developed an earlier and more severe disease than TCRα$^{-/-}$ mice, suggesting a protective role for B cells in this pathology (Mizoguchi et al. 1997a). Accordingly, cells from the MLN of B cell-deficient TCRα$^{-/-}$ mice, but not from TCRα$^{-/-}$ mice with B cells, induced UC upon adoptive transfer in RAG-1-deficient recipient mice (Mizoguchi et al. 1997a), and addition of B cells to the MLN cell preparation from B cell-deficient TCRα$^{-/-}$ mice suppressed their capacity to provoke disease (Mizoguchi et al. 1997a). This protective effect did not seem related to a specific B cell subset because B cells from bone marrow, or from a mixture of MLN and spleen cells, could similarly suppress disease in recipient mice upon adoptive transfer (Mizoguchi et al. 2000). However, the protective function of B cells in UC required their provision of IL-10 (Mizoguchi et al. 2002). Noteworthy, B cells were the major source of IL-10 in MLN of TCRα$^{-/-}$ mice with UC (Mizoguchi et al. 2002). IL-10 was absent in MLN B cells from healthy TCRα$^{-/-}$ mice, indicating that its expression was induced in MLN B cells upon the development of the disease as a counter-regulatory mechanism. The IL-10-mediated protective function of B cells was apparently independent of autoantibodies because autoantibody levels were not affected by a lack of IL-10 expression by B cells (Mizoguchi et al. 2002). It also did not operate through a down-modulation of the pathogenic IL-4 producing CD4^{+} T cell response, but was reflected at the level of the target organ in the colonic lamina propria by an inhibition of the local inflammatory reaction associated with a reduction of STAT3 activation and IL-1β expression (Mizoguchi et al. 1999).

The signals controlling the production of IL-10 by B cells in this model of UC are starting to be defined. CD1d expression was critical for the induction of IL-10 expression in MLN B cells, and for their protective activity against UC (Mizoguchi et al. 2002). The expressions of IL-10 and CD1d were to some degree correlated because both were up-regulated in MLN B cells upon development of colitis in TCRα$^{-/-}$ mice (Mizoguchi et al. 2002). CD1d might directly contribute to the induction of IL-10 expression in B cells because CD1d cross-linking was sufficient to trigger IL-10 expression in an intestinal epithelial cell line (Colgan et al. 1999). The way CD1d is engaged on MLN B cells during UC cannot involve invariant NK-T cells because these cells are lacking in TCRα$^{-/-}$ mice (Taniguchi et al. 1996), but might involve noninvariant CD1d-restricted T cells. During UC, the up-regulation of CD1d expression was uniform on MLN B cells, suggesting a global effect rather than a B cell subset-specific response. It is unknown whether all CD1d^{hi} B cells expressed IL-10 in this context. The up-regulation of CD1d occurred selectively on B cells from gut-associated lymphoid tissues (MLN, colonic lamina propria, and Peyer's patches), but was not noticeable in splenic B cells (Mizoguchi et al. 2002). The induction of suppressive function in B cells during an organ-specific inflammatory disease might therefore be anatomically localized, and in such a case it might be difficult to track the efficiency of this regulatory circuit by monitoring B cells from peripheral tissues such as blood.

The mechanism leading to up-regulation of CD1d and IL-10 by B cells in MLN of mice with UC remains to be characterized. The translocation of microbial products from the intestinal epithelium to the MLN might be implicated, but is probably insufficient to account for this process because B cells from MLN of IL-10$^{-/-}$ mice with IBD did not up-regulate CD1d (Mizoguchi et al. 2002), even though they certainly experienced increased bacterial translocation from gut to MLN during this disease. Importantly, the regulatory pathway controlled by CD1d did not account entirely for the suppressive function of B cells in UC, because B cell-deficient TCRα$^{-/-}$ mice developed disease earlier than CD1d-deficient TCRα$^{-/-}$ mice (Mizoguchi et al. 2002). Other receptors such as CD40, B7.2, and MHC-II, also contributed to the regulatory function of B cells during UC (Mizoguchi et al. 2000). These molecules might control the protective functions of B cells at different levels. For instance, MHC-II expression was required for the expansion of B cells in recipient mice, rather than for their suppressive function *per se*. Indeed MHC-II-deficient B cells failed to expand in recipient mice upon adoptive transfer, yet repeated administration of these cells protected recipient TCRα$^{-/-}$ mice from UC as efficiently as a single injection of wild-type B cells (Mizoguchi et al. 2002). It is also possible that some of these signaling pathways controlled the expression of other suppressive mediators than IL-10 by B cells.

In addition to MLN B cells, peritoneal cavity B1 cells can also protect mice from UC (Shimomura et al. 2008). The protective role of B1 cells in this disease was discovered following the observation that TCRα$^{-/-}$ mice maintained in conventional hygienic condition (CV) displayed a lower incidence of UC than TCRα$^{-/-}$ mice maintained in a specific pathogen free (SPF) animal facility (Shimomura et al. 2008). The lower disease incidence observed in CV compared to SPF facility was not observed in mice lacking B cells, and inversely correlated with the numbers of B1 cells in peritoneal cavity, and of IgM- and IgA-producing plasma cells in spleen of TCRα$^{-/-}$ mice (Shimomura et al. 2008). Noteworthy, B1 cells taken from mice kept in CV conditions protected recipient TCRα$^{-/-}$ mice maintained in a SPF facility from disease upon adoptive transfer (Shimomura et al. 2008). Although B1 cells were efficient IL-10 producers compared to other B cell subsets (O'Garra et al. 1992), their protective effect was not associated with higher levels of IL-10 in gut-associated lymphoid tissues, suggesting an alternative mechanism of protection. B1 cells are also a major source of antibodies. Antibodies can provide protection from UC: injection of antibodies purified from the serum of TCRα$^{-/-}$ mice decreased the severity of colitis in B cell-deficient TCRα$^{-/-}$ mice, and injection of a mixture of five monoclonal autoantibodies reacting against colonic tissue was sufficient to achieve a similar effect (Mizoguchi et al. 1997a). Antibodies might limit the severity of the disease by facilitating the clearance of apoptotic cells, which translocate their intracellular material on the cell surface during the apoptotic process (Gilligan et al. 1996). B cell-deficient TCRα$^{-/-}$ mice had markedly more apoptotic cells in colon, MLN, and spleen than TCRα$^{-/-}$ mice, and this difference was erased by passive transfer of serum immunoglobulin (Mizoguchi et al. 1997a). They also had more circulating colonic self-antigens in serum, which might lead to an exacerbation of the pathogenic CD4^{+} T cell

response (Mizoguchi et al. 1997a). It is therefore possible that B1 cells inhibited UC onset through the production of autoantibodies, although this remains to be formally proven. In any case, these data illustrate that B cells can suppress T_H2-mediated inflammatory disorders through the provision of IL-10, or via (auto) antibodies. These mechanisms apparently operate in parallel because autoantibody levels were not altered by a lack of CD1d or IL-10 expression by B cells (Mizoguchi et al. 2002).

IL-10 is also essential for protection from intestinal inflammatory diseases caused by T_H1 cells (Moran et al. 2013). Mice with a deficiency in *Il-10* gene often develop a spontaneous inflammatory disorder of the gut resembling CD (Kuhn et al. 1993). This disease is associated with an infiltration of lymphocytes and neutrophils in the intestinal mucosa, and results in weight loss as well as shortened life span of the mice (Kuhn et al. 1993). The onset of the disease can be prevented by administration of recombinant IL-10 in newborn mice, yet this treatment has only a moderate effect when applied later to animals with ongoing disease, indicating that different mechanisms are required to control the initiation versus progression of CD (Berg et al. 1996). $CD4^+$ T cells producing IFN-γ are particularly important for the initiation of this disease (Berg et al. 1996; Davidson et al. 1996). During IBD, B cells also undergo abnormal activation, leading to an elevated influx of B cells in the colon, and increased serum levels of antibodies, including colon-reactive antibodies (Davidson et al. 1996). A B cell-deficiency did not affect the course of colitis in IL-10-deficient mice (Davidson et al. 1996). The lack of disease aggravation in IL-10-deficient mice lacking B cells could result from the fact that B cells were unable to produce IL-10, or reflect an impossibility for B cells to control this type of inflammatory disorder. However, wild type peritoneal cavity B cells reduced weight loss and colitis severity in recipient IL-10-deficient mice upon adoptive transfer, compared to $IL\text{-}10^{-/-}$ peritoneal cavity B cells (Maseda et al. 2013), which correlated with reduced numbers of IFN-γ-producing $CD4^+$ T cells, and neutrophils (Maseda et al. 2013). Thus, peritoneal cavity B cells can limit T_H1-driven intestinal inflammation through IL-10 production in IL-10-deficient hosts. Peritoneal cavity B cells do not express high levels of CD1d, indicating that this receptor is not a universal marker of IL-10-producing immune suppressive B cells (Yanaba et al. 2008). Of note, peritoneal cavity B cells also reduced intestinal inflammation induced by adoptive transfer of $CD25^-CD45RB^{hi}CD4^+$ T cells in RAG-2-deficient mice, suggesting that their beneficial effect was not restricted to a particular model of CD-like disease (Maseda et al. 2013). It is unknown whether antibodies played any role in the protective function of B1 cells in these disease settings.

MLN B cells can also protect recipient mice from T_H1-driven intestinal inflammation upon adoptive transfer. This was shown in a model of IBD induced by adoptive transfer of Gαi2-deficient $CD3^+$ T cells in RAG-deficient mice (Wei et al. 2005). Gαi2 is a protein involved in adenyl cyclase inhibition, activation of phosphoinositide 3-kinase and voltage-independent calcium channels (Hamm and Gilchrist 1996), and a deficiency in the corresponding gene facilitates the development of colitis, in part because Gαi2-deficient T cells have increased

pro-inflammatory functions (Rudolph et al. 1995). The disease resulting from the administration of such T cells is associated with elevated numbers of $CD4^+$ T cells producing inflammatory cytokines, such as TNF-α, IFN-γ, and IL-17 (Wei et al. 2008). Remarkably, a single administration of MLN B cells completely protected recipient RAG-deficient mice from such disease via a mechanism involving IL-10 (Wei et al. 2005, 2008). In contrast, marginal zone B cells (i.e., splenic $CD1d^{hi}CD21^{hi}CD23^{lo}$ B cells) offered only a modest and transient protection from disease, highlighting the unique protective value of MLN B cells against such type of intestinal inflammation (Wei et al. 2008). The protective function of MLN B cells did not require their expression of MHC-II, CD1d, CD40, CD80, or CD86, suggesting that interaction between B cells and $CD4^+$ T cells, or NK-T cells was not required (McPherson et al. 2008). In contrast, B cells depended on expression of MHC-I and TAP-1, and required the presence of $CD8^+$ T cells to achieve protection, indicating that it involved direct interaction between B cells and $CD8^+$ T cells (McPherson et al. 2008; Wei et al. 2005, 2008). The efficient suppression observed in this model in the absence of CD1d indicates that IL-10-producing B cells can regulate immunity via different mechanisms depending on the disease characteristics, even when the targeted organ is the same. A possible explanation could be that distinct signals induce IL-10 expression in B cells in these different pathologies.

Most of the data discussed above on the protective functions of IL-10 produced by B cells in UC and CD indicate that MLN and peritoneal cavity B cells play particularly important roles in these processes. There is some evidence that splenic B cells can also control the severity of IBD via IL-10. Upon induction of IBD in mice by addition of dextran sulfate sodium (DSS) to the drinking water, B cells displayed a marked up-regulation of *Il-10* mRNA expression in spleen, but not in MLN, Peyer's patches, or intestinal lamina propria (Yanaba et al. 2011). This increased IL-10 expression was stronger in $CD1d^{hi}CD5^+$ than in non-$CD1d^{hi}CD5^+$ B cells, and wild-type $CD1d^{hi}CD5^+$ B cells markedly reduced the severity of IBD in CD19-deficient mice upon adoptive transfer. Of note, CD19-deficient mice displayed a defect in IL-10 expression by splenic B cells and developed exacerbated IBD compared to wild-type mice (Yanaba et al. 2011). The reason why B cell-mediated immune regulation occurs in spleen rather than in gut in this form of IBD is not known. A possibility is that DSS treatment results in extended tissue damage causing an inflammatory reaction in the entire colon (Okayasu et al. 1990). This case of extreme pathology might lead to systemic inflammation reaching the spleen, and prevent a normal induction of B cell regulatory function in gut-associated lymphoid organs.

Collectively, the data discussed above illustrate the diversity of the IL-10-mediated suppressive functions of B cells during inflammatory diseases. This diversity can be found at the level of the tissue where B cells express IL-10, of the phenotype of the B cells associated with the protection, and of the pleiotropic mechanisms through which IL-10 expressing B cells suppress unwanted immunity. This might have important implications for the therapeutic utilization of this regulatory mechanism. For instance, the fact that different B cell subsets showed

distinct capacity to control disease depending on the IBD model investigated, even though IL-10 was a common denominator for protection, raises an important question: Will it be possible to suppress the various forms of immunopathology represented by these different IBD models using a single B cell subset for adoptive cellular therapy? An identification of the phenotype(s) of the B cells that actually expressed IL-10 in vivo and were involved in disease suppression in these different models might help to overcome this difficulty.

3 Role of IL-10 Producing B Cells During Microbial Infections

3.1 Salmonella Typhimurium

Salmonella are Gram-negative bacterial pathogens that can infect human upon ingestion of contaminated food or water. Some *Salmonella* strain can provoke life-threatening diseases. For instance, *Salmonella typhi* is the causative agent for typhoid fever. This infection causes about 20 million cases of illnesses, and 600,000 deaths annually (Mittrucker and Kaufmann 2000). Infection with *Salmonella typhimurium* can induce in mice a disease with similarities to typhoid fever, and is widely accepted as an experimental model for this human disease (Mittrucker and Kaufmann 2000). Systemic infection with virulent *Salmonella typhimurium* results in a rapid activation of the mouse innate immune system, including neutrophils, NK cells, and macrophages. This innate response reduces the growth of the microbe, but is insufficient to control the infection, which is usually fatal within about 10 days after challenge. It is possible to increase the protection from such disease by prior vaccination, which promotes the development of a specific adaptive immune response. B cells can contribute positively to anti-*Salmonella* immunity through antibody production (Fillatreau 2011). However, B cells can also negatively regulate host resistance to this disease via secretion of IL-10 (Neves et al. 2010).

Administration of *Salmonella* via the systemic route led to the rapid development of IL-10-expressing B cells in infected mice (Neves et al. 2010). IL-10-expressing B cells were detectable in spleen already 24 h post-infection (p.i.), which was remarkable because B cells were classically thought to start playing a role in immune responses at later time points (Lampropoulou et al. 2012). B cells were the main cell type expressing IL-10 at this time after infection (Neves et al. 2010). The fact that no other cell type expressed IL-10 in spleen at this stage of the disease might explain the nonredundant role of B cell-derived IL-10 in the regulation of immunity. The relevance of IL-10 expression by B cells during *Salmonella* infection was investigated using mice in which only B cells could not express IL-10. Compared to mice with wild-type B cells, these animals displayed a prolonged survival after systemic infection with virulent *Salmonella*, which

correlated with a heightened activation of the innate immune system, particularly of neutrophils and NK cells. The effects of B cell-derived IL-10 on neutrophils and NK cells were noticeable already on day 2 p.i., in line with the expression of IL-10 by B cells on day 1 p.i.

The fast induction of IL-10 expression in B cells during *Salmonella* infection is typical of an innate response. Further emphasizing the innate aspect of this process, intrinsic signaling via MyD88 was crucial for these B cell-mediated regulatory activities. Indeed, mice with a deficiency in MyD88 restricted to B cells displayed a prolonged survival compared to mice with wild-type B cells after challenge with virulent *Salmonella*, and this correlated with a stronger activation of neutrophil and NK cell responses, as observed also in mice with a B cell-specific deficiency in IL-10 (Neves et al. 2010). Intrinsic MyD88 signaling was also required for IL-10 production by B cells in vitro upon co-culture with *Salmonella*, suggesting that IL-10 expression was triggered directly via TLR in B cells upon sensing of the bacteria during the infection (Neves et al. 2010). Taken together, the rapidity of this response, its dependence on intrinsic TLR signaling, and its effects on neutrophils and NK cells introduce IL-10-producing B cells as novel players in innate immunity.

The impact of IL-10 production by B cells on the adaptive immune response to *Salmonella* was investigated using a live attenuated bacterial strain that produced only a transient and nonlethal infection (Neves et al. 2010). Following infection, the bacteria-specific $CD4^+$ T cell response was detectable within about 1 week, and on day 21 after challenge mice lacking IL-10 expression in B cells displayed about two-fold more *Salmonella*-reactive IFN-γ- and TNF-α-producing $CD4^+$ T cells than mice with wild-type B cells, indicating that IL-10 from B cells dampened the pathogen specific inflammatory T cell responses during this infectious disease (Neves et al. 2010), as observed during autoimmune diseases (Fillatreau et al. 2002). The observation of a similar effect of B cell derived IL-10 in these two models suggest that a similar B cell-mediated regulatory circuit is at play during autoimmune and infectious diseases.

The phenotype of the B cells expressing IL-10 in vivo was characterized at the single cell level by flow cytometry using a reporter mouse in which the green fluorescent protein (GFP) gene was integrated immediately behind the STOP codon of the endogenous *Il10* coding sequence (Neves et al. 2010). These analyses showed that IL-10 producing B cells were not present in the spleen of naive mice, and that all the B cells expressing IL-10 at 24 h p.i. carried CD19 and CD138 on their cell surface (Neves et al. 2010). This result was unanticipated because CD138 is a marker characteristic for antibody-secreting cells (ASC), implying that ASC are the major source of B cell-derived IL-10 in vivo during *Salmonella* infection. The phenotype of the B cells expressing IL-10 in vivo can therefore differ from the phenotype of the B cells competent to express IL-10 in vitro after short-term stimulation with pharmacological agents, such as phorbol 12-myristate 13-acetate (PMA), ionomycin, and bacterial products. Because IL-10 produced by B cells down-modulates the antibacterial innate response, these data demonstrate that ASC can regulate immunity via antibody-independent mechanisms

particularly through provision of IL-10 (Neves et al. 2010). The concept that ASC regulate immunity through IL-10 might seem paradoxical given the positive role generally attributed to ASC in immunity. $CD19^{+}CD138^{+}$ cells were also the main source of B cell-derived IL-10 in a model of systemic lupus erythematosus (SLE) (Teichmann et al. 2012), indicating that the production of IL-10 by ASC is not a feature particular to *Salmonella* infection, but might be of more general relevance. A possible interpretation of this apparent paradox could be that plasma cells can through the production of IL-10 signal the innate immune system of the appearance of specific ASC, and this interaction might be important for the successful transition from a nonspecific system of host defense governed by innate immune receptors, such as TLR to an adaptive immune response controlled by specific antigen receptors, such as the BCR and TCR. Such a regulation of innate and nonspecific modes of immune activation upon the development of an adaptive immune response might be important to limit the risk of immunopathology.

3.2 Listeria Monocytogenes

Listeria monocytogenes is a Gram-positive bacteria that can cause severe infections in pregnant women, the elderly, and immune-compromised patients. The control of this infection requires in mice an innate reaction involving neutrophils, macrophages, NK cells, and the cytokines IL-6, IFN-γ, and TNF-α (Fillatreau 2011). The clearance of this infection is, however, dependent on the development of potent $CD4^{+}$ and $CD8^{+}$ T cell responses (Ladel et al. 1994). B cells can contribute to anti-*Listeria* immunity by increasing the formation of memory $CD8^{+}$ T cells (Shen et al. 2003), and by producing antibodies (Berche et al. 1990). However, they can also impair host defense against this bacteria through secretion of IL-10 (Horikawa et al. 2013; Lee and Kung 2012).

Administration of IL-10 strongly impaired host resistance to *Listeria* in mice (Kelly and Bancroft 1996), and B cells were a prominent source of this suppressive cytokine early after infection (Lee and Kung 2012). Indeed B cell-deficient mice displayed a better control of the bacteria at the early stage of the disease compared to control mice (Matsuzaki et al. 1999), which correlated with a lower production of IL-10 and an increased provision of IFN-γ in spleen, suggesting that IL-10 from B cells suppressed the inflammatory reaction induced by the bacteria (Lee and Kung 2012). Upon culture with heat-killed *Listeria*, marginal zone B cells secreted IL-10 in a TLR2- and/or TLR4-dependent manner (Lee and Kung 2012), and they markedly impaired via IL-10, the control of *Listeria* in recipient mice upon adoptive transfer, while follicular B cells had no effect (Lee and Kung 2012). The inhibitory effect of IL-10 produced by marginal zone B cells on the control of the bacterial growth was also observed in mice deficient in T and NK-T cells, implying that it directly targeted a distinct innate cell type (Lee and Kung 2012). As expected from this, a deficiency in CD1d did not result in loss of regulation by B cells (Lee and Kung 2012), and mice deficient in marginal zone B cells,

either as a result of a deficiency in CD19 or in the major Notch signaling adaptor RBP-J, more efficiently controlled *Listeria* growth, and displayed a better survival rate than control mice after infection (Horikawa et al. 2013; Lee and Kung 2012). Thus, intrinsic TLR signaling in B cells can lead to suppression of innate host defense against *Listeria* via IL-10.

The data collected in *Listeria* and *Salmonella* infections suggest that a similar process is at play during these two diseases. In both cases intrinsic TLR signaling in B cells leads to suppression of innate host defense via IL-10. In *Listeria* infection, marginal zone B cells are the precursors of IL-10 producing B cells, and plasma cells are the producers of IL-10 after challenge with *Salmonella*. It is tempting to propose as a model that in these two infections marginal zone B cells were rapidly stimulated via bacterial-derived agonists of TLR, and subsequently differentiated into IL-10-producing plasma cells. Indeed, microbes reaching the blood are promptly trapped by the marginal zone of the spleen, where antigen-reactive B cells are then rapidly activated to differentiate into IgM-producing plasma cells (Balazs et al. 2002). Antibodies produced by marginal zone B cells have important roles in defense against some microbial infections. For instance, a lack of marginal zone B cells has been associated to an impaired resistance against *Staphylococcus aureus*, and *Borrelia burgdorferi* (Belperron et al. 2007; Tanigaki et al. 2002). Moreover, marginal zone B cells improved via antibodies resistance against polyoma virus in recipient mice upon adoptive transfer (Guay et al. 2009). The dual roles of plasma cells derived from marginal zone B cells in protection from infection via antibodies and regulation of innate immunity via IL-10 can be reconciled by considering that the secretion of IL-10 by these cells serves at organizing the transition between an innate driven system of host defense to an immune response orchestrated by adaptive receptors, as discussed above. This might represent a general feature of antimicrobial immunity because several other pathogens were found to induce IL-10 expression by B cells including *Schistosoma mansoni, Borrelia hermsii, and Babesia microti* (Jeong et al. 2012; Mangan et al. 2004; Sindhava et al. 2010). This response can be dependent on intrinsic TLR signaling (Sindhava et al. 2010), result in suppression of host defense (Jeong et al. 2012), or confer protection from allergic hypersensitivity (Mangan et al. 2004). A similar process might be at play during autoimmune disorders since $CD1d^{hi}$ B cells mediated protective functions in various models of such diseases (Kalampokis et al. 2013). Are plasma cells the major source of B cell-derived IL-10 in autoimmune diseases?

4 IL-10-Producing B Cells in Human

The demonstration that endogenous B cells could dampen autoimmune pathogenesis through provision of IL-10 in mice immediately raised the question of whether human B cells could perform similar suppressive functions, and have protective

activities during autoimmune diseases. Answering this question is difficult because access to human B cells is often limited to those circulating in blood, and their functional relatedness to the B cells engaged in immune reactions in secondary lymphoid organs or in inflamed target tissues is unknown. Beyond this problem, a further complication stems from the difficulty of analyzing selectively antigen-specific B cells, either because the (auto)antigens driving the disease are unknown, or because it is technically challenging to identify these cells specifically. B cells expressing BCR of disease-relevant antigen specificity are likely rare in blood. In mouse, the regulatory functions of B cells were antigen specific (Fillatreau et al. 2002). Typically, experiments in humans are performed using polyclonal stimulation of complete blood B cells, regardless of their antigen specificity, for various length of time, and therefore assess whether the global responsiveness of the B cell compartment to the applied stimuli is changed during the disease. They provide an indirect assessment of the activity of B cells in the patients.

One of the first studies to compare IL-10 production by B cells from healthy donors (HD) and patients with autoimmune diseases was performed in the context of MS (Duddy et al. 2007). B cells from human blood produced more IL-10 upon single CD40 stimulation than after activation via both BCR and CD40, and B cells from MS patients secreted markedly less IL-10 than B cells from HD in both conditions (Duddy et al. 2007). This defect was observed for both patients with relapsing-remitting or secondary progressive MS (SPMS). Separation of peripheral blood B cells according to CD27 expression indicated that IL-10 was produced almost exclusively by $CD27^-$ cells in these assays (Duddy et al. 2007). This was, however, not the basis for the difference between MS patients and HD because they had similar ratios of $CD27^+$ and $CD27^-$ B cells in blood. Independent studies reported similar findings, emphasizing the generality of this phenomenon (Hirotani et al. 2010; Knippenberg et al. 2011; Quan et al. 2013). For instance, B cells from MS patients also secreted less IL-10 than B cells from HD upon activation via TLR9 in vitro (Hirotani et al. 2010). It was unlikely that this decreased IL-10 production affected only B cells expressing BCR with disease-relevant antigen specificities, because the frequencies of CNS antigen-reactive B cells were likely low in blood of MS patients, and the stimulations used in these studies were polyclonal. Nevertheless, these results suggested that a deficit in the capacity of B cells to produce IL-10 might facilitate MS progression. If so, it might be possible to improve the disease course by increasing IL-10 expression in B cells. The drug mitoxantrone, which is an approved chemotherapy for patients with severe MS, led to an increased aptitude of B cells from treated patients to produce IL-10 (Duddy et al. 2007). B cells might also contribute to the therapeutic effect of IFN-β. IFN-β augmented the production of IL-10 by B cells in vitro (Ramgolam et al. 2011), and elevated the percentage of $CD27^-$ B cells in blood of MS patients (Niino et al. 2009). Similarly, B cell depletion therapy resulted in a marked increase in the percentage of $CD27^-$ B cells in blood at 12 months after treatment, suggesting that it could transiently favor the predominance of B cells with a superior capacity to produce IL-10 (Duddy et al. 2007). Finally, B cells

from MS patients infected with helminth parasites displayed a restored capacity to produce IL-10, compared to noninfected patients, and this was associated with a reduced frequency of disease flares (Correale et al. 2008; Correale and Farez 2011). Importantly, the impaired secretion of IL-10 by MS B cells is paralleled by an increased capacity of these cells to produce IL-6, tumor necrosis factor (TNF), and lymphotoxin upon activation compared to HD (Bar-Or et al. 2010; Barr et al. 2012). MS progression might therefore be facilitated by an unbalanced production of pro- versus anti-inflammatory cytokines by B cells, which might be the basis for the pathogenic roles of these cells, and the beneficial effects of B cell-depletion therapy in this disease (Barr et al. 2012).

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with B cell involvement. A hyperactivity of the B cell compartment, with noticeable increases in number of circulating plasma cells, and blood levels of autoantibodies to nuclear antigens (ANA) are prominent immunological abnormalities in SLE (Jacobi et al. 2008; Sherer et al. 2004). ANA predominantly target double-stranded (ds) DNA and nucleosomes, and are quantified in the clinic to diagnose SLE. This autoreactive response is associated with defects in B cell tolerance (Wardemann and Nussenzweig 2007), and susceptibility to SLE has been linked with polymorphisms in genes coding for proteins involved in BCR signaling (Jarvinen et al. 2012), further highlighting a role for B cells as drivers of pathogenesis. However, two double-blinded, randomized, placebo controlled trials investigating the effect of B cell-depletion therapy in SLE failed to meet their primary and secondary endpoints (Merrill et al. 2010; Rovin et al. 2012). Cytokines also play important roles in SLE. SLE is associated with increased levels of type I IFN, IL-6, TNF, and B-cell activating factor (BAFF), which are thought to operate as amplifiers of the disease (Wahren-Herlenius and Dorner 2013). Few studies have explored whether the capacity of B cells to produce cytokines was altered in SLE. Indeed, B cells from patients with severe SLE produced less IL-6 and IL-10 than B cells from HD upon TLR9-stimulation (Zorro et al. 2009). TLR9 is likely a relevant stimulatory receptor in SLE, because this disease is associated with defects in clearance of apoptotic cells, which are an important source of nucleic acid complexes stimulatory for B cells. B cells from SLE patients also displayed a reduced capacity to secrete IL-10 upon CD40 engagement compared to HD (Blair et al. 2010). Of note, IL-10 was first considered as a pathogenic cytokine facilitating autoantibody production in SLE (Ishida et al. 1994; Llorente et al. 2000). It is therefore important to be cautious in discussing the function of IL-10 from B cells in this disease (Fillatreau et al. 2008). It is unknown whether B cells from SLE patients have an increased capacity to produce inflammatory cytokines upon stimulation, alike B cells from MS patients. This might be an important issue because it could be related to the fact that rituximab did not show the anticipated efficacy in SLE. If SLE B cells are generally defective in cytokine production, they may in fact have little pro-inflammatory functions, besides antibody production once this disease is established. Since the major mechanism explaining the beneficial effect of B cell-depletion therapy in EAE was the elimination of IL-6-producing B cells (Barr

et al. 2012), it is possible that rituximab therapy is less efficient in patients whose B cells do not produce increased amounts of pro-inflammatory cytokine(s).

It is important to note that the phenotype of the human B cells producing IL-10 and possibly mediating suppressive functions is controversially discussed. A classical approach to identify the cells ready to rapidly express a given cytokine in vivo is to perform a short-term (about 5 h) stimulation of cell suspensions with PMA and ionomycin ex vivo followed by intracellular cytokine staining. After such stimulation IL-10 was expressed in a small percentage of blood B cells corresponding to about 3 % of cells having a $CD24^{hi}CD27^{hi}$ phenotype (Iwata et al. 2011). This suggests that memory $CD27^{+}$ B cells, rather than naïve B cells, are the cells ready to produce IL-10 in vivo. Noteworthy, CD1d, CD5, CD25, and CD38 were expressed in similar manners by IL-10-positive and -negative cells in this assay. In fact, IL-10-positive B cells expressed heterogeneous levels of CD38 and IgD, possibly reflecting distinct activation status or B cell subsets. Using this protocol, IL-10-expressing B cells were also induced at similar frequency in cord blood, spleen, and tonsil as in adult blood, arguing against the notion that immature/transitional B cells, which are more abundant in cord blood, have a stronger competence to express IL-10 (Bouaziz et al. 2010; Iwata et al. 2011). In spleen, the B cells competent for IL-10 production were in majority $CD27^{+}$, yet did not differentially express CD24 compared to IL-10-negative cells (Iwata et al. 2011). The phenotype of the blood B cells ready to express IL-10 might therefore not be strictly identical to their counterparts in secondary lymphoid tissues.

In a distinct study, the phenotype of B cells producing IL-10 after stimulation via TLR9 was investigated using an IL-10 secretion assay based on a bi-specific anti-$IL\text{-}10^{+}$ anti-CD45 antibody construct to retain secreted IL-10 at the surface of the cells (van de Veen et al. 2013). These cells could subsequently be separated from non-IL-10-secreting B cells as live cells, and compared via global transcriptome analyses. With this approach, neither expression of CD24, CD27, or CD38 distinguished $IL\text{-}10^{+}$ from $IL\text{-}10^{-}$ B cells. In contrast, IL-10 secreting cells were preferentially found among $CD73^{-}CD25^{+}CD71^{+}$ B cells (van de Veen et al. 2013).

This brief survey of the literature on the phenotype of the human B cells capable of producing IL-10 upon activation ex vivo highlights that the possible phenotypes are highly diverse. It is difficult to reconcile these findings, except by considering that this heterogeneity reflects the nature of the stimuli and the durations of the cultures used in these studies. It will be important to characterize the cells actually expressing IL-10 in vivo, in absence of any re-stimulation ex vivo, to understand the relatedness of the culture assays described above to the actual process of suppression by IL-10-producing B cells in vivo. This will then allow a more physiological interpretation of these results, and the design of appropriate assays to estimate the strength of suppressive functions in vivo. Nevertheless, the data obtained so far provide supporting evidence that B cells might provide via IL-10 protection from autoimmune diseases in human.

5 Concluding Remarks

The concept that B cells can inhibit immunity through provision of IL-10 has now been confirmed in numerous experimental studies in mice. Evidence is accumulating that it might also be relevant in human. It has been proposed as an explanation for the fact that B cell depletion therapy resulted in increased immunopathology in some patients treated with rituximab. Conversely, it might be possible to suppress unwanted immunity by promoting the inhibitory functions of B cells. This could be achieved using "regulatory B cells" in adoptive cell therapy (Calderon-Gomez et al. 2011; Scott 2011), or by administration of drugs promoting the regulatory functions of endogenous B cells (Ding et al. 2011). Some drugs already used to treat patients with autoimmune diseases, such as IFN-β, might already recruit the suppressive functions of B cells for their therapeutic effect (Ramgolam et al. 2011). Therapies based on biologics might also be suitable to achieve such an effect. For instance, an antibody against T cell immunoglobulin and mucin domain (TIM)-1, a receptor expressed by 5–8 % of splenic B cells in naïve mice, markedly prolonged survival of pancreatic islets allografts in diabetic mice via a mechanism dependent on IL-10 secretion by B cells (Ding et al. 2011). This resulted in long-term engraftment of the transplanted islets in 30 % of recipient mice, while in all animals of the control group grafts were eliminated within 15 days. This treatment increased the frequency of B cells competent for fast IL-10 production, and TIM-1^+ B cells isolated from treated mice could prolong allograft survival in an IL-10-dependent and antigen-specific manner in recipient mice upon adoptive transfer. Such targeting of TIM-1 with a specific antibody also prolonged the survival of pancreatic islet allograft synergistically with an anti-CD45RB antibody (Lee et al. 2012). This combination therapy allowed maintenance of the grafted islets in all recipient mice for at least 100 days via a mechanism dependent on B cells. In this case also B cells isolated from successfully treated mice transferred allograft tolerance to recipient mice in an antigen-specific manner upon administration. These studies provide a proof of concept that it is possible to inhibit unwanted immune reactions by targeting endogenous B cells. Of note, the efficacy of this approach depended on the disease setting. Targeting TIM-1 promoted tolerance when C57BL/6 islets were transplanted into BALB/c mice (Ding et al. 2011), but was insufficient on its own to prolong the survival of BALB/c islets in C57BL/6 mice (Lee et al. 2012). It is now important to understand the mechanisms behind these protective effects, all the most that targeting TIM-1 with a different antibody clone resulted in an exacerbation of inflammation (Xiao et al. 2007).

A better understanding of B cell-mediated suppression is desirable to develop therapeutic strategies allowing the manipulation of this pathway in a safe and effective manner. The identification of the relevant B cell subsets and their targets are still at preliminary stages. There is currently no combination of cell surface receptors available to stringently isolate the B cells providing IL-10 in a regulatory manner. IL-10 expression by B cells is not always paralleled by immune suppression in vivo (Anderton and Fillatreau 2008). Moreover, in mice, only a small proportion of $CD1d^{hi}CD5^+$ B cells up-regulate IL-10 expression upon stimulation with PMA, inomycin, and LPS in vitro, and it is unknown whether the progeny of these cells, which suppresses immunity in

an IL-10-dependent manner in recipient mice upon adoptive transfer, maintains this phenotype (Lampropoulou et al. 2010). As an additional difficulty, the cell subsets so far ascribed with a superior competence for IL-10 production in vitro possess additional functions that might be stimulatory for immunity. For instance, CD1d^{hi} marginal zone B cells not only display the strongest capacity to express IL-10 upon TLR stimulation, but are also the most effective IL-6 producers compared to other splenic B cell subsets. Furthermore, they can also rapidly differentiate into plasma cells implicating that they also contribute to immunity through antibody production. The antibodies produced by marginal zone B cells can play critical roles in host defense against infectious diseases. It will be important to find strategies allowing the separation of the pleiotropic activities of these B cells. A better characterization of the cells that produce IL-10 in vivo might help to reach this goal.

References

Anderton SM, Fillatreau S (2008) Activated B cells in autoimmune diseases: the case for a regulatory role. Nat Clin Pract Rheumatol 4:657–666. doi:10.1038/ncprheum0950

Balazs M, Martin F, Zhou T, Kearney J (2002) Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. Immunity 17:341–352

Bar-Or A, Fawaz L, Fan B, Darlington PJ, Rieger A, Ghorayeb C, Calabresi PA, Waubant E, Hauser SL, Zhang J, Smith CH (2010) Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? Ann Neurol 67:452–461. doi:10.1002/ana.21939

Barr TA, Shen P, Brown S, Lampropoulou V, Roch T, Lawrie S, Fan B, O'Connor RA, Anderton SM, Bar-Or A, Fillatreau S, Gray D (2012) B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. J Exp Med 209:1001–1010. doi:10.1084/jem.20111675

Belperron AA, Dailey CM, Booth CJ, Bockenstedt LK (2007) Marginal zone B-cell depletion impairs murine host defense against Borrelia burgdorferi infection. Infect Immun 75:3354–3360. doi:10.1128/IAI.00422-07

Berche P, Reich KA, Bonnichon M, Beretti JL, Geoffroy C, Raveneau J, Cossart P, Gaillard JL, Geslin P, Kreis H et al (1990) Detection of anti-listeriolysin O for serodiagnosis of human listeriosis. Lancet 335:624–627

Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D (1996) Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. J Clin Invest 98:1010–1020. doi:10.1172/JCI118861

Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, 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. doi:10.1016/j.immuni.2009.11.009

Bouaziz JD, Calbo S, Maho-Vaillant M, Saussine A, Bagot M, Bensussan A, Musette P (2010) IL-10 produced by activated human B cells regulates CD4(+) T-cell activation in vitro. Eur J Immunol 40:2686–2691. doi:10.1002/eji.201040673

Calderon-Gomez E, Lampropoulou V, Shen P, Neves P, Roch T, Stervbo U, Rutz S, Kuhl AA, Heppner FL, Loddenkemper C, Anderton SM, Kanellopoulos JM, Charneau P, Fillatreau S (2011) Reprogrammed quiescent B cells provide an effective cellular therapy against chronic experimental autoimmune encephalomyelitis. Eur J Immunol 41:1696–1708. doi:10.1002/eji.201041041

Colgan SP, Hershberg RM, Furuta GT, Blumberg RS (1999) Ligation of intestinal epithelial CD1d induces bioactive IL-10: critical role of the cytoplasmic tail in autocrine signaling. Proc Natl Acad Sci U S A 96:13938–13943

Correale J, Farez M, Razzitte G (2008) Helminth infections associated with multiple sclerosis induce regulatory B cells. Ann Neurol 64:187–199. doi:10.1002/ana.21438

Correale J, Farez MF (2011) The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol 233:6–11. doi:10.1016/j.jneuroim.2011.01.002

Dass S, Vital EM, Emery P (2007) Development of psoriasis after B cell depletion with rituximab. Arthritis Rheum 56:2715–2718. doi:10.1002/art.22811

Davidson NJ, Leach MW, Fort MM, Thompson-Snipes L, Kuhn R, Muller W, Berg DJ, Rennick DM (1996) T helper cell 1-type $CD4^+$ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. J Exp Med 184:241–251

Ding Q, Yeung M, Camirand G, Zeng Q, Akiba H, Yagita H, Chalasani G, Sayegh MH, Najafian N, Rothstein DM (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. doi:10.1172/JCI46274

Duddy M, Niino M, Adatia F, Hebert S, Freedman M, Atkins H, Kim HJ, Bar-Or A (2007) Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J Immunol 178:6092–6099

Fillatreau S (2011) Novel regulatory functions for toll-like receptor-activated B cells during intracellular bacterial infection. Immunol Rev 240:52–71. doi:10.1111/j.1600-065X.2010.00991.x

Fillatreau S, Anderton SM (2007) B-cell function in CNS inflammatory demyelinating disease: a complexity of roles and a wealth of possibilities. Expert Rev Clin Immunol 3:565–578. doi:10.1586/1744666X.3.4.565

Fillatreau S, Gray D, Anderton SM (2008) Not always the bad guys: B cells as regulators of autoimmune pathology. Nat Rev Immunol 8:391–397. doi:10.1038/nri2315

Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM (2002) B cells regulate autoimmunity by provision of IL-10. Nat Immunol 3:944–950. doi:10.1038/ni833

Gilligan HM, Bredy B, Brady HR, Hebert MJ, Slayter HS, Xu Y, Rauch J, Shia MA, Koh JS, Levine JS (1996) Anti neutrophil cytoplasmic autoantibodies interact with primary granule constituents on the surface of apoptotic neutrophils in the absence of neutrophil priming. J Exp Med 184:2231–2241

Goetz M, Atreya R, Ghalibafian M, Galle PR, Neurath MF (2007) Exacerbation of ulcerative colitis after rituximab salvage therapy. Inflamm Bowel Dis 13:1365–1368. doi:10.1002/ibd.20215

Guay HM, Mishra R, Garcea RL, Welsh RM, Szomolanyi-Tsuda E (2009) Generation of protective T cell-independent antiviral antibody responses in SCID mice reconstituted with follicular or marginal zone B cells. J Immunol 183:518–523. doi:10.4049/jimmunol.0900068

Hamm HE, Gilchrist A (1996) Heterotrimeric G proteins. Curr Opin Cell Biol 8:189–196

Harris DP, Haynes L, Sayles PC, Duso DK, Eaton SM, Lepak NM, Johnson LL, Swain SL, Lund FE (2000) Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat Immunol 1:475–482. doi:10.1038/82717

Hirotani M, Niino M, Fukazawa T, Kikuchi S, Yabe I, Hamada S, Tajima Y, Sasaki H (2010) Decreased IL-10 production mediated by Toll-like receptor 9 in B cells in multiple sclerosis. J Neuroimmunol 221:95–100. doi:10.1016/j.jneuroim.2010.02.012

Hoehlig K, Shen P, Lampropoulou V, Roch T, Malissen B, O'Connor R, Ries S, Hilgenberg E, Anderton SM, Fillatreau S (2012) Activation of CD4(+) Foxp3(+) regulatory T cells proceeds normally in the absence of B cells during EAE. Eur J Immunol 42:1164–1173. doi:10.1002/eji.201142242

Horikawa M, Weimer ET, DiLillo DJ, Venturi GM, Spolski R, Leonard WJ, Heise MT, Tedder TF (2013) Regulatory B cell (B10 Cell) expansion during Listeria infection governs innate and cellular immune responses in mice. J Immunol 190:1158–1168. doi:10.4049/jimmunol.1201427

Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, Howard M (1994) Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J Exp Med 179:305–310

Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, Szabolcs PM, Bernstein SH, Magro CM, Williams AD, Hall RP, St Clair EW, Tedder TF (2011) Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood 117:530–541. doi:10.1182/blood-2010-07-294249

Jacobi AM, Reiter K, Mackay M, Aranow C, Hiepe F, Radbruch A, Hansen A, Burmester GR, Diamond B, Lipsky PE, Dorner T (2008) Activated memory B cell subsets correlate with disease activity in systemic lupus erythematosus: delineation by expression of CD27, IgD, and CD95. Arthritis Rheum 58:1762–1773. doi:10.1002/art.23498

Jarvinen TM, Hellquist A, Zucchelli M, Koskenmies S, Panelius J, Hasan T, Julkunen H, D'Amato M, Kere J (2012) Replication of GWAS-identified systemic lupus erythematosus susceptibility genes affirms B-cell receptor pathway signalling and strengthens the role of IRF5 in disease susceptibility in a Northern European population. Rheumatology (Oxford) 51:87–92. doi:10.1093/rheumatology/ker263

Jeong YI, Hong SH, Cho SH, Lee WJ, Lee SE (2012) Induction of IL-10-producing CD1dhighCD5$^+$ regulatory B cells following Babesia microti-infection. PLoS ONE 7:e46553. doi:10.1371/journal.pone.0046553

Jin G, Hamaguchi Y, Matsushita T, Hasegawa M, Le Huu D, Ishiura N, Naka K, Hirao A, Takehara K, Fujimoto M (2013) B-cell linker protein expression contributes to controlling allergic and autoimmune diseases by mediating IL-10 production in regulatory B cells. J Allergy Clin Immunol 131:1674–1682. doi:10.1016/j.jaci.2013.01.044

Kalampokis I, Yoshizaki A, Tedder TF (2013) IL-10-producing regulatory B cells (B10 cells) in autoimmune disease. Arthritis Res Ther 15(Suppl 1):S1. doi:10.1186/ar3907

Kaser A, Zeissig S, Blumberg RS (2010) Inflammatory bowel disease. Annu Rev Immunol 28:573–621. doi:10.1146/annurev-immunol-030409-101225

Kelly JP, Bancroft GJ (1996) Administration of interleukin-10 abolishes innate resistance to *Listeria monocytogenes*. Eur J Immunol 26:356–364. doi:10.1002/eji.1830260214

Knippenberg S, Peelen E, Smolders J, Thewissen M, Menheere P, Cohen Tervaert JW, Hupperts R, Damoiseaux J (2011) Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is accompanied by a reduced naive/memory Breg ratio during a relapse but not in remission. J Neuroimmunol 239:80–86. doi:10.1016/j.jneuroim.2011.08.019

Koretzky GA, Abtahian F, Silverman MA (2006) SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. Nat Rev Immunol 6:67–78. doi:10.1038/nri1750

Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274

Ladel CH, Flesch IE, Arnoldi J, Kaufmann SH (1994) Studies with MHC-deficient knock-out mice reveal impact of both MHC I- and MHC II-dependent T cell responses on *Listeria monocytogenes* infection. J Immunol 153:3116–3122

Lampropoulou V, Calderon-Gomez E, Roch T, Neves P, Shen P, Stervbo U, Boudinot P, Anderton SM, Fillatreau S (2010) Suppressive functions of activated B cells in autoimmune diseases reveal the dual roles of Toll-like receptors in immunity. Immunol Rev 233:146–161. doi:10.1111/j.0105-2896.2009.00855.x

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

Lampropoulou V, Shen P, Hilgenberg E, Ries S, Opitz C, Fillatreau S (2012) Functional interactions between B lymphocytes and the innate immune system. Infect Disord Drug Targets 12:191–199

Lee CC, Kung JT (2012) Marginal zone B cell is a major source of Il-10 in *Listeria monocytogenes* susceptibility. J Immunol 189:3319–3327. doi:10.4049/jimmunol.1201247

Lee KM, Kim JI, Stott R, Soohoo J, O'Connor MR, Yeh H, Zhao G, Eliades P, Fox C, Cheng N, Deng S, Markmann JF (2012) Anti-CD45RB/anti-TIM-1-induced tolerance requires regulatory B cells. Am J Transplant 12:2072–2078. doi:10.1111/j.1600-6143.2012.04055.x

Llorente L, Richaud-Patin Y, Garcia-Padilla C, Claret E, Jakez-Ocampo J, Cardiel MH, Alcocer-Varela J, Grangeot-Keros L, Alarcon-Segovia D, Wijdenes J, Galanaud P, Emilie D (2000) Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. Arthritis Rheum 43:1790–1800. doi:10.1002/1529-0131(200008)43:8<1790:AID-ANR15>3.0.CO;2-2

Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, Fallon PG (2004) Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol 173:6346–6356

Maseda D, Candando KM, Smith SH, Kalampokis I, Weaver CT, Plevy SE, Poe JC, Tedder TF (2013) Peritoneal cavity regulatory B cells (B10 cells) modulate IFN-gamma$^+$ CD4$^+$ T cell numbers during colitis development in mice. J Immunol 191:2780–2795. doi:10.4049/jimmunol.1300649

Matsumoto M, Fujii Y, Baba A, Hikida M, Kurosaki T, Baba Y (2011) The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. Immunity 34:703–714. doi:10.1016/j.immuni.2011.03.016

Matsushita T, Fujimoto M, Hasegawa M, Komura K, Takehara K, Tedder TF, Sato S (2006) Inhibitory role of CD19 in the progression of experimental autoimmune encephalomyelitis by regulating cytokine response. Am J Pathol 168:812–821. doi:10.2353/ajpath.2006.050923

Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF (2008) Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest 118:3420–3430. doi:10.1172/JCI36030

Matsuzaki G, Vordermeier HM, Hashimoto A, Nomoto K, Ivanyi J (1999) The role of B cells in the establishment of T cell response in mice infected with an intracellular bacteria, *Listeria monocytogenes*. Cell Immunol 194:178–185. doi:10.1006/cimm.1999.1503

McPherson M, Wei B, Turovskaya O, Fujiwara D, Brewer S, Braun J (2008) Colitis immunoregulation by CD8$^+$ T cell requires T cell cytotoxicity and B cell peptide antigen presentation. Am J Physiol Gastrointest Liver Physiol 295:G485–G492. doi:10.1152/ajpgi.90221.2008

Merrill JT, Neuwelt CM, Wallace DJ, Shanahan JC, Latinis KM, Oates JC, Utset TO, Gordon C, Isenberg DA, Hsieh HJ, Zhang D, Brunetta PG (2010) Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. Arthritis Rheum 62:222–233. doi:10.1002/art.27233

Mielke F, Schneider-Obermeyer J, Dorner T (2008) Onset of psoriasis with psoriatic arthropathy during rituximab treatment of non-Hodgkin lymphoma. Ann Rheum Dis 67:1056–1057. doi:10.1136/ard.2007.080929

Mittrucker HW, Kaufmann SH (2000) Immune response to infection with *Salmonella typhimurium* in mice. J Leukoc Biol 67:457–463

Mizoguchi A, Mizoguchi E, Bhan AK (1999) The critical role of interleukin 4 but not interferon gamma in the pathogenesis of colitis in T-cell receptor alpha mutant mice. Gastroenterology 116:320–326

Mizoguchi A, Mizoguchi E, Chiba C, Spiekermann GM, Tonegawa S, Nagler-Anderson C, Bhan AK (1996a) Cytokine imbalance and autoantibody production in T cell receptor-alpha mutant mice with inflammatory bowel disease. J Exp Med 183:847–856

Mizoguchi A, Mizoguchi E, Tonegawa S, Bhan AK (1996b) Alteration of a polyclonal to an oligoclonal immune response to cecal aerobic bacterial antigens in TCR alpha mutant mice with inflammatory bowel disease. Int Immunol 8:1387–1394

Mizoguchi A, Mizoguchi E, Smith RN, Preffer FI, Bhan AK (1997a) Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. J Exp Med 186:1749–1756

Mizoguchi E, Mizoguchi A, Chiba C, Niles JL, Bhan AK (1997b) Anti neutrophil cytoplasmic antibodies in T-cell receptor alpha-deficient mice with chronic colitis. Gastroenterology 113:1828–1835

Mizoguchi E, Mizoguchi A, Preffer FI, Bhan AK (2000) Regulatory role of mature B cells in a murine model of inflammatory bowel disease. Int Immunol 12:597–605

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

Moran CJ, Walters TD, Guo CH, Kugathasan S, Klein C, Turner D, Wolters VM, Bandsma RH, Mouzaki M, Zachos M, Langer JC, Cutz E, Benseler SM, Roifman CM, Silverberg MS, Griffiths AM, Snapper SB, Muise AM (2013) IL-10R polymorphisms are associated with very-early-onset ulcerative colitis. Inflamm Bowel Dis 19:115–123. doi:10.1002/ibd.22974

Moulin V, Andris F, Thielemans K, Maliszewski C, Urbain J, Moser M (2000) B lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. J Exp Med 192:475–482

Neves P, Lampropoulou V, Calderon-Gomez E, Roch T, Stervbo U, Shen P, Kuhl AA, Loddenkemper C, Haury M, Nedospasov SA, Kaufmann SH, Steinhoff U, Calado DP, Fillatreau S (2010) Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during *Salmonella typhimurium* infection. Immunity 33:777–790. doi:10.1016/j.immuni.2010.10.016

Niino M, Hirotani M, Miyazaki Y, Sasaki H (2009) Memory and naive B-cell subsets in patients with multiple sclerosis. Neurosci Lett 464:74–78. doi:10.1016/j.neulet.2009.08.010

O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M (1992) Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. Eur J Immunol 22:711–717. doi:10.1002/eji.1830220314

Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 98:694–702

Quan C, Yu H, Qiao J, Xiao B, Zhao G, Wu Z, Li Z, Lu C (2013) Impaired regulatory function and enhanced intrathecal activation of B cells in neuromyelitis optica: distinct from multiple sclerosis. Mult Scler 19:289–298. doi:10.1177/1352458512454771

Ramgolam VS, Sha Y, Marcus KL, Choudhary N, Troiani L, Chopra M, Markovic-Plese S (2011) B cells as a therapeutic target for IFN-beta in relapsing-remitting multiple sclerosis. J Immunol 186:4518–4526. doi:10.4049/jimmunol.1000271

Rovin BH, Furie R, Latinis K, Looney RJ, Fervenza FC, Sanchez-Guerrero J, Maciuca R, Zhang D, Garg JP, Brunetta P, Appel G, Group LI (2012) Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. Arthritis Rheum 64:1215–1226. doi:10.1002/art.34359

Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Boulay G, Bradley A, Birnbaumer L (1995) Ulcerative colitis and adenocarcinoma of the colon in G alpha i2-deficient mice. Nat Genet 10:143–150. doi:10.1038/ng0695-143

Scott DW (2011) Transduced B cells: B is for 'beneficial'! Eur J Immunol 41:1528–1530. doi:10.1002/eji.201141649

Shen H, Whitmire JK, Fan X, Shedlock DJ, Kaech SM, Ahmed R (2003) A specific role for B cells in the generation of CD8 T cell memory by recombinant *Listeria monocytogenes*. J Immunol 170:1443–1451

Sherer Y, Gorstein A, Fritzler MJ, Shoenfeld Y (2004) Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum 34:501–537

Shimomura Y, Mizoguchi E, Sugimoto K, Kibe R, Benno Y, Mizoguchi A, Bhan AK (2008) Regulatory role of B-1 B cells in chronic colitis. Int Immunol 20:729–737. doi:10.1093/intimm/dxn031

Sindhava V, Woodman ME, Stevenson B, Bondada S (2010) Interleukin-10 mediated autoregulation of murine B-1 B-cells and its role in Borrelia hermsii infection. PLoS ONE 5:e11445. doi:10.1371/journal.pone.0011445

Takahashi I, Kiyono H, Hamada S (1997) $CD4^+$ T-cell population mediates development of inflammatory bowel disease in T-cell receptor alpha chain-deficient mice. Gastroenterology 112:1876–1886

Tanigaki K, Han H, Yamamoto N, Tashiro K, Ikegawa M, Kuroda K, Suzuki A, Nakano T, Honjo T (2002) Notch-RBP-J signaling is involved in cell fate determination of marginal zone B cells. Nat Immunol 3:443–450. doi:10.1038/ni793

Taniguchi M, Koseki H, Tokuhisa T, Masuda K, Sato H, Kondo E, Kawano T, Cui J, Perkes A, Koyasu S, Makino Y (1996) Essential requirement of an invariant V alpha 14 T cell antigen receptor expression in the development of natural killer T cells. Proc Natl Acad Sci U S A 93:11025–11028

Teichmann LL, Kashgarian M, Weaver CT, Roers A, Muller W, Shlomchik MJ (2012) B cell-derived IL-10 does not regulate spontaneous systemic autoimmunity in MRL.Fas(lpr) mice. J Immunol 188:678–685. doi:10.4049/jimmunol.1102456

van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, Ruckert B, Akdis CA, Akdis M (2013) IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. J Allergy Clin Immunol 131:1204–1212. doi:10.1016/j.jaci.2013.01.014

Wahren-Herlenius M, Dorner T (2013) Immunopathogenic mechanisms of systemic autoimmune disease. Lancet 382:819–831. doi:10.1016/S0140-6736(13)60954-X

Wardemann H, Nussenzweig MC (2007) B-cell self-tolerance in humans. Adv Immunol 95:83–110. doi:10.1016/S0065-2776(07)95003-8

Wei B, McPherson M, Turovskaya O, Velazquez P, Fujiwara D, Brewer S, Braun J (2008) Integration of B cells and $CD8^+$ T in the protective regulation of systemic epithelial inflammation. Clin Immunol 127:303–312. doi:10.1016/j.clim.2008.01.001

Wei B, Velazquez P, Turovskaya O, Spricher K, Aranda R, Kronenberg M, Birnbaumer L, Braun J (2005) Mesenteric B cells centrally inhibit $CD4^+$ T cell colitis through interaction with regulatory T cell subsets. Proc Natl Acad Sci U S A 102:2010–2015. doi:10.1073/pnas.0409449102

Wen L, Roberts SJ, Viney JL, Wong FS, Mallick C, Findly RC, Peng Q, Craft JE, Owen MJ, Hayday AC (1994) Immunoglobulin synthesis and generalized autoimmunity in mice congenitally deficient in alpha beta(+) T cells. Nature 369:654–658. doi:10.1038/369654a0

Xiao S, Najafian N, Reddy J, Albin M, Zhu C, Jensen E, Imitola J, Korn T, Anderson AC, Zhang Z, Gutierrez C, Moll T, Sobel RA, Umetsu DT, Yagita H, Akiba H, Strom T, Sayegh MH, DeKruyff RH, Khoury SJ, Kuchroo VK (2007) Differential engagement of Tim-1 during activation can positively or negatively costimulate T cell expansion and effector function. J Exp Med 204:1691–1702. doi:10.1084/jem.20062498

Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF (2008) A regulatory B cell subset with a unique $CD1dhiCD5^+$ phenotype controls T cell-dependent inflammatory responses. Immunity 28:639–650. doi:10.1016/j.immuni.2008.03.017

Yanaba K, Kamata M, Ishiura N, Shibata S, Asano Y, Tada Y, Sugaya M, Kadono T, Tedder TF, Sato S (2013) Regulatory B cells suppress imiquimod-induced, psoriasis-like skin inflammation. J Leukoc Biol 94:563–573. doi:10.1189/jlb.1112562

Yanaba K, Yoshizaki A, Asano Y, Kadono T, Tedder TF, Sato S (2011) IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. Am J Pathol 178:735–743. doi:10.1016/j.ajpath.2010.10.022

Yoshizaki A, Miyagaki T, DiLillo DJ, Matsushita T, Horikawa M, Kountikov EI, Spolski R, Poe JC, Leonard WJ, Tedder TF (2012) Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature 491:264–268. doi:10.1038/nature11501

Zorro S, Arias M, Riano F, Paris S, Ramirez LA, Uribe O, Garcia LF, Vasquez G (2009) Response to ODN-CpG by B Cells from patients with systemic lupus erythematosus correlates with disease activity. Lupus 18:718–726. doi:10.1177/0961203309103098

Pathogen Manipulation of cIL-10 Signaling Pathways: Opportunities for Vaccine Development?

Meghan K. Eberhardt and Peter A. Barry

Abstract Interleukin-10 (IL-10) is a tightly regulated, pleiotropic cytokine that has profound effects on all facets of the immune system, eliciting cell-type-specific responses within cells expressing the IL-10 receptor (IL-10R). It is considered a master immune regulator, and imbalances in IL-10 expression, resulting from either inherent or infectious etiologies, have far reaching clinical ramifications. Regarding infectious diseases, there has been accumulating recognition that many pathogens, particularly those that establish lifelong persistence, share a commonality of their natural histories: manipulation of IL-10-mediated signaling pathways. Multiple viral, bacterial, protozoal, and fungal pathogens appear to have evolved mechanisms to co-opt normal immune functions, including those involving IL-10R-mediated signaling, and immune effector pathways away from immune-mediated protection toward environments of immune evasion, suppression, and tolerance. As a result, pathogens can persist for the life of the infected host, many of whom possess otherwise competent immune systems. Because of pathogenic avoidance of immune clearance, persistent infections can exact incalculable physical and financial costs, and represent some of the most vexing challenges for improvements in human health. Enormous benefits could be gained by the development of efficient prevention and/or therapeutic strategies that block primary infection, or clear the infection. There are now precedents that indicate that modalities focusing on pathogen-mediated manipulation of IL-10 signaling may have clinical benefit.

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Contents

1 Introduction

A central element of immunological homeostasis is the self-regulation of immune responses to ensure an orchestrated activation followed by temporally appropriate termination of effector functions and generation of immunological memory. Under ideal circumstance, this process leads to clearance of the pathogen or infected cells without the generation of immunopathological processes, such as the damaging effects brought on by a prolonged proinflammatory state. Mammals have evolved elaborate mechanisms to control the type, magnitude, and duration of immune activation and suppression, and the evolution of these normal immunological processes has ensured host survival in environments of microbial challenge that begin at birth. Conversely, innate and adaptive immunity present a high threshold for infectious pathogens to surmount in order to successfully complete their natural histories. As a result, pathogens have evolved strategies that counteract and exploit aspects of the immune system to enable them to infect, replicate, and be transmitted to the next immune competent host, thereby maintaining the microbial species. Simply put, pathogens have co-opted normal immune functions, which evolved to protect the host from infection and disease, to alter the pathogen–host balance. This struggle between host and pathogen has, undoubtedly, been ongoing since the first appearance of a primordial self-replicating cell, and has resulted in a genetic yin-yang in which host and pathogens have shaped each other's genome in response to selective pressures exerted by the other. Because of this Darwinian survival of the fittest, infectious diseases are one of the leading causes of death worldwide and represent the leading cause of death in low-income countries (WHO 2008).

Significant reductions in morbidity and mortality associated with infectious diseases will be gained by the development of strategies that (1) prevent primary infection, primarily through the development of pathogen-specific vaccines, and (2) therapeutically treat ongoing persistent infections. For the latter, development of therapeutic vaccines remains a "Holy Grail" for vaccinologists (Hilleman 2004), but the efforts are justified to treat the tens of millions to billions of people persistently infected with chronic pathogens, such as Human Immunodeficiency

virus (HIV), *Mycobacteria tuberculosis*, malaria (*Plasmodium spp.*), Hepatitis B and C Viruses, or Human Papillomavirus. While the pathogenic mechanisms differ for the specific disease, there is increasing evidence that many of the end pathologies arise by a common theme. Recent studies suggest that a major regulator of host immune responses, IL-10, is the salient target in infection/disease-mediated alteration of protective immune homeostasis. Current research has implicated imbalances in IL-10-mediated signaling in the establishment and maintenance of persistent infections. Targeting of the pathogenic components responsible for dysregulating IL-10-mediated signaling holds promise for vaccines to prevent primary infection and, potentially, for vaccines to treat established infections.

2 Infectious Exploitation of IL-10 Signaling

Since the seminal characterization in 1989 of the cytokine expressed from Th2 cell clones "that inhibits the synthesis of several cytokines by Th1 clones" (Fiorentino et al. 1989), almost 21,000 articles have been published discussing either "interleukin-10" or "cytokine synthesis inhibitory factor" (CSIF). An extrapolation from a brief survey of those papers published in 2012 (>1,500) leads to an estimate that 15–20 % of all papers about IL-10 are in conjunction with an infectious agent, particularly in relation to the immunological phenotype resulting from engagement of IL-10R. Many of these papers are based on human or non-human animal natural history studies, animal models, and/or in vitro studies using lymphoid cells. The diversity in the categories and sheer numbers of the infectious agents identified to date is quite remarkable. An incomplete summary in Table 1 lists examples from viruses, protozoa, pathogenic and commensal bacteria, helminths, and fungi. This summary complements and partially overlaps those examples noted in previous reviews by Duell et al. (Duell et al. 2012) (Table 2), Redpath et al. (Redpath et al. 2001) (Table 3), and Slobedman et al. (Slobedman et al. 2009) (Table 4). In all cases, except for those listed in Table 4, the ligand for IL-10R is cellular IL-10 (cIL-10) following activation of cIL-10 expression by some component(s) of the infectious agent. In contrast, certain representatives of the *Herpesvirales* Order and *Poxviridae* Family of viruses encode viral orthologs (vIL-10) of cIL-10 (Table 4), the subject of which has been previously reviewed in depth (Slobedman et al. 2009). While detailed studies are limited in some cases, all vIL-10 orthologs appear to activate effector functions in myeloid cells consistent with engagement of the host IL-10R. The convergent targeting of IL-10R-mediated signaling by evolutionarily divergent pathogens and commensal organisms emphasizes that IL-10 is a critical nexus in infectious agent–host interactions.

That viruses and microbes would target the IL-10 pathway should not be surprising since the initial sequencing of the murine CSIF cDNA clones revealed strong sequence identity with what was then an uncharacterized open reading frame (ORF) in Epstein-Barr Virus (EBV) (Moore et al. 1990). The authors highlighted the *sine*

Table 1 Microbial manipulation of cIL-10 signaling (references noted below)

Bacteria—pathogenic		
Aggregatibacter actinomycetemcomitans Saygili et al. (2012)		
Bordetella bronchiseptica Nagamatsu et al. (2009), Pilione and Harvill (2006), Skinner et al. (2005)	*B. parapertussis* Wolfe et al. (2010)	*B. pertussis* Coleman et al. (2012)
Borrelia hermsii Sindhava et al. (2010)	*Brucella abortus* Spera et al. (2006)	*Brudia pahangi* Gillan et al. (2005)
Chlamydia trachomatis Azenabor et al. (2011), Marks et al. (2010)	*Coxiella burnetii* Layez et al. (2012), Meghari et al. (2008)	*Francisella tularensis* Hunt et al. (2012), MacKenzie et al. (2013), Obermajer and Kalinski (2012)
Lysteria monocytogenes Horikawa et al. (2013)	*Mycobacterium tuberculosis* Cyktor et al. (2013a, b), Gerosa et al. (1999), Howard and Zwilling (1998), Mahajan et al.(2012), O'Leary et al. (2011), Redford et al. (2010, 2011), Remoli et al. (2011)	*Salmonella enterica* Wei et al. (2012)
Staphylococcus aureus Parcina et al. (2013), Wang et al. (2012)	*Yersinia pseudotuberculosis* McPhee et al. (2010, 2012), Nemeth and Straley (1997), Trulzsch et al. (2004)	*Yersinia spp.* Reithmeier-Rost et al. (2004), Sing et al. (2002)
Bacteria—commensal		
Bacteroides fragilis Cohen-Poradosu et al. (2011), Manuzak et al. (2012), Round and Mazmanian (2010)	*Bifidobacterium spp.* Jeon et al. (2012), Konieczna et al. (2012), Lopez et al. (2011)	*Clostridium butyricum* Gao et al. (2012)
Lactobacillus reuteri Levkovich et al. (2013), Livingston et al. (2010)		
Helminths and fungi		
Babesia microti Jeong et al. (2012)	*Candida albicans* Netea et al. (2004)	*Paracoccidioides brasiliensis* Felonato et al. (2012), Ferreira et al. (2010), Ulsenheimer et al. (2003)
Schistosoma mansoni Sun et al. (2012), van der Kleij et al. (2002)		

(continued)

Table 1 (continued)

Protozoa		
Leishmania donovani Mukherjee et al. (2012), Stager et al. (2006)	*L. major* Pagan et al. (2013)	*Plasmodium spp.* Abel et al. (2012), Bueno et al. (2010), Medina et al. (2011), Sarfo et al. (2011), Scholzen et al. (2009)
Trypanosoma cruzi Arce-Fonseca et al. (2011), Flores-Garcia et al. (2011)		
Viruses		
Cytomegalovirus HCMV or RhCMV, Avdic et al. (2011), Chang and Barry (2010), Chang et al. (2009, 2004), Jaworowski et al. (2009), Jenkins et al. (2008a, b), Kotenko et al. (2000), Lockridge et al. (2000), Nachtwey and Spencer (2008), Raftery et al. (2004), Spencer et al. (2008), Yamamoto-Tabata et al. (2004), MCMV, Campbell et al. (2008), Humphreys et al. (2007), Mandaric et al. (2012)	Dengue Ubol et al. (2010)	Epstein–Barr Virus Incrocci et al. (2013), Liu et al. (1997), Moore et al. (1990), Vieira et al. (1991)
Hepatitis B Virus Barboza et al. (2009)	Hepatitis C Virus Kaplan et al. (2008), Ulsenheimer et al. (2003)	HIV Elrefaei et al. (2006), Saitoh et al. (2012), Schols and De Clercq (1996), Siewe et al. (2013)
Lymphocytic Choriomeningitis Virus Brooks et al. (2008), Brooks et al. (2006a, b), Brooks et al. (2010), Ejrnaes et al. (2006), Richter et al. (2013), Wilson et al. (2012)		Orf Virus Fleming et al. (2007, 1997), Haig et al. (2002a, b), Wise et al. (2007)

Table 2 Microbial Manipulation of cIL-10 Signaling (Duell et al. 2012) (see references therein) (known activators of cIL-10 expression are indicated in parentheses)

Bacteria		
B. fragilis	*Borrelia burgdorferi*	*B. turicatae*
Burkholderia pseudomallei	*Chlamydia pneumonia* (MOMP)	*C. trachomatis*
Chlamydia muridarum	*Escherichia coli*	*Helicobacter pylori*
Legionella pneumophila	*Leptospira biflexa*	*L. interrogans*
Mycobacterium avium	*M. leprae*	*M. tuberculosis*
Neisseria gonorrhoeae	*N. meningitidis*	*Orientia tsutsugamushi*
Pseudomonas aeruginosa	*Rickettsia conorii*	*Salmonella Group B*
S. enterica (FliC)	*S. muenchen*	*S. aureus*
Streptococcus pneumonia	*S. pyogenes* (M protein, peptidoglycan)	*S. agalactiae*
Yersinia enterocolitica		
Helminths and fungi		
Heligmosomoides polygyrus	*P. brasiliensis*	*P. brasiliensis* vaccine
Schistosoma japonicum		
Protozoa		
Leishmania amazonensis	*L. braziliensis*	*L. chagasi*
L. donovani	*L. guyanensis*	*L. major* (Leif)
Plasmodium spp.	*Toxoplasma gondii*	*Trypanosoma brucei*
T. cruzi		
Viruses		
Chikungunya Virus	Crimean-Congo Hemorrhagic Fever Virus	Cytomegalovirus
Epstein–Barr Virus	Herpesvirus-6	HIV
Human Papilloma Virus	Tacaribe Virus	

FliC Flagellin, *Leif* Leishmania Eukaryotic initiation factor, *MOMP* Major outer membrane protein

Table 3 Microbial manipulation of cIL-10 signaling (Redpath et al. 2001) (see references therein)

Viruses		
Cytomegalovirus	Equine Herpesvirus-2	HIV
Respiratory Syncytial Virus	Rhinovirus	
Bacteria		
L. pneumophila	*Listeria monocytogenes*	*M. avium*
Mycobacterium leprae	*M. tuberculosis*	

qua non of infectious natural histories when they speculated that, "*an intriguing possibility is that EBV has exploited the biological activity of the product of a captured cytokine gene to manipulate the immune response against virally infected cells, thereby promoting survival of the virus*." Since the same rationale can be applied to those infectious agents that up-regulate cIL-10 expression in the absence of a microbe-encoded IL-10 ortholog, it stands to reason that blockade of microbial exploitation of IL-10/IL-10R signaling in naïve hosts could protect against primary infection and, potentially, could therapeutically alter the pathogen–host

Table 4 Virally-encoded cIL-10 Orthologs (vIL-10) (Slobedman et al. 2009) (see references therein, except as noted); sequence identities of vIL-10 to that of their host's cIL-10 are shown in parentheses)%)

Herpesviridae
Betaherpesvirinae: Human Cytomegalovirus (HCMV) (25 %), Rhesus CMV (RhCMV) (25 %), African green monkey CMV (27 %), Baboon CMV (29 %)
Gammaherpesvirinae: Epstein-Barr Virus (EBV) (90 %), Rhesus Lymphocryptovirus (84 %), Equine Herpesvirus 2 (84 %), Ovine Herpesvirus 2 (41 %)
Alloherpesviridae
Koi Herpesvirus (20 %)
European eel (50 %) (van Beurden et al. 2011)
Poxviridae
Chordopoxvirinae: Orf virus (91 %), Sheeppox virus (38 %), Goatpox virus (45 %), Lumpy skin disease virus (39 %)
Parapoxvirinae: Camel Pseudocowpoxvirus (69 %) (Nagarajan et al. 2013), Bovine papular stomatitis virus (85 %) (Nagarajan et al. 2013), Reindeer Parapoxvirus (83 %) (Nagarajan et al. 2013)

balance in those with ongoing infections. There are, at least, three general prevention/therapeutic approaches based on such an assumption: (1) prevention vaccines specifically targeting the pathogenic component(s) responsible for IL-10R-mediated signaling (i.e., targeting either the pathogenic factor inducing increased cIL-10 expression, or the vIL-10); (2) a live attenuated pathogen vaccine deleted of either the pathogenic factor inducing increased cIL-10 expression or the vIL-10; and (3) therapeutic modalities that restore protective IL-10 homeostasis. Such approaches are justified only if alteration of IL-10 signaling is truly a virulence factor for pathogen infection, replication, and dissemination.

3 Mechanisms of Microbial-Induced IL-10 Signaling

Multiple cell types express cIL-10 during the course of protective immunity, including macrophages (Mø), dendritic cells (DC), T cells, B cells, neutrophils, eosinophils, and mast cells, and most immune cell types express the cIL-10 receptor tetramer complex (IL-10R) endogenously or in an inducible manner. Examples abound in which the very same cell types that produce cIL-10 are exploited during infections to increase cIL-10 expression at inappropriate times and/or levels, eliciting downstream cIL-10-induced effector responses in cells expressing IL-10R (Tables 1, 2, 3, 4 and 5 and Fig. 1). Infection-stimulated increases can be found in both innate (Mø and DC) and adaptive cells (T and B), and the phenotypic effects of aberrant cIL-10 expression are dependent, in part, on the stage at which cIL-10 is expressed. During early stages of infection, the data present a model in which there is an infection-related dampening of innate immune functions and decreases in phagocytic activity, inflammation, antigen presentation in antigen-presenting cells (APC), and microbicidal functions. Moreover, since DC represent the

Table 5 Mechanisms of pathogenic induction of IL-10 signaling (see Table 1 for references)

Pathogen	Pathogenic mediator	Pathway	Phenotype
Bacteria (Pathogenic)			
Aggregatibacter actinomycetemcomitans	GroEL (heat-shock protein)		↑ cIL-10/IFNγ T-bet + $CD4^+$ cells
B. bronchiseptica	BopN	Translocates to nucleus,↓MAPK and MAPK kinase signaling pathways, alters nuclear trafficking of p50, p65	↓ IFNγ responses ↓ PMN infiltration
Bordetella parapertussis			↑ cIL-10/IFNγ ratio in T cells
Bordetella pertussis	CyaA/LPS or CpG complex	Modulates TLR4 activation	↓ DC maturation ↑ Treg
Borreliahermsii			↑ B cell cIL-10
B. abortus	PrpA		↑ B cell proliferation and cIL-10 expression
Brugia pahangi			↑ B cell cIL-10
C. trachomatis			↑ cIL-10/IFNγ ratio in T cells (Jurkat) ↑ cIL-10 in CD11c(+) CD11b(+) DC in lower genital tract
C. burnetii			↑ $FoxP3^+$ $CD4^+$ T cells
F. tularensis	*F. tularensis* Macrophage Supernatant (FTMøSN contains an unidentified protein > 10 kDa)	PGE2 induces FTMøSN that induces Mø IL-10 expression	↓ $CD4^+$ IFNγ responses ↑ suppressor DC ↑ Mϕ cIL-10 ↓ Mϕ Ag presentation
L. monocytogenes			↑ B cell cIL-10
M. tuberculosis		Modulation of PPARγ and Testicular Receptor 4	↑ immunosuppressive $CD8^+$ T cells expressing IL-10 ↓ Mϕ innate responses ↑ Mϕ cIL-10

(continued)

Table 5 (continued)

S. aureus	Lipoproteins	TLR2 activation on pDC and subsequent interactions with B cells	↑ B cell cIL-10
	Heat-killed S aureus (SAC)		↓ T cell IL-2 production; ↓ HLA-DR & CD86, ↑ IL-10 in Mø
		TLR2 activation	
Yersinia spp.	YopM;	Phosphorylates RSK1 and PFK2, altering their signaling to promote IL-10	↑ cIL-10 production by innate effector cells
	LcrV		
		TLR2 and CD14 activation	
Bacteria (commensal)	**Pathogenic mediator**	**Pathway**	**Phenotype**
B. fragilis	polysaccharide A (PSA)		↑ FoxP3$^+$ CD4$^+$ T cells
Bifidobacterium bifidum			↓ IL-12/IL-10 ratio
			↑ FoxP3$^+$ CD4$^+$ T cells
Bifidobacterium breve			↑ cIL-10 in colonic Tr1 CD4$^+$ cells
C. butyricum			↑ cIL-10 production
L. reuteri			↑ FoxP3$^+$ T cells
Helminth/Fungal	**Pathogenic mediator**	**Pathway**	**Phenotype**
B. microti			↑ CD1dhighCD5$^+$ Bregs
C. albicans		TLR2 activation	↓ candidacidal Mϕ
			↑ Treg survival, ↑ susceptibility to candida dissemination
Schistosoma mansoni	Schistosome-specific phosphatidylserine	TLR2 activation on DC	↑ Tregs, ↑ cIL-10
Protozoa	**Pathogenic mediator**	**Pathway**	**Phenotype**
L. donovani		TLR2/6 heterodimer activation	↑ cIL-10 production in Mϕ
			↑ cIL-10/IFNγ CD4$^+$ T cells
Leishmania major			↑ cIL-10/IFNγ CD4$^+$ T cells
T. cruzi	SSP4 (surface glycoprotein)		↑ cIL-10/IFNγ CD4$^+$ T cells

(continued)

Table 5 (continued)

Viruses	Pathogenic mediator	Pathway	Phenotype
Primate CMV	vIL-10 ortholog in CMVs from African green monkeys, baboons, humans (cmvIL-10, LA-cmvIL-10), and rhesus macaques (rhcmvIL-10) but not chimp CMV	IL-10R activation	↓ DC activation, maturation and Ag presentation
			Mϕ Ag presentation, ↑ Mϕ pro-phagocytic phenotype
			↓ B and T cell education
			↓ Antiviral activity
			↓ pro-inflammatory cytokines and chemokines
Murine CMV (MCMV)			↑ cIL-10 production in $CD4^+$
			↓ NK/DC cross-talk
			↓ $CD4^+$ IFNγ responses in salivary glands
EBV	IL-10 ortholog (ebvIL-10; BCRF1)	IL-10R activation	↑ B cell infectivity
	LMP2A		↑ cIL-10 production
Orf Virus (zoonotic disease)	IL-10 ortholog (orfIL-10)	IL-10R activation	↓ DC maturation and Ag presentation
			↓ pro-inflammatory cytokine production in Mϕ and monocytes
Dengue			↑ cIL-10 production in highly virulent strains
Hepatitis B			↑ cIL-10 expression in CD4 T cells
Hepatitis C			↓ IFNγ expression in $CD4^+$ T cells but maintains cIL-10 expression
HIV		↓ PMN NET formation	↑ Treg activity stimulates ↑cIL-10 production in monocytes
			↑ cIL-10 production in NK, T and B cells
			↓ HIV-specific CD4 T cell activation, ↓IFNγ and IL-2
			↑ CD209-dependent ↑cIL-10 production in DC
LCMV			↑ cIL-10 production
			↑ Imunoregulatory APC
			↑ T cell anergy
			↓ $CD4^+$ effector/memory responses

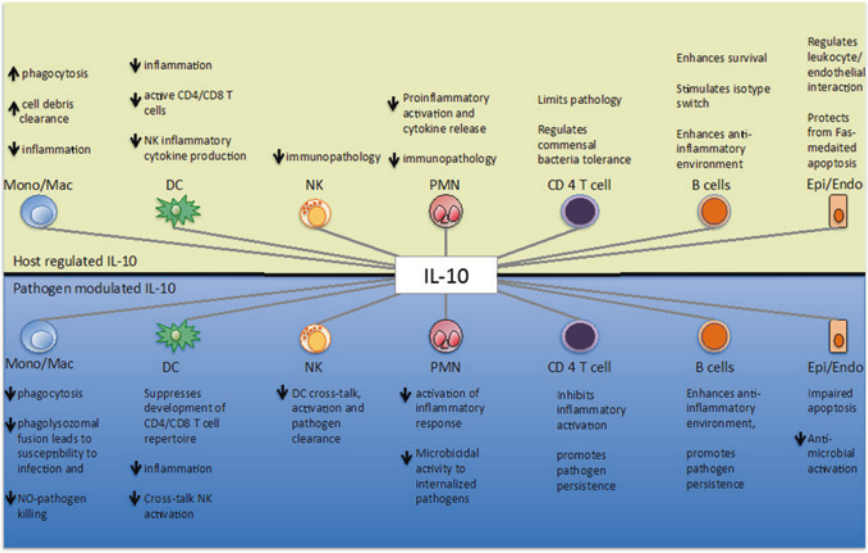

Fig. 1 Host-regulated versus pathogen-modulated phenotypes of IL-10 signaling

immunological link between innate and adaptive immune responses, perturbation of cytokine responses by early activation of IL-10 signaling in DC would likely alter the type of adaptive responses generated during priming of naïve T cells (discussed further below in relation to cytomegalovirus (CMV)-encoded vIL-10). During later stages of infection, increased cIL-10 expression is observed in regulatory B (Bregs) and T cells (FoxP3$^+$ CD4$^+$ Tregs). Notably, both cell types are associated with tolerance, and their increased functionality during infection provides a mechanistic basis for failure to clear persistent infections. Detailed reviews of these cells, including during healthy states, diseases, and infections, are presented elsewhere (Belkaid and Rouse 2005; Mauri and Bosma 2012; McGuirk and Mills 2002; Sanchez et al. 2012). A unifying theme of the infectious agents identified in Tables 1, 2, 3, 4, and 5 is the redirection or co-option of inherent protective immune responses.

For those infectious agents that do not encode a cIL-10 ortholog, the mechanisms of cIL-10 induction are incompletely defined. Some microbial inducers have been identified and include the following secreted or released proteins from bacteria, helminths, and protozoa: outer proteins that are part of Type III Secretion Systems of *Bordetella* and *Yersinia* (BopN, and YopM and LcrV, respectively) (McPhee et al. 2010; Nagamatsu et al. 2009); the adenylate cyclase virulence toxin (CyaA) of *B. pertussis* (Boyd et al. 2005; Hickey et al. 2008; Ross et al. 2004); the cercarial secreted Sj16 protein of *S. japonicum* (Sun et al. 2012); a phospholipid (phosphatidylserine) released from the membranes of *S. mansoni* (Furlong and Caulfield 1989; van der Kleij et al. 2002); the GPI-anchored SSP4 membrane glycoprotein of *T. cruzi* (Flores-Garcia et al. 2011); the anti-inflammatory

Polysaccharide A (PSA) of the commensal *B. fragilis* (Mazmanian et al. 2008; Round and Mazmanian 2010); and the GroEL heat-shock protein (AaGroEL) of *A. actinomycetemcomitans* (Saygili et al. 2012). While it is not known if AaGroEL is secreted, the GroEL of *H. pylori* has been demonstrated to be a secreted protein (Gonzalez-Lopez et al. 2013). Additional activators of cIL-10 expression have been previously reviewed (Duell et al. 2012), including the major outer membrane proteins of *C. pneumonia*; the major virulence protein of *Streptococcus pyogenes* (the M protein), peptidoglycan, a cell wall component of *S. aureus*; flagella of *Salmonella* and *Yersinia*; and the *Leishmania* Eukaryotic Initiation Factor.

Multiple signaling pathways have been identified that converge on induction of cIL-10 expression. Two pattern recognition receptors, toll-like receptor (TLR)-2 and TLR-4, which primarily recognize lipoproteins, lipopolysaccharides, and glycolipids found in Gram-positive and Gram-negative bacterial cell walls, are activated by bacteria, as well as some viruses, fungi, and helminths (Table 5). Activation of the two TLR is often in conjunction with additional synergistic factors (e.g., TLR-2 with lipid sensing nuclear receptors peroxisome proliferator-activated receptor γ (PPARγ) and testicular receptor 4 (TR4) in *M. tuberculosis* (*Mtb*) (Mahajan et al. 2012); TLR-2/CD14 in *Yersinia spp*. (Reithmeier-Rost et al. 2004; Sing et al. 2002), TLR-4 by CyaA and lipopolysaccharide of *B. pertussis* (Ross et al. 2004); and TLR-2/6 heterodimer stimulation by *L. donovani* (Mukherjee et al. 2012). Mechanistic studies of cIL-10 activation are predominated by evidence of TLR-2 activation in myeloid cells, as well as T cells, suggesting that TLR-2 signaling is frequently exploited for cIL-10 induction. In addition, prostaglandin E2 (PGE2) in combination with TLR agonists induces a regulatory phenotype shift in macrophages and DC, giving rise to production of high levels of cIL-10 (MacKenzie et al. 2013; Obermajer and Kalinski 2012). Studies in *F. tularensis*, *Mtb*, and *Salmonella typhi*, among others, reveal PGE2 activation and subsequent cIL-10 production in infected cell cultures (Hunt et al. 2012; Mahajan et al. 2012; Yusof et al. 1993). Studies have also found that macrophages infected with *F. tularensis* elicit cIL-10 production in bystander cells through the excretion into the supernatant of a 10 kDa protein, termed PGE2-inducible factor (Hunt et al. 2012).

Differences in the particular mechanisms activated by microbes can determine the phenotypic outcome of infection, as illustrated for the commensal bacterium *B. fragilis*. A distinguishing characteristic of commensals is a lack of immune activation and the maintenance of an anti-inflammatory environment. Ectopic infection with *B. fragilis* outside of its commensal niche within the gut, such as the peritoneal cavity, induces immune responses typical of a pathogenic infection, stimulating high levels of inflammation in regions outside of the gut (Cohen-Poradosu et al. 2011). Studies of commensal bacteria have revealed that $CD4^+$ T cells are induced to mature into a tolerant phenotype, most commonly thought to be cIL-10 expressing $FoxP3^+$ $CD25^+$ $CD4^+$ T cells, stimulated, in the case of *B. fragilis*, by PSA on the bacterial cell surface as well as other as yet unidentified antigens (Manuzak et al. 2012; Round and Mazmanian 2010). *B. fragilis* appears to stimulate a local $CD4^+$ T cell population that is constantly re-stimulated by bacterial interaction and maintains a cIL-10-rich, tolerant mucosal environment. Studies

displacing *B. fragilis* have revealed that while *B. fragilis* is still able to stimulate cIL-10 production outside of the colon, cIL-10 production is stimulated mainly in macrophages via a TLR2/MYD88-dependant pathway (Cohen-Poradosu et al. 2011). This pathway does not induce tolerance, and inflammation is not controlled. Commensal infection with *B. fragilis* also illustrates distinctions between local versus systemic induction of cIL-10, particularly for persistent pathogens.

Sites of pathogen persistence, such as the salivary glands for cytomegalovirus, the skin for *L. major*, and the lungs for *Mtb*, are consistent with induction of local immune privilege or tolerance to the infection, which is facilitated by the local development of cIL-10 expressing effector $CD4^+$ T cells (Campbell et al. 2008; Gerosa et al. 1999; Henson and Strano 1972; Howard and Zwilling 1998; Loughman and Hunstad 2012; Okwor and Uzonna 2008; Pagan et al. 2013). Infection of mice with murine CMV (MCMV) by multiple routes of inoculation leads to systemic dissemination and replication within multiple tissues. Local replication is rapidly controlled primarily by MCMV-specific $CD8^+$ T cells in all tissues except the salivary gland where high viral loads can persist (Reddehase et al. 2008). MCMV infection in the salivary glands is often unnoticed by the immune system, with local $CD8^+$ T cells having no effect on actively infected glandular epithelial cells while $CD4^+$ T cells showed unusually high cIL-10:IFNγ expression ratios (Campbell et al. 2008; Cavanaugh et al. 2003; Henson and Strano 1972; Humphreys et al. 2007; Jonjic et al. 1990). Tolerance in this specific niche has been attributed to local cIL-10 responses of $CD4^+$ T cells as abrogation of IL-10 signaling results in clearance of infected cells (Humphreys et al. 2007), similar to what has been reported for lymphocytic choriomeningitis virus (LCMV) (Brooks et al. 2008, 2006a, b, 2010) (discussed further below).

Following dermal inoculation, *L. major* causes cutaneous lesions that maintain a low-level persistent infection at the lesion site even after the lesion has healed due to the accumulation of $CD4^+$ $CD25^+$ T cells that suppress effector T cells from clearing the infection (Belkaid et al. 2002a, b). Similar to what was observed in MCMV studies, the main source of cIL-10 is derived from local effector $CD4^+$ T cell responses that are modulated to produce cIL-10 during initial antigen stimulation. A study by Pagan et al. (Pagan et al. 2013) investigated local versus systemic T cell populations in mice infected with *L. major* and boosted with peptides. Initial infection of mice stimulated Treg cell recruitment to the ear (site of inoculation) within 1–2 weeks of inoculation. However, that population was transient and quickly declined, being replaced by cIL-10/IFNγ producing $CD4^+$ T cells. Within 3–4 weeks post inoculation, mature effector cIL-10/IFN-γ CD4 $^+$ T cells became the predominant and permanent local source of cIL-10. This response was unique to the local immune system, with samples from the spleen (a region of high concentration of parasite-specific IFN-γ $CD4^+$ T cells) displaying extremely transient populations of cIL-10 expressing $CD4^+$ T cells. The authors conclude that antigen encounter at the site of infection drives long-term local cIL-10 production by effector $CD4^+$ T cells, allowing parasite persistence. Similar results were observed with *L. donovani* and *Mtb* in which the development of local specific

cIL-10/IFN-γ $CD4^+$ T cells correlated with the shift from acute to persistent infection (Howard and Zwilling 1998; Stager et al. 2006).

Based on the examples listed in Tables 1, 2, 3, and 4, a central issue is whether induction of cIL-10 is a virulence factor for pathogenic infections or a requisite step for commensal bacterial infections. Many studies are correlative in nature, relating the presence of an organism with changes in cIL-10 levels in cell populations, disease states, and/or animal models. Multiple studies, however, have used a variety of techniques to demonstrate that abrogation of cIL-10 induction and signaling attenuates infection and/or disease (summarized in Table 6). Most studies have used mouse models of human pathogens, taking advantage of mouse genetics to study infection of a pathogen in transgenic mouse strains (either gene knockouts or knockins) and the ability to modify the genomes of pathogens to delete specific ORF.

4 Induction of IL-10 Signaling as a Virulence Factor for Microbial Infection

cIL-10 (−/−) knockout strains of mice have been extensively used to compare the patterns of bacterial infections (*B. pertussis*, *B. bronchiseptica*, *B. parapertussis*, *C. trachomatis*, *Y. pseudotuberculosis*) in the presence and absence of functional cIL-10 signaling. In general, the absence of a functional cIL-10 resulted in decreased pathogen-mediated pathogenesis and/or colonization (Mahajan et al. 2012; McPhee et al. 2010, 2012; Mukherjee et al. 2012; Sing et al. 2002), and, for *C. trachomatis*, the absence of cIL-10 was associated with a notable increase in protective Th1 cells in the lower genital tract (Marks et al. 2010). Conversely, infection of a transgenic mouse strain that constitutively overexpresses cIL-10 with *C. burnetii* resulted in reduced anti-infectious competence of the mouse, compared to the parental non-transgenic mouse (Meghari et al. 2008). A recent study noted that pulmonary infection with *Mtb* of a mouse strain susceptible to chronic *Mtb* infection (CBA/J) was associated with an increase in immunosuppressive $CD8^+$ T cells ($PD\text{-}1^+$ $Tim\text{-}3^+$ $CD122^+$) expressing cIL-10 compared with *Mtb* infection in an *Mtb*-resistant strain (C57BL/6) (Cyktor et al. Cyktor et al. 2013a, b). The authors further noted that the expansion of this population of $CD8^+$ cells expressing an immunosuppressive phenotype was not dependent on cIL-10 as a similar effect of these cells was observed in a cIL-10 knockout strain of CBA/J. Other knockout mouse strains have also been used to delineate signaling pathways and partial characterization of the microbial inducer of cIL-10 (Hunt et al. 2012; Ross et al. 2004).

A complementary approach to the use of gene-manipulated mouse models to study microbial mechanisms of pathogenesis is use of site-directed mutations within the genomes of pathogenic organisms to delete microbial activators of cIL-10. Using this approach, studies have evaluated the pathogenic and/or immunological profile of gene-deleted variants compared to infection with the wild-type parental organism (Table 6). These studies include the *B. bronchiseptica* ΔBopN and Type Three Secretion System mutants, *B. abortus* ΔprpA, *B. fragilis* ΔPSA

Table 6 Modulation of IL-10 signaling as a virulence factor

Mouse transgenic/Gene knockout/Models or Mouse strains

- IL-10 overproducer mice → *C. burnetii* → ↓ anti-infectious competence of mouse (Meghari et al. 2008)
- TLR-4 K/O → *B. pertussis* → ↓ CyaA-mediated induction of IL-10 induction only in conjunction with LPS and functional TLR4 (Ross et al. 2004)
- WT *B. bronchiseptica* in IL-10 (−/−) → ↓ pathogenesis (Nagamatsu et al. 2009)
- WT *B. bronchiseptica* in IL-10 (−/−) → ↓ colonization (Skinner et al. 2005)
- WT *B. parapertussis* in IL-10 (−/−) → ↓ pathogenesis (Wolfe et al. 2010)
- WT *C. trachomatis* in IL-10 (−/−) → ↓ induction of IL-10$^+$ CD11c$^+$ CD11b$^+$ DC in lower genital tract (Marks et al. 2010)
- FTMøSN treatment of Mø from IL-10 (−/−) mice → comparable Mø IL-10 induction as with from WT mice (Hunt et al. 2012) *F. tularensis*
- *Mtb* infection in susceptible (CBA/J) or resistant (C57BL/6) strains → ↑ pulmonary immunosuppressive CD8$^+$ T cells (PD-1$^+$ Tim-3$^+$ CD122$^+$) expressing IL-10 in CBA/J mice (Cyktor et al. 2013a, b)
- Breg adoptive transfer from IL-10 (−/−) → ↓ colonization (Sindhava et al. 2010) *B. hermsii*
- WT *L. monocytogenes* in CD19 (−/−) → ↓ IL-10 → ↓ colonization (Horikawa et al. 2013)
- WT *Y. enterocolitica* in IL-10 (−/−) → ↓ colonization; ↓ pathogenesis; ↑ survival (Sing et al. 2002)
- TLR-2 K/O → *C. albicans* → severely impaired IL-10 production along with a 50 % decrease in Tregs; ↓ colonization (Netea et al. 2004)
- LCMV clone 13 in IL-10 (−/−) → ↑ viral clearance (Brooks 2006a, b)
- MCMV in IL-10 (−/−) → ↓ viral burden in salivary glands but ↑ burden in liver leading to animal mortality; ↑ immunopathology (Campbell et al. 2008; Mandaric et al. 2012)

Neutralization of IL-10 signaling

- WT *B. pahangi* infection with αIL-10R Ab block → ↑ antigen-specific CD4$^+$ T cell proliferation (Gillan et al. 2005)
- LCMV Clone 13 infection with αIL-10R Ab block → ↑ viral clearance; ↑ anti-viral T cell responses (Brooks et al. 2006a, b; Ejrnaes et al. 2006)
- Murine CMV infection with αIL-10R Ab block → ↑ viral clearance in salivary glands; ↑ anti-viral T cell responses (Humphreys et al. 2007)

Depletion of Tregs/Bregs

- αCD25 → ↓ Tregs → ↓ Disease severity of *P braziliensis*; Depletion of Tregs is "beneficial to the progressive forms of paracoccidioimycosis" in mice (Felonato et al. 2012)
- αCD25 → ↓ Tregs → "Depletion of CD25 + cells significantly reduced the rate of bacterial clearance following *B. pertussis* challenge, but only in IL-10-defective (IL-10 −/−) mice" (Coleman et al. 2012)
- αCD25 → ↓ Tregs → 10-fold ↓ in fungal outgrowth from Kidneys (Netea et al. 2004) *C. albicans*
- αCD20 or αCD22 → ↓ B cells → ↓ *L. monocytogenes* colonization → Breg adoptive transfer after B cell depletion → ↑ colonization (Horikawa et al. 2013)
- IL-10$^+$ Bregs adoptively transferred during *B. microti* infection → ↑ parasitemia (Jeong et al. 2012)
- *B. microti* infection of B-cell deficient μMT mice → ↓ IL-10; slower and less severe parasitemia development; ↑ Treg formation (Jeong et al. 2012)

Phenotypic recapitulation with purified subunit

- Purified AaGroEL → ↑ IL-10$^+$/IFN-γ CD4$^+$ cells → ↑ Induction of immune regulatory phenotype (Saygili et al. 2012)
- Purified BaGroEL → Vx augmented PA-mediated protective efficacy (Sinha and Bhatnagar 2013) *Bacillus anthracis*

(continued)

Table 6 (continued)

- Purified CyaA/LPS → effect of both noted only in TLR-4-defective mice (C3HeJ) (Ross et al. 2004) *B. pertussis*
- Recombinant LcrV → TLR2/CD14 signaling → ↑ IL-10 (Sing et al. 2002) *Y. enterocolitica*
- Isolated schistosomal PS → ↑ IL-10 producing T cells (van der Kleij et al. 2002) *Schistosome mansoni*
- Recombinant SSP4 → ↑ IL-10$^+$/IFN-γ CD4$^+$ cells → ↑ Induction of immune regulatory phenotype (Flores-Garcia et al. 2011) *T. cruzi*
- Recombinant cmvIL-10 → ↑ immunosuppressive phenotypes (Lockridge et al. 2000) cytomegalovirus
- Recombinant orfIL-10 → ↑ immunosuppressive phenotypes (Wise et al. 2007) Orf virus
- Recombinant ebvIL-10 → ↑ immunosuppressive phenotypes (Vieira et al. 1991) Epstein-Barr virus
- LMP2A-Tg expressing B cells → ↑ IL-10 production twofold in mitogen stimulated B cells (Incrocci et al. 2013) Epstein-Barr virus

Vaccination

- Purified BaGroEL → Vx augmented PA-mediated protective efficacy (Sinha and Bhatnagar 2013) *Bacillus anthracis*
- ΔBopN *B. bronchiseptica* → ↓ pathogenesis compared to WT (Nagamatsu et al. 2009)
- TcSSP4 cDNA → ↓ parasitemia; ↓ pathogenesis (Arce-Fonseca et al. 2011) *T. cruzi*
- Mutant rhcmvIL-10 DNA/protein → ↓ viral shedding (publication pending) Cytomegalovirus

Gene deletion

- ΔBopN → ↓ IL-10 induction in vitro; → ↓ IL-10 induction and ↑ survival of infected mice (Nagamatsu et al. 2009) *B. bronchiseptica*
- TTSS-mutant *B. bronchiseptica* → ↓ colonization and ↓ IL-10 (Skinner et al. 2005)
- ΔprpA *B. abortus* → ↑ Splenocyte responsiveness in infected mice (Spera et al. 2006)
- vorfIL-10ko → ↓clinical viral loads; → ↓pathology in infected sheep (Fleming et al. 2007) orf virus
- YopM mutant *Yersinia peudotuberculosis* → ↓ IL-10, ↓ colonization and ↑ survival of infected mice → growth advantage of WT YopM was abrogated in IL-10 (−/−) mice(McPhee et al. 2010; McPhee et al. 2012)
- ΔrhcmvIL-10 → ↓ viral burden at site of inoculation; ↑ specificity and magnitude of early and adaptive immune responses (Chang and Barry 2010)

genes, *B. pertussis* ΔCyaA, and *Y. pseudotuberculosis* ΔYopM (Gross et al. 1992; McPhee et al. 2010, 2012; Nagamatsu et al. 2009; Round and Mazmanian 2010; Skinner et al. 2005; Spera et al. 2006). Notable features following infection of wild-type mice with deleted variants included decreased bacterial colonization and increased survival of infected mice, compared to infection with the wild-type bacteria (McPhee et al. 2010, 2012; Nagamatsu et al. 2009; Skinner et al. 2005), coincident with decreased cIL-10 induction (Nagamatsu et al. 2009; Round and Mazmanian 2010; Skinner et al. 2005), decreased cIL-10$^+$ Tregs (Round and Mazmanian 2010), and increased splenocyte responsiveness (Spera et al. 2006). In sum, the results of these studies are all consistent with the notion that induction of cIL-10 is requisite for the full pathogenic phenotype, a conclusion that also holds for viruses encoding cIL-10 orthologs (vIL-10). In particular, deletion of the vIL-10 genes from Orf virus (ORFV), rhesus CMV (RhCMV), and human CMV

(HCMV) markedly alters the profiles of infection compared to those observed with the parental, vIL-10-expressing viral variants.

ORFV, which normally infects sheep and goats but can zoonotically infect humans (CDC 2012), encodes a transduced sheep cIL-10 (orfIL-10) gene that is 80 % identical to sheep cIL-10 and 67 % identical to human cIL-10 (Fleming et al. 1997). Unlike sheep cIL-10, the orfIL-10 gene contains no introns. Most non-conserved coding changes are concentrated near the N-terminus of the protein, yet retains the tertiary structure of sheep cIL-10, including 6 alpha-helical segments and 6 core cysteine residues (Wise et al. 2007). orfIL-10 retains similar function as cIL-10, limiting pro-inflammatory cytokine production and inhibiting DC maturation, although it lacks certain function of cIL-10, including the ability to inhibit monocyte proliferation (Chan et al. 2006; Haig et al. 2002a, b; Wise et al. 2007; Yoon et al. 2006). Structural analysis found that orfIL-10 lacks 2 of the 3 amino acids essential for IL-10R2 activation of the STAT3 pathway in humans, possibly accounting for deficits in function (Gesser et al. 1997; Wise et al. 2007; Yoon et al. 2006). The ORFV genome was engineered to delete the orfIL-10 gene (creating vorfIL-10ko), and lambs were inoculated with the wild-type parental ORFV and a variant in which the deleted orfIL-10 gene was repaired (Fleming et al. 2007). vorfIL10ko was distinguished by a 100-1,000-fold reduced clinical severity, compared to lesions induced by the parental and repaired viruses, and the authors concluded that orfIL-10 is a virulence factor for ORFV. The authors speculated that one of the functions of orfIL-10 could be the suppression of innate responses at the site of inoculation. ORFV, like human CMV (HCMV) and RhCMV, can reinfect immune host. Another clinical phenotype of vorfIL-10ko was reduced clinical severity following reinfection, compared to reinfection with the wild-type parental ORFV (Fleming et al. 2007). This leads to the possibility that one element in preventing HCMV reinfection, the clinically relevant issue during pregnancy due to congenital HCMV infection, might be boosting immune responses to the HCMV-encoded IL-10 (discussed further below).

Primate CMVs encode and express vIL-10 orthologs of their host's cIL-10 (Table 4) (Kotenko et al. 2000; Lockridge et al. 2000; Slobedman et al. 2009) with the exception of chimpanzee CMV, which does not (Davison et al. 2003). Assessments of whether the vIL-10 genes encoded by RhCMV (rhcmvIL-10) and HCMV (cmvIL-10) alter the infectious process have been reported. In an in vivo study involving rhesus macaques, naïve animals were inoculated with low doses of either the 68-1 strain of RhCMV, expressing rhcmvIL-10, or with a variant of RhCMV (ΔUL111A) lacking a functional rhcmvIL-10 protein (Chang and Barry 2010). Compared to naive monkeys inoculated with the parental rhcmvIL-10-expressing variant, animals inoculated with ΔUL111A were noted for prominent changes in both acute and long-term immune responses. Acute changes included increased inflammatory infiltrates at the site of subcutaneous inoculation as well as qualitative changes in the nature of the cellular infiltrate at the inoculation site. In addition, increases in dendritic cells in the draining lymph nodes were observed in the ΔUL111A-inoculated animals, and there were increased numbers of RhCMV-specific T cells secreting IFN-γ, TNF-α,

and IL-2 in the axillary lymph node of animals inoculated with ΔUL111A. Long-term changes included increases in T and B cell responses to viral antigens, compared to animals inoculated with 68-1 RhCMV. The results of RhCMV reinforce the interpretation of the results observed with deletion of orfIL-10 (Fleming et al. 1997) in that rhcmvIL-10 acts as a virulence factor by subverting the induction of protective innate and adaptive immune responses (Chang and Barry 2010).

HCMV infection is strictly species-specific, and, thus, there are no available animal models to test directly what role the HCMV-encoded vIL-10 (cmvIL-10) exerts during infection in vivo. However, critical insights have been gained using clinically relevant cell types in vitro. Long-term HCMV infection in humans is noted for the presence of transcriptionally repressed, non-replicating HCMV genomes within circulating $CD34^+$ myeloid progenitor cells, and fully productive viral gene expression can be induced upon activated differentiation of the cells into myeloid DC and macrophages (Soderberg-Naucler et al. 1997, 2001; Taylor-Weideman et al. 1991; Zhuravskaya et al. 1997). The establishment of latency is also observed following HCMV infection of purified myeloid progenitor cells in vitro, although the state of transcription of the infected cells is determined in large part by expression of cmvIL-10. Avdic et al. demonstrated that infection with cmvIL-10-expressing viruses inhibited the differentiation of latently infected cells into myeloid DC, compared to engineered HCMV variants in which cmvIL-10 had been deleted (Avdic et al. 2011). The authors speculated that the function of cmvIL-10 was to suppress the infected host's ability to clear reservoirs of infected cells.

Taken together, studies of bacterial and viral manipulation of IL-10 signaling provide compelling support for the argument that activation of cIL-10 expression or engagement of IL-10R with vIL-10 confers greater fitness for microbial natural history in immune competent hosts. Acceptance of this premise logically leads to the idea that targeted disruption of microbial exploitation of IL-10 signaling could lead to clinically relevant options for preventing primary infection or for clearing persistent infectious reservoirs. There are now numerous animal model precedents that the establishment and/or restoration of protective IL-10 signaling homeostasis can generate markedly reduced infectious burdens within the infected host. These include (1) targeted depletion of cIL-10-expressing cells, (2) disruption of IL-10 signaling, (3) and vaccine targeting of "microbial triggers" (Duell et al. 2012) of IL-10 signaling.

5 Enhanced Pathogen Clearance Following Depletion of Tregs or Bregs

There are numerous publications cited in Table 5 demonstrating increased levels of regulatory T and B cells (Treg and Breg, respectively) following microbial infection. Depletion of these cells in infected mice have demonstrated that (1) induction of these cells are essential for maintenance of high pathogens loads

and pathogenesis, and (2) depletion of these cells in established infections can shift the pathogen–host balance in favor of the host (Table 6). Anti-CD25 monoclonal antibodies (MAb) were passively infused into mice previously infected with *P. brasiliensis* (Felonato et al. 2012) to deplete $CD4^+Foxp3^+CD25^+$ Treg cells. This study included the use of two strains of mice that exhibited differential susceptibility to *P braziliensis* pathogenesis. As described by the authors, treated mice were "*rescued* [from] *progressive disease and precocious mortality*." The clinical improvement did not result from an immune-mediated induction of sterilizing immunity. Instead, reduced tissue pathology resulted from enhanced control of fungal replication due to increased effector T cell functions. The authors concluded that, "*the manipulation of Treg cells may prove to be a potential new therapy against this systemic mycosis*." However, CD25 is a marker for other activated $CD4^+$ T cell subpopulations, as recognized by the authors of this paper, and depletion of $CD25^+$ cells with a CD25-specific MAb may not be justified in other types of infections. In fact, evidence has been presented that *B. pertussis* induces the accumulation of cIL-10 producing $CD4^+Foxp3^+$CD25-negative Treg cells in the lungs of infected mice (Coleman et al. 2012).

Dietze et al. used a novel transgenic mouse model to selectively deplete $FoxP3^+$ Treg cells chronically infected with Friend retrovirus (FV) (Dietze et al. 2011). The DEREG (depletion of regulatory T cell) mouse strain expresses the diphtheria toxin (DT) receptor under the control of the Treg-specific Foxp3 promoter, and treatment of mice with DT leads to the complete but temporary loss of $FoxP3^+$ Treg cells. Especially important for translation to chronic infections in humans, the authors found that a short-term course of Treg depletion was followed by a sustained reduction in FV loads, although FV was not completely eliminated. However, this study and others highlight that changes in the immune microenvironment can stimulate protective immune responses, either through the de novo acquisition of pathogen-specific immune responses or by the expansion of preexisting memory responses. The authors of this study also noted that therapeutic treatments that worked in other infectious mouse models did not work against FV and suggested that therapies will likely need to be tailored to the specificities of the pathogen–host interactions.

L. monocytogenes infection in mice induces expansion of a rare population of regulatory B (B10) cells that secrete cIL-10, impairing bacterial clearance (Horikawa et al. 2013). B10 cells were depleted 1-day after acute *L. monocytogenes* infection by treatment of mice with an anti-CD22 MAb, which is expressed on all mature B lymphocytes. CD22-treatment of infected mice was associated with increased macrophage effector functions (increased IFN-γ and TNF-α production, and increased phagocytosis in ex vivo assays) and decreased bacterial loads. The authors emphasized the role of early bacterial manipulation of B10 cells to shape the "magnitude and duration" of the innate and adaptive responses to bacterial infection, leading to the implication that prevention of pathogenic alteration of aberrant cIL-10 expression at the earliest stage of infection would enhance both innate and adaptive immunity.

6 Therapeutic Neutralization of IL-10 Signaling

The downstream effects of IL-10 signaling are operative only after engagement of IL-10R by the cIL-10 or vIL-10 ligands, and, therefore, abrogation of IL-10/IL-10R interaction would block downstream transduction pathways. This concept has been elegantly applied to address how blockade of IL-10 signaling alters the pathogen–host balance in mice infected with either LCMV or MCMV. Mice infected with either virus were treated with passive transfer of an IL-10R-specific MAb that blocked or neutralized engagement of the receptor with ligand. Infection of mice with the clone 13 variant of the LCMV Armstrong strain results in progressive T cell impairment and establishment of a persistent infection (Brooks et al. 2008; Ejrnaes et al. 2006). Treatment of infected mice with IL-10R MAb during either the acute or persistent phases of infection results in restoration of T cell effector functions, reduced pathogenesis (weight loss), and dramatic reductions in LCMV viral loads. Both of these studies (Brooks et al. 2006a, b; Ejrnaes et al. 2006) highlight the plasticity of the host immune responses to ongoing viral infection once the immune suppressing catalyst (i.e., engagement of IL-10R) is blocked. Similar conclusions have been reached in a study of MCMV infection in mice. MCMV replicates acutely in multiple tissues following primary infection, but adaptive T cells largely clear tissues of actively infected cells, except in the salivary glands (SG). MCMV persists and replicates for an extended period of time in the SG, and this particular site is considered an "immune privileged" site for MCMV persistence (Campbell et al. 2008). The state of immune privilege or tolerance to MCMV in the SG is due to accumulation of cIL-10-expressing $CD4^+$ cells (Humphreys et al. 2007). As with LCMV infection, treatment of MCMV-infected mice with IL-10R MAb resulted in an increase in $CD4^+$ T cells in the SG secreting IFN-γ and a significant reduction in MCMV loads in the SG.

The use of IL-10R blockade as a therapeutic treatment for persistent pathogen infections is bolstered by a study in mice characterizing loss of T cell function during persistent LCMV antigenic stimulation (Brooks et al. 2006a, b). Of particular note, the authors concluded that, "*the functional programming of T cell effector and memory responses* in vivo *in mice is not hardwired during priming but is alterable and responsive to continuous instruction from the antigenic environment*." Given this ability of dysfunctional antigen-specific T cells to be "instructed" back to immune competence, it might seem reasonable that protective immunity could be restored by appropriate exposure to protective antigens during chronic infection. One study demonstrated that immune therapeutic strategies may not work in the presence of an immune suppressive environment engendered by continued activation of IL-10 signaling (Brooks et al. 2008). Administration of a DNA vaccine against the LCMV glycoprotein to LCMV-infected mice together with the neutralizing MAb to IL-10R significantly increased T cell responsiveness and decreased LCMV loads compared to mice treated with the DNA vaccine alone, or mice treated with the IL-10R MAb alone. In other words, T cells regained functional competence following antigenic stimulation only in the

absence of IL-10 signaling. This work has been extended through analysis of the effects of acute LCMV infection in $CD4^+$ and $CD8^+$ T cell responses. During acute LCMV infection, $CD4^+$ cells, but not $CD8^+$ cells, are specifically suppressed. However, this LCMV-mediated suppressive phenotype is abrogated when IL-10R is administered immediately prior to infection and again 5-days after infection. The authors of this study proposed the inclusion of cIL-10 blockade as a vaccine adjuvant to boost protective immune responses, particularly those involving $CD4^+$ (Brooks et al. 2008).

Other studies have explored the validity of specifically vaccinating against microbial-encoded activators of IL-10 signaling to prevent primary infection and/or pathogenesis, in contrast to the therapeutic blockade strategies of IL-10R-mediated signaling described above for acute or established LCMV and MCMV infections. Mixed results have been observed in terms of stimulating protective efficacy, but there are also encouraging results that warrant optimism that vaccination against microbial activators of IL-10 signal is a potential vaccine option.

7 Preventive Vaccination Against Microbial Activators of IL-10 Signaling

There is a relative paucity of studies addressing vaccine targeting of microbial antigens that modulate host immune responses. Of those addressing microbial activation of IL-10 signaling, a spectrum of outcomes have been observed following vaccination and challenge, including (1) failure to induce protective immunity, (2) induction of protective immunity in the context of the antigen delivery method, (3) increased protective immunity following co-vaccination against other virulence factors, and (4) induction of protective immunity. For this discussion, the term "protective immunity" is broadly defined as vaccine-induced immune responses that attenuate microbial infection and/or pathogenesis. Despite the limited number of studies (summarized below), the results offer a paradigm for moving this field forward.

Absence of protective immunity Yersinia species (*pestis*, *enterocolitica, and pseudotuberculosis*) express multiple plasmid-encoded proteins that facilitate bacterial replication and dissemination in the infected mammalian host, and because they act as virulence factors, these proteins have been evaluated as vaccine antigens (Williamson 2012). The *Y. pestis* YopM protein was purified to homogeneity and used to immunize mice in complete Freund's adjuvant by the intraperitoneal route. Despite the induction of high binding antibody titers, immunization against YopM did not elicit any protective efficacy against lethal *Y. pestis* challenge (Nemeth and Straley 1997). However, the authors of this study did make note of the fact that neutralizing antibodies against YopM function were not measured. They speculated that, "*It is possible that YopM is not neutralized by antibody because its main target in the host is intracellular*," perhaps, because "*YopM*

might enter host cells by the well-established vectorial translocation mechanism," thereby protecting YopM from antibody-mediated neutralization. In support of this idea, it has been reported that purified YopM can undergo "autonomous translocation" into the interior of mammalian cells (Ruter et al. 2010). Vaccination/challenge results comparable to those observed for YopM were noted for immunization with the PrpA virulence factor of *B. abortus* (Lowry et al. 2011), a protein that acts as a B cell mitogen and stimulates expression of cIL-10 (Spera et al. 2006). Despite eliciting high binding antibody titers, mice immunized with wild-type PrpA were not protected from *B. abortus* challenge. As with the YopM study, only binding antibody titers were measured, and it is unknown whether neutralizing antibodies were generated. The mechanism by which PrpA activates cIL-10 expression and stimulates B cell replication are not resolved, but the immunization with fully functional proteins that can undergo "autonomous translocation" as described for YopM (Ruter et al. 2010), may facilitate protein entry into cells in ways that preclude the ability for antibody neutralization. There is precedent that the ways in which antigens are presented to cells may influence the generation of protective immune responses.

Protection in the context of antigen delivery A single immunization of mice with the amastigote-specific surface protein (SSP4) of *T. cruzi* has been reported to increase both parasite loads in the blood and lethality following challenge with *T. cruzi* trypomastigotes (Arce-Fonseca et al. 2011). While the mechanism for increased pathogenesis following SSP4 immunization are not known, it is likely that interaction of functional SSP4 protein with susceptible cells may skew the immune system in ways that facilitate parasite replication and pathogenesis. Another study demonstrated that three immunizations with purified SSP4 stimulates the generation of $CD4^+$ T cells expressing both cIL-10 and IFN-γ, two cytokines associated with *T. cruzi* pathogenesis during Chagas disease (Flores-Garcia et al. 2011). In marked contrast to the increased parasite pathogenesis following challenge of mice immunized with SSP4 protein, intramuscular immunization of mice with a plasmid expression vector for SSP4 resulted in significant reductions in parasitemia and long-term pathogenic outcomes (Arce-Fonseca et al. 2011). As noted by the authors of this paper, "*the antigen encoded by plasmid DNA is processed in the cytoplasm by transfected cells and presented to elicit an immune response*." Together, these results suggest that vaccine targeting of the microbial activators of cIL-10 should use engineered variant forms of the protein that lack immune modulating function but retain immunogenicity in antigen-presenting cells.

Increased protection against other virulence factors Pathogens have, undoubtedly, evolved multiple overlapping and redundant functions to infect and replicate within immune competent hosts. Accordingly, it is unlikely that single-antigen targeting of those pathogen-encoded mediators of cIL-10 induction can stimulate complete protection against pathogen challenge. Instead, establishment of clinically relevant levels of protective immunity will probably require immunization with additional antigens. Support for this idea comes from a paper investigating the protective efficacy of the GroEL protein of *Bacillus anthracis* (Sinha

and Bhatnagar 2013), a strong immunogen in *B. anthracis*-infected mice (Chitlaru et al. 2007). Although there have been no reports to date of cIL-10 induction by *B. anthracis* GroEL, a recent study reported that human PBMC exposed to GroEL of *A. actinomycetemcomitans* differentiate into IFN-γ/IL-10 double-positive T-bet^{+} cells, consistent with an immune regulatory phenotype (Saygili et al. 2012). Current licensed vaccines for *B. anthracis* induce antibodies to the Protective Antigen (PA), the *anthracis*-encoded protein essential for binding of the bacterial Edema and Lethal Toxins to the infected host's cell membranes (Sinha and Bhatnagar 2013). Notably, both toxins skew chemokine and cytokine expression, including IL-10, IL-12, and TNF-α, of human DC (Cleret-Buhot et al. 2012). Immunization of mice with recombinant *B. anthracis* GroEL did not reduce mortality following challenge of immunized mice with *B. anthracis* spores, although mean time to death was significantly increased, compared to controls (Sinha and Bhatnagar 2013). However, combined immunization with GroEL and PA significantly increased protection against lethal spore challenge above that observed with PA immunization alone. The authors propose an important modification for *B. anthracis* vaccination: "*it can be concluded that the immune response generated by GroEL when combined with that of PA could be an effective candidate for suppressing infection, as well as pathogenesis and virulence*."

Induction of Protective Immunity Multivalent vaccines, as suggested above for *B. anthracis*, may not always be required for some pathogens. The CyaA toxin of *B. pertussis* is expressed as an inactive protoxin that requires the CyaC protein for conversion into an active form, and CyaA is highly immunogenic in *B. pertussis* in infected humans (Betsou et al. 1993; Farfel et al. 1990; Hormozi et al. 1999). Immunization of mice with the active form of CyaA stimulates high titers of binding antibodies (neutralizing titers were not evaluated) that significantly protects against intranasal challenge as measured by reductions in pathogenic outcomes (weight loss) and bacterial loads (Hormozi et al. 1999). Similarly, immunization with the inactive form of CyaA stimulated anti-CyaA IgG also conferred protective efficacy. Whereas the development of protective immune responses to SSP4 may be dependent on the intracellular location of SSP4 (described above), immunization with CyaA appears to efficiently target epitopes into the MHC class I and class II pathways. In fact, the CyaA protein has been engineered to express heterologous epitopes because the N-terminus of CyaA, which contains the catalytic domain, efficiently directs the protein into the cytosol (Schlecht et al. 2004).

An alternative approach to immunization with recombinant subunit vaccines is the use of attenuated vectors deleted of virulence factors. Such an approach has been used to immunize against pathogenic *B. bronchiseptica* challenge. Nagamatsu et al. engineered *B. bronchiseptica* to delete the BopN virulence factor (ΔBopN) (Nagamatsu et al. 2009). In addition to demonstrating that BopN acts as a virulence factor through the induction of cIL-10, the authors of this study demonstrated that preinfection of mice with *B. bronchiseptica* ΔBopN protected mice against lethal challenge infection with wild-type *B. bronchiseptica*. A critical insight into the mechanism of protection conferred by preinfection with ΔBopN was the observation that the pattern of cytokine induction after

wild-type *B. bronchiseptica* challenge was distinctly different from that observed in *B. bronchiseptica* infection of naïve mice. IFN-γ expression increased and cIL-10 expression decreased following challenge of vaccinated animals, the opposite phenotype of what was observed following *B. bronchiseptica* infection in unimmunized mice. The results of this study emphasize the importance of the cytokine milieu influencing the quality of the immune response between protective and subprotective anti-pathogen immunity. As noted by the authors, "*these results confirm that the up-regulation of IL-10 by the BopN effector, resulting in the suppression of IFN-γ signaling, is a significant stealth strategy by which Bordetella evades the host immune system*" (Nagamatsu et al. 2009). This is likely to be especially critical for pathogens that establish persistent infections where the premature exposure of antigen-presenting cells to either cIL-10 or vIL-10 may skew the priming of adaptive immune responses in ways that facilitate a lifelong homeostasis between pathogen and host. Support for this comes from a recent study addressing the protective immunity conferred against the vIL-10 encoded by rhesus CMV (RhCMV) (Eberhardt et al. 2013).

8 Vaccination Against vIL-10

The potential benefits of therapeutic interventions to change the pathogen–host balance during persistent and/or pathogenic infections through depletion of specific cIL-10-producing lymphoid subpopulations or neutralizations of IL-10R (described above) may be counterbalanced by the increased risks of proinflammatory-mediated immunopathogenic outcomes in the absence of cIL-10 suppression. Prevention vaccines targeting microbial-encoded activators of IL-10 signaling, as just described, obviously avoid this potentiality. Viral orthologs of cIL-10 (Table 4) offer opportunities for significantly disrupting viral natural histories by targeting them by vaccination due to their role as virulence factors for infection (described above). However, there are elements of caution in adopting this approach for all viral orthologs due to the potential to stimulate cross-reactive immune responses to cIL-10. The basis for this issue is because the viral orthologs are evolutionary remnants of ancient transduction events whereby the host cIL-10 gene was incorporated into the viral genome and expressed in the context of viral infection (Slobedman et al. 2009). Depending on the extent of genetic drift of the vIL-10 from the cIL-10 of its host, there may still be extensive sequence identity precluding the simple design of vIL-10-specific vaccines. The issue is best illustrated for the vIL-10 proteins encoded by HCMV and RhCMV (cmvIL-10 and rhcmvIL-10, respectively).

Herpesviridae, including CMV, are ancient viruses that evolved from a progenitor more the 200 M years ago (McGeoch et al. 1995). As might be expected for a virus with ancient origins, a large portion of CMV ORF is dispensable for replication in vitro in fibroblasts, and epithelial and endothelial cells. More than half of the open reading frames (ORF) of the HCMV and RhCMV genomes can be

deleted without impairing replication in fibroblasts (Dunn et al. 2003; Lilja et al. 2008; Yu et al. 2003). The ORFs that can be deleted without affecting replication in fibroblasts encode functions that modulate host innate and adaptive immune responses through the disruption of antigen presentation, and alteration of cell trafficking, signaling, activation, and viability. Following the evolutionary divergence of primates and rodents, a progenitor primate CMV transduced the cIL-10 gene of its progenitor primate host. The viral IL-10 gene is still extant within HCMV, RhCMV, and other monkey CMVs (Lockridge et al. 2000), although it was apparently deleted during the evolution of chimp CMV (Davison et al. 2003). As each primate CMV co-speciated with its host, the viral IL-10 genes underwent extreme genetic drift from the cIL-10 gene of their host such that the viral IL-10 proteins share only 25–27 % identity with their host's cIL-10 (Lockridge et al. 2000). The extent of genetic drift in the viral orthologs is highlighted by the facts that (i) primate cIL-10 proteins share >95 % identity, and (ii) the viral IL-10 orthologs are as divergent from each other as they are from the cIL-10 of their host. While sharing only 31 % amino acid identity, both cmvIL-10 and rhcmvIL-10 are highly stable in sequence (>98 % identity) among different strains of HCMV and RhCMV, respectively (Barry and Chang 2007; Cunningham et al. 2010). The functionalities of cmvIL-10 are almost identical to those of cIL-10 (Chang and Barry 2010; Chang et al. 2009, 2007, 2004; Eberhardt et al. 2012; Jones et al. 2002; Kotenko et al. 2000; Logsdon et al. 2011; Raftery et al. 2004; Spencer et al. 2008, 2002). There is no evidence that cmvIL-10 has evolved new IL-10R-mediated signaling responses. Inter-specific drift of the primate CMV vIL-10 proteins from the cIL-10 of their host was likely driven as a compensatory selection to some aspect of its host's evolution. Jones et al. demonstrated that the binding affinity of cmvIL-10/IL-10R exceeds that of cIL-10/IL-10R (Jones et al. 2002), which presents a potential mechanistic basis for the evolutionary selections that occurred during evolution of the primate CMV vIL-10 proteins. Since the IL-10R proteins of the different primate hosts are highly conserved, genetic drift of the primate CMV vIL-10 proteins was not a compensatory drift reflective of the drift in IL-10R. Instead, it seems likely that maintaining a higher binding affinity to the host IL-10R was probably critical in shaping the particular viral IL-10 sequence. Put another way, selection may have been driven by a greater replication fitness conferred by vIL-10 outcompeting cIL-10 for IL-10R. Whatever selective forces might have been operative, the results is that the original transduced cIL-10 has drifted from what was once a "self" protein, expressed in the context of CMV infection, to one that is now very much a "non-self" protein. The probable disadvantage of evolving into a non-self protein was likely outweighed by the advantage gained by rapidly binding to IL10R and altering host immunity in ways that prevent effective clearance of viral genomes.

The extent of sequence drift for other vIL-10 proteins has been quite variable. vIL-10 orthologs range in sequence identity to the host's cIL-10 from 20 % (Koi herpesvirus) to ≥90 % (Epstein-Barr Virus—EBV; and Orf Virus) (Table 4). There do not appear to be any papers investigating vaccination against highly conserved vIL-10 orthologs, including that of the clinically relevant human pathogen EBV.

However, one study measured antibody responses against IL-10 in chronically infected EBV patients by ELISA and immunoprecipitation (Tanner et al. 1997). This study showed that elevated titers of IgG antibodies to ebvIL-10 are detected in patients with clinically active EBV disease, including chronic infectious mononucleosis (CIM), nasopharyngeal carcinoma, and EBV-associated lymphoproliferative disease, compared to EBV-seronegative and asymptomatic EBV-seropositive individuals. They also showed that there were concomitant elevated IgG antibodies to cIL-10 in CIM patients, and for one CIM patient, anti-cIL-10 antibodies neutralized cIL-10 function. The clinical relevance of cross-reactive antibodies between ebvIL-10 and cIL-10 requires additional study, but one implication is that anti-ebvIL-10 vaccine strategies will require approaches that do not lead to reactivity to endogenous cIL-10.

Serologic analyses of seroreactivity to divergent vIL-10 proteins have only been addressed for HCMV and RhCMV. cmvIL-10 protein, which is 25 % identical to human cIL-10, is secreted from infected cells in culture at the late stage of infection (Chang et al. 2004). de Lemos Rieper et al. developed a radioimmunoassay to determine the frequency of HCMV-seropositive individuals that had antibodies to cmvIL-10, and, if so, whether cmvIL-10-specific IgG cross-reacted with human cIL-10 (de Lemos Rieper et al. 2011). This study showed that 28 % of HCMV-seropositive Danish blood donors developed IgG binding antibodies to cmvIL-10. Competition studies using competitor unlabeled IL-10 proteins demonstrated that cmvIL-10 antibodies exhibited no cross-reactivity to human cIL-10. Importantly for vaccine development, cmvIL-10 antibodies were capable of neutralizing cmvIL-10 function in an in vitro bioassay. The absence of evidence for cross-immunogenicity between cmvIL-10 and cIL-10 in this study is bolstered by another study investigating cross-reactivity of anti-cIL-10 monoclonal antibodies with cmvIL-10 and ebvIL-10. Brodeur and Spencer observed that six of seven monoclonal antibodies for human cIL-10 cross-reacted with ebvIL-10 and neutralized its function (Brodeur and Spencer 2010). In contrast, none of the cIL-10 MAb recognized cmvIL-10. In addition, polyclonal antibodies to ebvIL-10 bound to cIL-10 but not cmvIL-10, whereas polyclonal sera to cmvIL-10 did not bind to either cIL-10 or ebvIL-10.

Serological analysis of healthy, long-term RhCMV-infected rhesus macaques, in which the seroprevalence of RhCMV is almost 100 % in corral-housed animals >1 year of age (Vogel et al. 1994), indicates that all RhCMV-infected animals develop binding antibodies to rhcmvIL-10 (Eberhardt et al. 2012). rhcmvIL-10 antibodies develop early after infection and remain elevated for the life of the infected host. Almost all animals with binding antibodies to rhcmvIL-10 also have antibodies that neutralize rhcmvIL-10 bioactivity. RhCMV is endemic in breeding cohorts of rhesus macaques, and infectious virus is transmitted horizontally between animals in bodily fluids, such as saliva and urine (Früh et al. 2013). RhCMV infection is subclinical following either natural infection or experimental inoculation, and there has never been any demonstration that RhCMV-infected animals develop any autoimmune conditions that might result from development of rhcmvIL-10 antibodies that cross-react with rhesus cIL-10. Plasma

samples from RhCMV-infected animals that neutralize rhcmvIL-10 function do not demonstrate any detectable neutralizing activity against rhesus cIL-10 (Eberhardt et al. 2012). In sum, this study demonstrated that rhcmvIL-10 is a strong immunogen during infection and that antibodies generated in the context of infection do not cross-react with cIL-10.

Since both cmvIL-10 and rhcmvIL-10 exhibit extensive sequence divergence from human and rhesus cIL-10, respectively, the vIL-10 orthologs present potential vaccine targets. As studies of vaccinating against microbial activators of cIL-10 have produced mixed results in terms of inducing protective immunity (described above), immunization with functional forms of cmvIL-10 or rhcmvIL-10 might be detrimental to the host and potentially skew vaccine-induced immune responses. Accordingly, Logsdon et al. used structural biology to engineer a minimal number of amino acid changes into rhcmvIL-10 to ablate functionality while retaining the immunogenicity of native rhcmvIL-10 (Logsdon et al. 2011). Two mutated version were constructed (M1 and M2) each of which contained two amino acid changes to abolish (1) any binding activity to IL-10R and (2) immunosuppressive activity on LPS-activated rhesus PBMC. RhCMV-infected macaques were immunized with M1 and M2 to determine whether the loss of functional activity in M1 and M2 resulted in loss of immunogenicity. Immunization with M1 and M2 protein stimulated large increases in rhcmvIL-10 binding antibodies in six out of six vaccinees and increased neutralizing antibody titers in five of the six vaccinees. Further, booster immunizations in RhCMV-infected animals did not lead to the development of cross-neutralizing activity against rhesus cIL-10.

To determine whether immunization with nonfunctional rhcmvIL-10 M1 and M2 confers protective immunity against RhCMV challenge, naïve macaques were immunized with a DNA prime/protein boost regimen and longitudinally evaluated for rhcmvIL-10 immune responses (Eberhardt et al. 2013). Immunized animals developed both binding and neutralizing antibodies to wild-type rhcmvIL-10, and there were no detectable antibodies that cross-neutralized rhesus cIL-10. Thus, de novo induction of immune responses by immunization with M1 and M2 were specific to rhcmvIL-10. Vaccinated animals and mock-vaccinated controls were challenged with a low titer of an epitheliotropic strain of RhCMV (1,000 plaque forming units of UCD59) delivered by a subcutaneous route of inoculation. Vaccinated animals were noted for both acute and long-term control of RhCMV infection. A biopsy of the inoculation site seven days after inoculation showed that the vaccinees had less inflammation and fewer RhCMV-infected cells than observed in the controls. In addition, the inflammatory infiltrate in the vaccinees was predominantly mononuclear, whereas there was a mixed infiltrate in the controls, consisting of mononuclear cells and neutrophils. These results were consistent with acute control of challenge virus infection. To measure whether immunization stimulated immune responses that altered the pattern of long-term infection, saliva and urine were longitudinally evaluated by qPCR for RHCMV DNA. Prolonged shedding of RhCMV in saliva and urine is a characteristic of animals naturally infected with wild-type RhCMV (Huff et al. 2003) or experimentally inoculated with epitheliotropic strains of RhCMV, including UCD59 (Oxford

et al. 2008), and reductions in shedding in vaccinated/RhCMV-challenged animals has proven useful as a marker of vaccine protective efficacy (Wussow et al. 2013). Whereas RhCMV shedding generally persisted in the saliva and urine of unvaccinated controls once shedding began, RhCMV DNA was notably absent in the saliva and urine in 50 and 75 %, respectively, of the vaccinees throughout 26 weeks post challenge. The results of this study show that immunization against rhcmvIL-10, using nonfunctional but immunogenic forms of the protein, can elicit long-term changes in the acute and long-term profiles of challenge virus infection (Eberhardt et al. 2013).

9 Conclusions

It is no coincidence that the natural histories of pathogenic and commensal organisms converge on the IL-10 signaling pathway as a central nexus during acute and chronic infections and disease outcomes, given the role of cIL-10 as a central immune regulator. Accumulating precedents in animal models now provide compelling justification for pursuing translational studies aimed at maintaining and/or restoring protective cIL-10 homeostasis for infectious pathogens.

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References

Abel S, Luckheide N, Westendorf AM, Geffers R, Roers A, Muller W, Sparwasser T, Matuschewski K, Buer J, Hansen W (2012) Strong impact of CD4 + Foxp3 + regulatory T cells and limited effect of T cell-derived IL-10 on pathogen clearance during Plasmodium yoelii infection. J Immunol 188:5467–5477

Arce-Fonseca M, Ramos-Ligonio A, Lopez-Monteon A, Salgado-Jimenez B, Talamas-Rohana P, Rosales-Encina JL (2011) A DNA vaccine encoding for TcSSP4 induces protection against acute and chronic infection in experimental Chagas disease. Int J Biol Sci 7:1230–1238

Avdic S, Cao JZ, Cheung AK, Abendroth A, Slobedman B (2011) Viral interleukin-10 expressed by human cytomegalovirus during the latent phase of infection modulates latently infected myeloid cell differentiation. J Virol 85:7465–7471

Azenabor AA, Cintron-Cuevas J, Schmitt H, Bumah V (2011) *C. trachomatis* induces anti-inflammatory effect in human macrophages by attenuation of immune mediators in Jurkat T-cells. Immunobiology 216:1248–1255

Barboza L, Salmen S, Peterson DL, Montes H, Colmenares M, Hernandez M, Berrueta-Carrillo LE, Berrueta L (2009) Altered T cell costimulation during chronic hepatitis B infection. Cell Immunol 257:61–68

Barry P, Chang W-L (2007) Primate Betaherpesviruses. In: Arvin A, Campadielli G, Moore P, Mocarski E, Roizman B, Whitley R, Yamanishi K (eds) Human herpesviruses: biology, therapy and immunoprophylaxis. Cambridge University Press, Cambridge, pp 1051–1075

Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL (2002a) CD4 + CD25+ regulatory T cells control *L. major* persistence and immunity. Nature 420:502–507

Belkaid Y, Rouse BT (2005) Natural regulatory T cells in infectious disease. Nat Immunol 6:353–360

Belkaid Y, Von Stebut E, Mendez S, Lira R, Caler E, Bertholet S, Udey MC, Sacks D (2002b) CD8 + T cells are required for primary immunity in C57BL/6 mice following low-dose, intradermal challenge with Leishmania major. J Immunol 168:3992–4000

Betsou F, Sebo P, Guiso N (1993) CyaC-mediated activation is important not only for toxic but also for protective activities of *B. pertussis* adenylate cyclase-hemolysin. Infect Immun 61:3583–3589

Boyd AP, Ross PJ, Conroy H, Mahon N, Lavelle EC, Mills KH (2005) *B. pertussis* adenylate cyclase toxin modulates innate and adaptive immune responses: distinct roles for acylation and enzymatic activity in immunomodulation and cell death. J Immunol 175:730–738

Brodeur ND, Spencer JV (2010) Antibodies to human IL-10 neutralize ebvIL-10-mediated cytokine suppression but have no effect on cmvIL-10 activity. Virus Res 153:265–268

Brooks DG, Lee AM, Elsaesser H, McGavern DB, Oldstone MB (2008) IL-10 blockade facilitates DNA vaccine-induced T cell responses and enhances clearance of persistent virus infection. J Exp Med 205:533–541

Brooks DG, McGavern DB, Oldstone MB (2006a) Reprogramming of antiviral T cells prevents inactivation and restores T cell activity during persistent viral infection. J Clin Invest 116:1675–1685

Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB (2006b) Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 12:1301–1309

Brooks DG, Walsh KB, Elsaesser H, Oldstone MB (2010) IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection. Proc Natl Acad Sci USA 107:3018–3023

Bueno LL, Morais CG, Araujo FF, Gomes JA, Correa-Oliveira R, Soares IS, Lacerda MV, Fujiwara RT, Braga EM (2010) Plasmodium vivax: induction of CD4 + CD25 + FoxP3+ regulatory T cells during infection are directly associated with level of circulating parasites. PLoS ONE 5:e9623

Campbell AE, Cavanaugh VJ, Slater JS (2008) The salivary glands as a privileged site of cytomegalovirus immune evasion and persistence. Med Microbiol Immunol 197:205–213

Cavanaugh VJ, Deng Y, Birkenbach MP, Slater JS, Campbell AE (2003) Vigorous innate and virus-specific cytotoxic T-lymphocyte responses to murine cytomegalovirus in the submaxillary salivary gland. J Virol 77:1703–1717

CDC (2012) Human Orf virus infection from household exposures—United States, 2009–2011. MMWR Morb Mortal Wkly Rep 61:245–248

Chan A, Baird M, Mercer AA, Fleming SB (2006) Maturation and function of human dendritic cells are inhibited by orf virus-encoded interleukin-10. J Gen Virol 87:3177–3181

Chang WL, Barry PA (2010) Attenuation of innate immunity by cytomegalovirus IL-10 establishes a long-term deficit of adaptive antiviral immunity. Proc Nat Acad Sci USA 107:22647–22652

Chang WL, Barry PA, Szubin R, Wang D, Baumgarth N (2009) Human cytomegalovirus suppresses type I interferon secretion by plasmacytoid dendritic cells through its interleukin 10 homolog. Virology 390:330–337

Chang WL, Baumgarth N, Eberhardt MK, Lee CY, Baron CA, Gregg JP, Barry PA (2007) Exposure of myeloid dendritic cells to exogenous or endogenous IL-10 during maturation determines their longevity. J Immunol 178:7794–7804

Chang WL, Baumgarth N, Yu D, Barry PA (2004) Human cytomegalovirus-encoded interleukin-10 homolog inhibits maturation of dendritic cells and alters their functionality. J Virol 78:8720–8731

Chitlaru T, Gat O, Grosfeld H, Inbar I, Gozlan Y, Shafferman A (2007) Identification of in vivo-expressed immunogenic proteins by serological proteome analysis of the Bacillus anthracis secretome. Infect Immun 75:2841–2852

Cleret-Buhot A, Mathieu J, Tournier JN, Quesnel-Hellmann A (2012) Both lethal and edema toxins of Bacillus anthracis disrupt the human dendritic cell chemokine network. PLoS ONE 7:e43266

Cohen-Poradosu R, McLoughlin RM, Lee JC, Kasper DL (2011) *B. fragilis*-stimulated interleukin-10 contains expanding disease. J Infect Dis 204:363–371

Coleman MM, Finlay CM, Moran B, Keane J, Dunne PJ, Mills KH (2012) The immunoregulatory role of CD4(+) FoxP3(+) CD25(-) regulatory T cells in lungs of mice infected with *B. pertussis*. FEMS Immunol Med Microbiol 64:413–424

Cunningham C, Gatherer D, Hilfrich B, Baluchova K, Dargan DJ, Thomson M, Griffiths PD, Wilkinson GW, Schulz TF, Davison AJ (2010) Sequences of complete human cytomegalovirus genomes from infected cell cultures and clinical specimens. J Gen Virol 91:605–615

Cyktor JC, Carruthers B, Beamer GL, Turner J (2013a) Clonal expansions of CD8 + T cells with IL-10 secreting capacity occur during chronic *M. tuberculosis* infection. PLoS ONE 8:e58612

Cyktor JC, Carruthers B, Kominsky RA, Beamer GL, Stromberg P, Turner J (2013b) IL-10 inhibits mature fibrotic granuloma formation during *M. tuberculosis* infection. J Immunol 190:2778–2790

Davison AJ, Dolan A, Akter P, Addison C, Dargan DJ, Alcendor DJ, McGeoch DJ, Hayward GS (2003) The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. J Gen Virol 84:17–28

de Lemos Rieper C, Galle P, Pedersen BK, Hansen MB (2011) Characterization of specific antibodies against cytomegalovirus (CMV)-encoded interleukin 10 produced by 28 % of CMV-seropositive blood donors. J Gen Virol 92:1508–1518

Dietze KK, Zelinskyy G, Gibbert K, Schimmer S, Francois S, Myers L, Sparwasser T, Hasenkrug KJ, Dittmer U (2011) Transient depletion of regulatory T cells in transgenic mice reactivates virus-specific CD8 + T cells and reduces chronic retroviral set points. Proc Natl Acad Sci USA 108:2420–2425

Duell BL, Tan CK, Carey AJ, Wu F, Cripps AW, Ulett GC (2012) Recent insights into microbial triggers of interleukin-10 production in the host and the impact on infectious disease pathogenesis. FEMS Immunol Med Microbiol 64:295–313

Dunn W, Chou C, Li H, Hai R, Patterson D, Stolc V, Zhu H, Liu F (2003) Functional profiling of a human cytomegalovirus genome. Proc Natl Acad Sci USA 100:14223–14228

Eberhardt M, Deshpande A, Chang W-L, Barthold S, Walter M, Barry P (2013) Vaccination against a virally-encoded cytokine significantly restricts viral challenge. (Submitted)

Eberhardt MK, Chang WL, Logsdon NJ, Yue Y, Walter MR, Barry PA (2012) Host immune responses to a viral immune modulating protein: immunogenicity of viral interleukin-10 in rhesus cytomegalovirus-infected rhesus macaques. PLoS ONE 7:e37931

Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, von Herrath MG (2006) Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 203:2461–2472

Elrefaei M, Barugahare B, Ssali F, Mugyenyi P, Cao H (2006) HIV-specific IL-10-positive CD8 + T cells are increased in advanced disease and are associated with decreased HIV-specific cytolysis. J Immunol 176:1274–1280

Farfel Z, Konen S, Wiertz E, Klapmuts R, Addy PA, Hanski E (1990) Antibodies to *B. pertussis* adenylate cyclase are produced in man during pertussis infection and after vaccination. J Med Microbiol 32:173–177

Felonato M, Pina A, de Araujo EF, Loures FV, Bazan SB, Feriotti C, Calich VL (2012) Anti-CD25 treatment depletes Treg cells and decreases disease severity in susceptible and resistant mice infected with *P. brasiliensis*. PLoS ONE 7:e51071

Ferreira MC, de Oliveira RT, da Silva RM, Blotta MH, Mamoni RL (2010) Involvement of regulatory T cells in the immunosuppression characteristic of patients with paracoccidioidomycosis. Infect Immun 78:4392–4401

Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170:2081–2095

Fleming SB, Anderson IE, Thomson J, Deane DL, McInnes CJ, McCaughan CA, Mercer AA, Haig DM (2007) Infection with recombinant orf viruses demonstrates that the viral interleukin-10 is a virulence factor. J Gen Virol 88:1922–1927

Fleming SB, McCaughan CA, Andrews AE, Nash AD, Mercer AA (1997) A homolog of interleukin-10 is encoded by the poxvirus orf virus. J Virol 71:4857–4861

Flores-Garcia Y, Rosales-Encina JL, Satoskar AR, Talamas-Rohana P (2011) IL-10-IFN-gamma double producers CD4 + T cells are induced by immunization with an amastigote stage specific derived recombinant protein of *T. cruzi*. Int J Biol Sci 7:1093–1100

Früh K, Malouli D, Oxford K, Barry P (2013) Non-human-primate models of cytomegalovirus infection, prevention, and therapy. In: Reddehase M (ed.) Cytomegaloviruses: from molecular pathogenesis to therapy. Caister Academic Press/Horizon. **II,** Norfolk

Furlong ST, Caulfield JP (1989) *S. mansoni*: synthesis and release of phospholipids, lysophospholipids, and neutral lipids by schistosomula. Exp Parasitol 69:65–77

Gao Q, Qi L, Wu T, Wang J (2012) An important role of interleukin-10 in counteracting excessive immune response in HT-29 cells exposed to *C. butyricum*. BMC Microbiol 12:100

Gerosa F, Nisii C, Righetti S, Micciolo R, Marchesini M, Cazzadori A, Trinchieri G (1999) CD4(+) T cell clones producing both interferon-gamma and interleukin-10 predominate in bronchoalveolar lavages of active pulmonary tuberculosis patients. Clin Immunol 92:224–234

Gesser B, Leffers H, Jinquan T, Vestergaard C, Kirstein N, Sindet-Pedersen S, Jensen SL, Thestrup-Pedersen K, Larsen CG (1997) Identification of functional domains on human interleukin 10. Proc Natl Acad Sci 94:14620–14625

Gillan V, Lawrence RA, Devaney E (2005) B cells play a regulatory role in mice infected with the L3 of Brugia pahangi. Int Immunol 17:373–382

Gonzalez-Lopez MA, Velazquez-Guadarrama N, Romero-Espejel ME, de Jesus Olivares-Trejo J (2013) *H. pylori* secretes the chaperonin GroEL (HSP60), which binds iron. FEBS Lett

Gross MK, Au DC, Smith AL, Storm DR (1992) Targeted mutations that ablate either the adenylate cyclase or hemolysin function of the bifunctional cyaA toxin of *B. pertussis* abolish virulence. Proc Natl Acad Sci USA 89:4898–4902

Haig DM, Thomson J, McInnes C, McCaughan C, Imlach W, Mercer A, Fleming S (2002a) Orf virus immuno-modulation and the host immune response. Vet Immunol Immunopathol 87:395–399

Haig DM, Thomson J, McInnes CJ, Deane DL, Anderson IE, McCaughan CA, Imlach W, Mercer AA, Howard CJ, Fleming SB (2002b) A comparison of the anti-inflammatory and immuno-stimulatory activities of orf virus and ovine interleukin-10. Virus Res 90:303–316

Henson D, Strano AJ (1972) Mouse cytomegalovirus. Necrosis of infected and morphologically normal submaxillary gland acinar cells during termination of chronic infection. Am J Pathol 68:183–202

Hickey FB, Brereton CF, Mills KH (2008) Adenylate cycalse toxin of *B. pertussis* inhibits TLR-induced IRF-1 and IRF-8 activation and IL-12 production and enhances IL-10 through MAPK activation in dendritic cells. J Leukoc Biol 84:234–243

Hilleman MR (2004) Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. Proc Natl Acad Sci USA 101(Suppl 2):14560–14566

Horikawa M, Weimer ET, DiLillo DJ, Venturi GM, Spolski R, Leonard WJ, Heise MT, Tedder TF (2013) Regulatory B cell (B10 Cell) expansion during Listeria infection governs innate and cellular immune responses in mice. J Immunol 190:1158–1168

Hormozi K, Parton R, Coote J (1999) Adjuvant and protective properties of native and recombinant *B. pertussis* adenylate cyclase toxin preparations in mice. FEMS Immunol Med Microbiol 23:273–282

Howard AD, Zwilling BS (1998) Cytokine production by CD4 and CD8 T cells during the growth of *M. tuberculosis* in mice. Clin Exp Immunol 113:443–449

Huff JL, Eberle R, Capitanio J, Zhou SS, Barry PA (2003) Differential detection of B virus and rhesus cytomegalovirus in rhesus macaques. J Gen virol 84:83–92

Humphreys IR, de Trez C, Kinkade A, Benedict CA, Croft M, Ware CF (2007) Cytomegalovirus exploits IL-10-mediated immune regulation in the salivary glands. J Exp Med 204:1217–1225

Hunt D, Wilson JE, Weih KA, Ishido S, Harton JA, Roche PA, Drake JR (2012) *F. tularensis* elicits IL-10 via a PGE(2)-inducible factor, to drive macrophage MARCH1 expression and class II down-regulation. PLoS ONE 7:e37330

Incrocci R, McCormack M, Swanson-Mungerson M (2013) Epstein-Barr virus LMP2A increases IL-10 production in mitogen-stimulated primary B-cells and B-cell lymphomas. J Gen Virol 94:1127–1133

Jaworowski A, Cheng WJ, Westhorpe CL, Abendroth A, Crowe SM, Slobedman B (2009) Enhanced monocyte Fc phagocytosis by a homologue of interleukin-10 encoded by human cytomegalovirus. Virology 391:20–24

Jenkins C, Garcia W, Abendroth A, Slobedman B (2008a) Expression of a human cytomegalovirus latency-associated homolog of interleukin-10 during the productive phase of infection. Virology 370:285–294

Jenkins C, Garcia W, Godwin MJ, Spencer JV, Stern JL, Abendroth A, Slobedman B (2008b) Immunomodulatory properties of a viral homolog of human interleukin-10 expressed by human cytomegalovirus during the latent phase of infection. J Virol 82:3736–3750

Jeon SG, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, Tsuji NM, Kiyono H, Ma JS, Kusu T, Okumura R, Hara H, Yoshida H, Yamamoto M, Nomoto K, Takeda K (2012) Probiotic *B. breve* induces IL-10-producing Tr1 cells in the colon. PLoS Pathog 8:e1002714

Jeong YI, Hong SH, Cho SH, Lee WJ, Lee SE (2012) Induction of IL-10-producing CD1dhighCD5 + regulatory B cells following *B. microti*-infection. PLoS ONE 7:e46553

Jones BC, Logsdon NJ, Josephson K, Cook J, Barry PA, Walter MR (2002) Crystal structure of human cytomegalovirus IL-10 bound to soluble human IL-10R1. Proc Natl Acad Sci USA 99:9404–9409

Jonjic S, Pavic I, Lucin P, Rukavina D, Koszinowski UH (1990) Efficacious control of cytomegalovirus infection after long-term depletion of CD8 + T lymphocytes. J Virol 64:5457–5464

Kaplan DE, Ikeda F, Li Y, Nakamoto N, Ganesan S, Valiga ME, Nunes FA, Rajender Reddy K, Chang KM (2008) Peripheral virus-specific T-cell interleukin-10 responses develop early in acute hepatitis C infection and become dominant in chronic hepatitis. J Hepatol 48:903–913

Konieczna P, Akdis CA, Quigley EM, Shanahan F, O'Mahony L (2012) Portrait of an immunoregulatory Bifidobacterium. Gut Microbes 3:261–266

Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, Pestka S (2000) Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci 97:1695–1700

Layez C, Brunet C, Lepolard C, Ghigo E, Capo C, Raoult D, Mege JL (2012) Foxp3(+)CD4(+) CD25(+) regulatory T cells are increased in patients with *C. burnetii* endocarditis. FEMS Immunol Med Microbiol 64:137–139

Levkovich T, Poutahidis T, Smillie C, Varian BJ, Ibrahim YM, Lakritz JR, Alm EJ, Erdman SE (2013) Probiotic bacteria induce a 'glow of health'. PLoS ONE 8:e53867

Lilja AE, Chang WL, Barry PA, Becerra SP, Shenk TE (2008) Functional genetic analysis of rhesus cytomegalovirus: Rh01 is an epithelial cell tropism factor. J Virol 82:2170–2181

Liu Y, de Waal Malefyt R, Briere F, Parham C, Bridon JM, Banchereau J, Moore KW, Xu J (1997) The EBV IL-10 homologue is a selective agonist with impaired binding to the IL-10 receptor. J Immunol 158:604–613

Livingston M, Loach D, Wilson M, Tannock GW, Baird M (2010) Gut commensal *L. reuteri* 100-23 stimulates an immunoregulatory response. Immunol Cell Biol 88:99–102

Lockridge KM, Zhou SS, Kravitz RH, Johnson JL, Sawai ET, Blewett EL, Barry PA (2000) Primate cytomegaloviruses encode and express an IL-10-like protein. Virol 268:272–280

Logsdon NJ, Eberhardt MK, Allen CE, Barry PA, Walter MR (2011) Design and analysis of rhesus cytomegalovirus IL-10 mutants as a model for novel vaccines against human cytomegalovirus. PLoS ONE 6:e28127

Lopez P, Gonzalez-Rodriguez I, Gueimonde M, Margolles A, Suarez A (2011) Immune response to *B. bifidum* strains support Treg/Th17 plasticity. PLoS ONE 6:e24776

Loughman JA, Hunstad DA (2012) Induction of indoleamine 2,3-dioxygenase by uropathogenic bacteria attenuates innate responses to epithelial infection. J Infect Dis 205:1830–1839

Lowry JE, Isaak DD, Leonhardt JA, Vernati G, Pate JC, Andrews GP (2011) Vaccination with *B. abortus* recombinant in vivo-induced antigens reduces bacterial load and promotes clearance in a mouse model for infection. PLoS ONE 6:e17425

MacKenzie KF, Clark K, Naqvi S, McGuire VA, Noehren G, Kristariyanto Y, van den Bosch M, Mudaliar M, McCarthy PC, Pattison MJ, Pedrioli PG, Barton GJ, Toth R, Prescott A, Arthur JS (2013) PGE(2) induces macrophage IL-10 production and a regulatory-like phenotype via a protein kinase A-SIK-CRTC3 pathway. J Immunol 190:565–577

Mahajan S, Dkhar HK, Chandra V, Dave S, Nanduri R, Janmeja AK, Agrewala JN, Gupta P (2012) *M. tuberculosis* modulates macrophage lipid-sensing nuclear receptors PPARgamma and TR4 for survival. J Immunol 188:5593–5603

Mandaric S, Walton SM, Rulicke T, Richter K, Girard-Madoux MJ, Clausen BE, Zurunic A, Kamanaka M, Flavell RA, Jonjic S, Oxenius A (2012) IL-10 suppression of NK/DC cross-talk leads to poor priming of MCMV-specific CD4 T cells and prolonged MCMV persistence. PLoS Pathog 8:e1002846

Manuzak J, Dillon S, Wilson C (2012) Differential interleukin-10 (IL-10) and IL-23 production by human blood monocytes and dendritic cells in response to commensal enteric bacteria. Clin Vaccine Immunol 19:1207–1217

Marks E, Tam MA, Lycke NY (2010) The female lower genital tract is a privileged compartment with IL-10 producing dendritic cells and poor Th1 immunity following *C. trachomatis* infection. PLoS Pathog 6:e1001179

Mauri C, Bosma A (2012) Immune regulatory function of B cells. Annu Rev Immunol 30:221–241

Mazmanian SK, Round JL, Kasper DL (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 453:620–625

McGeoch DJ, Cook S, Dolan A, Jamieson FE, Telford EA (1995) Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses. J Mol Biol 247:443–458

McGuirk P, Mills KH (2002) Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. Trends Immunol 23:450–455

McPhee JB, Mena P, Bliska JB (2010) Delineation of regions of the Yersinia YopM protein required for interaction with the RSK1 and PRK2 host kinases and their requirement for interleukin-10 production and virulence. Infect Immun 78:3529–3539

McPhee JB, Mena P, Zhang Y, Bliska JB (2012) Interleukin-10 induction is an important virulence function of the Yersinia pseudotuberculosis type III effector YopM. Infect Immun 80:2519–2527

Medina TS, Costa SP, Oliveira MD, Ventura AM, Souza JM, Gomes TF, Vallinoto AC, Povoa MM, Silva JS, Cunha MG (2011) Increased interleukin-10 and interferon-gamma levels in Plasmodium vivax malaria suggest a reciprocal regulation which is not altered by IL-10 gene promoter polymorphism. Malar J 10:264

Meghari S, Bechah Y, Capo C, Lepidi H, Raoult D, Murray PJ, Mege JL (2008) Persistent *C. burnetii* infection in mice overexpressing IL-10: an efficient model for chronic Q fever pathogenesis. PLoS Pathog 4:e23

Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science 248:1230–1234

Mukherjee S, Mukherjee B, Mukhopadhyay R, Naskar K, Sundar S, Dujardin JC, Das AK, Roy S (2012) Imipramine is an orally active drug against both antimony sensitive and resistant *L. donovani* clinical isolates in experimental infection. PLoS Negl Trop Dis 6:e1987

Nachtwey J, Spencer JV (2008) HCMV IL-10 suppresses cytokine expression in monocytes through inhibition of nuclear factor-kappaB. Viral Immunol 21:477–482

Nagamatsu K, Kuwae A, Konaka T, Nagai S, Yoshida S, Eguchi M, Watanabe M, Mimuro H, Koyasu S, Abe A (2009) Bordetella evades the host immune system by inducing IL-10 through a type III effector, BopN. J Exp Med 206:3073–3088

Nagarajan G, Swami SK, Dahiya SS, Sivakumar G, Narnaware SD, Tuteja FC, Patil NV (2013) Comparison of virokine from camel pseudocowpoxvirus (PCPV) with Interleukin 10 of the Dromedary camel (Camelus dromedarius). Cytokine 61:356–359

Nemeth J, Straley SC (1997) Effect of Yersinia pestis YopM on experimental plague. Infect Immun 65:924–930

Netea MG, Sutmuller R, Hermann C, Van der Graaf CA, Van der Meer JW, van Krieken JH, Hartung T, Adema G, Kullberg BJ (2004) Toll-like receptor 2 suppresses immunity against *C. albicans* through induction of IL-10 and regulatory T cells. J Immunol 172:3712–3718

O'Leary S, O'Sullivan MP, Keane J (2011) IL-10 blocks phagosome maturation in *M. tuberculosis*-infected human macrophages. Am J Respir Cell Mol Biol 45:172–180

Obermajer N, Kalinski P (2012) Generation of myeloid-derived suppressor cells using prostaglandin E2. Transplant Res 1:15

Okwor I, Uzonna J (2008) Persistent parasites and immunologic memory in cutaneous leishmaniasis: implications for vaccine designs and vaccination strategies. Immunol Res 41:123–136

Oxford KL, Eberhardt MK, Yang KW, Strelow L, Kelly S, Zhou SS, Barry PA (2008) Protein coding content of the U(L)b' region of wild-type rhesus cytomegalovirus. Virology 373:181–188

Pagan AJ, Peters NC, Debrabant A, Ribeiro-Gomes F, Pepper M, Karp CL, Jenkins MK, Sacks DL (2013) Tracking antigen-specific CD4 + T cells throughout the course of chronic Leishmania major infection in resistant mice. Eur J Immunol 43:427–438

Parcina M, Miranda-Garcia MA, Durlanik S, Ziegler S, Over B, Georg P, Foermer S, Ammann S, Hilmi D, Weber KJ, Schiller M, Heeg K, Schneider-Brachert W, Gotz F, Bekeredjian-Ding I (2013) Pathogen-triggered activation of plasmacytoid dendritic cells induces IL-10-producing B cells in response to *S. aureus*. J Immunol 190:1591–1602

Pilione MR, Harvill ET (2006) The *B. bronchiseptica* type III secretion system inhibits gamma interferon production that is required for efficient antibody-mediated bacterial clearance. Infect Immun 74:1043–1049

Raftery MJ, Wieland D, Gronewald S, Kraus AA, Giese T, Schonrich G (2004) Shaping phenotype, function, and survival of dendritic cells by cytomegalovirus-encoded IL-10. J Immunol 173:3383–3391

Reddehase MJ, Simon CO, Seckert CK, Lemmermann N, Grzimek NK (2008) Murine model of cytomegalovirus latency and reactivation. Curr Top Microbiol Immunol 325:315–331

Redford PS, Boonstra A, Read S, Pitt J, Graham C, Stavropoulos E, Bancroft GJ, O'Garra A (2010) Enhanced protection to *M. tuberculosis* infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung. Eur J Immunol 40:2200–2210

Redford PS, Murray PJ, O'Garra A (2011) The role of IL-10 in immune regulation during M. tuberculosis infection. Mucosal Immunol 4:261–270

Redpath S, Ghazal P, Gascoigne NR (2001) Hijacking and exploitation of IL-10 by intracellular pathogens. Trends Microbiol 9:86–92

Reithmeier-Rost D, Bierschenk S, Filippova N, Schroder-Braunstein J, Sing A (2004) Yersinia V antigen induces both TLR homo- and heterotolerance in an IL-10-involving manner. Cell Immunol 231:63–74

Remoli ME, Giacomini E, Petruccioli E, Gafa V, Severa M, Gagliardi MC, Iona E, Pine R, Nisini R, Coccia EM (2011) Bystander inhibition of dendritic cell differentiation by *M. tuberculosis*-induced IL-10. Immunol Cell Biol 89:437–446

Richter K, Perriard G, Oxenius A (2013) Reversal of chronic to resolved infection by IL-10 blockade is LCMV strain dependent. Eur J Immunol 43:649–654

Ross PJ, Lavelle EC, Mills KH, Boyd AP (2004) Adenylate cyclase toxin from *B. pertussis* synergizes with lipopolysaccharide to promote innate interleukin-10 production and enhances the induction of Th2 and regulatory T cells. Infect Immun 72:1568–1579

Round JL, Mazmanian SK (2010) Inducible Foxp3 + regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci USA 107:12204–12209

Ruter C, Buss C, Scharnert J, Heusipp G, Schmidt MA (2010) A newly identified bacterial cell-penetrating peptide that reduces the transcription of pro-inflammatory cytokines. J Cell Sci 123:2190–2198

Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, Uehata T, Iwasaki H, Omori H, Yamaoka S, Yamamoto N, Akira S (2012) Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. Cell Host Microbe 12:109–116

Sanchez AM, Zhu J, Huang X, Yang Y (2012) The development and function of memory regulatory T cells after acute viral infections. J Immunol 189:2805–2814

Sarfo BY, Wilson NO, Bond VC, Stiles JK (2011) Plasmodium berghei ANKA infection increases Foxp3, IL-10 and IL-2 in CXCL-10 deficient C57BL/6 mice. Malar J 10:69

Saygili T, Akincilar SC, Akgul B, Nalbant A (2012) *A. actinomycetemcomitans* GroEL protein promotes conversion of human CD4 + T cells into IFNgamma IL10 producing Tbet + Th1 cells. PLoS ONE 7:e49252

Schlecht G, Loucka J, Najar H, Sebo P, Leclerc C (2004) Antigen targeting to CD11b allows efficient presentation of CD4 + and CD8 + T cell epitopes and in vivo Th1-polarized T cell priming. J Immunol 173:6089–6097

Schols D, De Clercq E (1996) Human immunodeficiency virus type 1 gp120 induces anergy in human peripheral blood lymphocytes by inducing interleukin-10 production. J Virol 70:4953–4960

Scholzen A, Mittag D, Rogerson SJ, Cooke BM, Plebanski M (2009) Plasmodium falciparum-mediated induction of human CD25Foxp3 CD4 T cells is independent of direct TCR stimulation and requires IL-2, IL-10 and TGFbeta. PLoS Pathog 5:e1000543

Siewe B, Stapleton JT, Martinson J, Keshavarzian A, Kazmi N, Demarais PM, French AL, Landay A (2013) Regulatory B cell frequency correlates with markers of HIV disease progression and attenuates anti-HIV CD8 + T cell function in vitro. J Leukoc Biol 93:811–818

Sindhava V, Woodman ME, Stevenson B, Bondada S (2010) Interleukin-10 mediated autoregulation of murine B-1 B-cells and its role in *B. hermsii* infection. PLoS ONE 5:e11445

Sing A, Rost D, Tvardovskaia N, Roggenkamp A, Wiedemann A, Kirschning CJ, Aepfelbacher M, Heesemann J (2002) Yersinia V-antigen exploits toll-like receptor 2 and CD14 for interleukin 10-mediated immunosuppression. J Exp Med 196:1017–1024

Sinha K, Bhatnagar R (2013) Recombinant GroEL enhances protective antigen-mediated protection against Bacillus anthracis spore challenge. Med Microbiol Immunol 202:153–165

Skinner JA, Pilione MR, Shen H, Harvill ET, Yuk MH (2005) Bordetella type III secretion modulates dendritic cell migration resulting in immunosuppression and bacterial persistence. J Immunol 175:4647–4652

Slobedman B, Barry PA, Spencer JV, Avdic S, Abendroth A (2009) Virus-encoded homologs of cellular interleukin-10 and their control of host immune function. J Virol 83:9618–9629

Soderberg-Naucler C, Fish KN, Nelson JA (1997) Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. Cell 91:119–126

Soderberg-Naucler C, Streblow DN, Fish KN, Allan-Yorke J, Smith PP, Nelson JA (2001) Reactivation of latent human cytomegalovirus in CD14(+) monocytes is differentiation dependent. J Virol 75:7543–7554

Spencer JV, Cadaoas J, Castillo PR, Saini V, Slobedman B (2008) Stimulation of B lymphocytes by cmvIL-10 but not LAcmvIL-10. Virology 374:164–169

Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, Schall TJ (2002) Potent immunosuppressive activities of cytomegalovirus- encoded interleukin-10. J Virol 76:1285–1292

Spera JM, Ugalde JE, Mucci J, Comerci DJ, Ugalde RA (2006) A B lymphocyte mitogen is a *B. abortus* virulence factor required for persistent infection. Proc Natl Acad Sci USA 103:16514–16519

Stager S, Maroof A, Zubairi S, Sanos SL, Kopf M, Kaye PM (2006) Distinct roles for IL-6 and IL-12p40 in mediating protection against *L. donovani* and the expansion of IL-10 + CD4 + T cells. Eur J Immunol 36:1764–1771

Sun XJ, Li R, Sun X, Zhou Y, Wang Y, Liu XJ, Lu Q, Zhou CL, Wu ZD (2012) Unique roles of *S. japonicum* protein Sj16 to induce IFN-gamma and IL-10 producing CD4(+)CD25(+) regulatory T cells in vitro and in vivo. Parasite Immunol 34:430–439

Tanner JE, Diaz-Mitoma F, Rooney CM, Alfieri C (1997) Anti-interleukin-10 antibodies in patients with chronic active Epstein-Barr virus infection. J Infect Dis 176:1454–1461

Taylor-Weideman J, Sissons JGP, Borysiewicz LK, Sinclair JH (1991) Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J Gen Virol 72:2059–2064

Trulzsch K, Sporleder T, Igwe EI, Russmann H, Heesemann J (2004) Contribution of the major secreted yops of *Y. enterocolitica* O:8 to pathogenicity in the mouse infection model. Infect Immun 72:5227–5234

Ubol S, Phuklia W, Kalayanarooj S, Modhiran N (2010) Mechanisms of immune evasion induced by a complex of dengue virus and preexisting enhancing antibodies. J Infect Dis 201:923–935

Ulsenheimer A, Gerlach JT, Gruener NH, Jung MC, Schirren CA, Schraut W, Zachoval R, Pape GR, Diepolder HM (2003) Detection of functionally altered hepatitis C virus-specific CD4 T cells in acute and chronic hepatitis C. Hepatology 37:1189–1198

van Beurden SJ, Forlenza M, Westphal AH, Wiegertjes GF, Haenen OL, Engelsma MY (2011) The alloherpesviral counterparts of interleukin 10 in European eel and common carp. Fish Shellfish Immunol 31:1211–1217

van der Kleij D, Latz E, Brouwers JF, Kruize YC, Schmitz M, Kurt-Jones EA, Espevik T, de Jong EC, Kapsenberg ML, Golenbock DT, Tielens AG, Yazdanbakhsh M (2002) A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. J Biol Chem 277:48122–48129

Vieira P, De Waal-Malefyt R, Dang M-N, Johnson KE, Kastelein R, Fiorentino DF, DeVries JE, Roncarolo M-G, Mosmann TR, Moore KW (1991) Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. Proc Natl Acad Sci USA 88:1172–1176

Vogel P, Weigler BJ, Kerr H, Hendrickx A, Barry PA (1994) Seroepidemiologic studies of cytomegalovirus infection in a breeding population of rhesus macaques. Lab Anim Sci 44:25–30

Wang J, Roderiquez G, Norcross MA (2012) Control of adaptive immune responses by *S. aureus* through IL-10, PD-L1, and TLR2. Sci Rep 2:606

Wei L, Wu S, Li Y, Chu Y, Huang R (2012) *S. enterica* serovar Typhi plasmid impairs dendritic cell responses to infection. Curr Microbiol 65:133–140

WHO (2008) The top 10 causes of death

Williamson ED (2012) The role of immune correlates and surrogate markers in the development of vaccines and immunotherapies for plague. Adv Prev Med 2012:365980

Wilson EB, Kidani Y, Elsaesser H, Barnard J, Raff L, Karp CL, Bensinger S, Brooks DG (2012) Emergence of distinct multiarmed immunoregulatory antigen-presenting cells during persistent viral infection. Cell Host Microbe 11:481–491

Wise L, McCaughan C, Tan CK, Mercer AA, Fleming SB (2007) Orf virus interleukin-10 inhibits cytokine synthesis in activated human THP-1 monocytes, but only partially impairs their proliferation. J Gen Virol 88:1677–1682

Wolfe DN, Karanikas AT, Hester SE, Kennett MJ, Harvill ET (2010) IL-10 induction by *B. parapertussis* limits a protective IFN-gamma response. J Immunol 184:1392–1400

Wussow F, Yue Y, Martinez J, Deere JD, Longmate J, Herrmann A, Barry PA, Diamond DJ (2013) A vaccine based on the Rhesus Cytomegalovirus UL128 complex induces broadly Neutralizing antibodies in Rhesus Macaques. J Virol 87:1322–1332

Yamamoto-Tabata T, McDonagh S, Chang HT, Fisher S, Pereira L (2004) Human cytomegalovirus interleukin-10 downregulates metalloproteinase activity and impairs endothelial cell migration and placental cytotrophoblast invasiveness in vitro. J Virol 78:2831–2840

Yoon SI, Logsdon NJ, Sheikh F, Donnelly RP, Walter MR (2006) Conformational changes mediate interleukin-10 receptor 2 (IL-10R2) binding to IL-10 and assembly of the signaling complex. J Biol Chem 281:35088–35096

Yu D, Silva MC, Shenk T (2003) Functional map of human cytomegalovirus AD169 defined by global mutational analysis. Proc Natl Acad Sci USA 100:12396–12401

Yusof WN, Nagaratnam M, Koh CL, Puthucheary S, Pang T (1993) Release of prostaglandin E2 by human mononuclear cells exposed to heat-killed *S. typhi*. Microbiol Immunol 37:667–670

Zhuravskaya T, Maciejewski JP, Netski DM, Bruening E, Mackintosh FR, St Jeor S (1997) Spread of human cytomegalovirus (HCMV) after infection of human hematopoietic progenitor cells: model of HCMV latency. Blood 90:2482–2491

IL-10: Achieving Balance During Persistent Viral Infection

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Abstract The clearance of viral infections is reliant on the coordination and balance of inflammatory factors necessary for viral destruction and immunoregulatory mechanisms necessary to prevent host pathology. In the case of persistent viral infections, immunoregulatory pathways prevent the immune response from clearing the virus, resulting in a long-term equilibrium between host and pathogen. Consequently, negative immune regulators are being considered as a therapeutic target to treat persistent and chronic viral infections. In this review, we will highlight the current understanding of the important negative immune regulator interleukin-10 (IL-10) in persistent viral infection. Though its main role for the host is to limit immune-mediated pathology, IL-10 is a multifunctional cytokine that differentially regulates a number of different hematopoietic cell types. IL-10 has been shown to play a role in a number of infectious diseases and many viral pathogens specifically exploit the IL-10 pathway to help evade host immunity. Recent advances have demonstrated that manipulation of IL-10 signaling during persistent viral infection can alter T cell responses *in vivo* and that this manipulation can lead to the clearance of persistent viral infection. Furthermore, there have been crucial advances in the understanding of factors that induce IL-10. We summarize lessons learned about IL-10 in model organisms and human persistent infections and conclude with the potential use of IL-10 to treat persistent viral infections.

Contents

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1 Introduction

Persistent viral infections are infections in which the host immune response is unable to clear the primary infection, resulting in a long-term equilibrium between the host and the virus. The equilibrium is the result of a number of factors that include host immune regulation, active suppression, immune evasion, and the level of viral replication. These factors are not static and can change over time depending on the actions of host immunity and the viral pathogen. As is apparent with HIV, the equilibrium can eventually be tipped in favor of the virus. Due to the disease severity of persistent infections, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), much research has been directed at elucidating the events that lead to persistent infection and how factors might be manipulated to tip the balance in favor of the host rather than the virus.

There are a variety of mechanisms to achieve persistence. Some viruses such as the herpes simplex viruses utilize latency in which the virus lies dormant within host cells to escape immunity resulting in low or no viral antigen except during periods of reactivation in which the virus reemerges. These reactivation periods are generally quickly controlled by the immune response. Other viral infections such as HIV, Hepatitis B virus (HBV), hepatitis C virus (HCV), and lymphocytic choriomeningitis virus (LCMV) rely on interfering with the function of the immune cells necessary for orchestrating anti-viral responses and clearing viral infection. These chronic infections are characterized by high levels of viral replication and high expression of negative immune regulators resulting in immune suppression of CD4 and CD8 T cell responses. Disruption in the generation of T cells results in delayed and/or failed clearance of virus and abrogation of T cell function contributes to the inability to control multiple persistent infections (Wherry 2011).

Murine infection with the enveloped RNA virus LCMV is a widely-used model to investigate T cell suppression during persistent/chronic viral infection. Developed in the 1980s, there are two well-characterized strains of LCMV used to compare and contrast immunologic factors unique to either acute or persistent viral infection, Armstrong 53b (ARM) and Clone 13 (Cl-13) (reviewed in (Dutko and Oldstone 1983; Oldstone and Campbell 2011)). These two strains differ by only three amino acids, but can result in very different disease outcomes. ARM induces a robust T cell response that clears the infection by 10 days post infection (Ahmed et al. 1984). Comparatively, Cl-13 replicates to much higher titers, inducing multiple host-based suppressive pathways, thereby generating a systemic persistent viremic infection that is not cleared from most tissues until 2–3 months later.

1.1 T Cell Immunity During Viral Infection

At the onset of a viral infection, the presence of virus is detected via innate immune receptors such as toll-like receptors which trigger an inflammatory response leading to the activation of the adaptive immune response. Antigen presenting cells such as dendritic cells (DCs) pick up the viral antigen and travel back to secondary lymphoid tissue (lymph nodes, spleen, peyer's patches, etc.) where they present captured antigen on MHC I and MHCII to activate virus-specific CD8 and CD4 T cells. Clearance or control of viral infection is facilitated by effective anti-viral CD4+ and CD8+ T cell responses (Berger et al. 2000; Matloubian et al. 1994; Tishon et al. 1993, 1995). Naïve CD8 T cells mature into cytotoxic T cells [CTLs] that express inflammatory and antiviral cytokines and kill infected cells. Naïve CD4 T cells differentiate into one of several types of helper T cells that have a variety of functions that include expression of inflammatory cytokines, activation/expansion of CTLs, and regulating B cell class switching. The magnitude of the T cell response is controlled by the balance of factors that increase the activation and activity of immune cells (i.e., positive immune regulators) and factors that inhibit or decrease activity (i.e., negative immune regulators). In viral infections that become persistent, T cell responses are fairly normal at the initial stages of infection. In the acute phase of Cl-13 infection at 5 dpi, the numbers of LCMV-specific T cells and their cytokine production resemble those of ARM. Anti-viral effector CD4 and CD8 T cells produce IFNγ, TNFα, and IL-2 in response to antigen stimulation and CD8 T cells exhibit the ability to lyse infected cells. However, soon after, negative regulation triggers a hyporesponsive state in T cells termed "exhaustion" which was first described in the LCMV system (Zajac et al. 1998). The exhaustive state is characterized by an hierarchical diminishment of functional capacity that includes loss of proliferative ability and progresses to a decreased ability to produce key antiviral and immune stimulatory cytokines, and decreased cytolytic activity (Brooks et al. 2005; Wherry et al. 2003; Zajac et al. 1998). It is a state separate from anergy or tolerance with a distinct pattern of cellular and transcriptional expression. By 9 dpi in persistent Cl-13 infected mice, there are significantly fewer LCMV-specific T cells, illustrating physical deletion. Those anti-viral T cells that remain express fewer cytokines and have lost their cytolytic abilities, exhibiting severe hypofunctionality (Wherry 2011). The degeneration of anti-viral T cell function is compounded by the decreased ability of the immune system to mount de novo immune responses, thereby hampering the generation of new anti-viral T cells as well as disabling the host's ability to fight secondary infections in most cases (Ahmed et al. 1984; Oldstone et al. 1988).

The development of exhaustion directly contributes to impaired control of viral infection, consequently, motivating extensive examination of T cell exhaustion. Transcriptional profiling comparing functionally active CTLs versus "exhausted" CTLs has uncovered differences in expression of a variety of genes for inhibitory receptors, transcription factors, metabolic pathways, chemotaxins, and migration factors (Wherry et al. 2007). Although many factors were found to be associated with T cell exhaustion, only two factors have been shown to have therapeutic impact when

either genetically deleted or neutralized alone *in vivo*: programmed death-1 (PD-1) and interleukin-10 (IL-10). PD-1 is a member of the CD28 receptor family expressed on activated T cells and negatively regulates these cells by binding its cognate ligands PD-L1 and PD-L2 found on the surface of other cell types including DCs and non-hematopoietic cells (Sharpe et al. 2007). High levels of PD-1 are found on the surface of exhausted CD4+ and CD8+ cells during HIV (Day et al. 2006; Trautmann et al. 2006), HBV (Peng et al. 2008), HCV (Urbani et al. 2006), and persistent LCMV infection (Barber et al. 2006). *Ex vivo* blockade results in resurrection of T cell function (Nakamoto et al. 2009; Trautmann et al. 2006; Tzeng et al. 2012; Urbani et al. 2006) and *in vivo* blockade in persistent LCMV leads to early clearance (Barber et al. 2006).

IL-10 is also a potent regulator of T cell exhaustion which we will focus on for the remainder of this review. IL-10 was first described in 1989 as a factor secreted by Th2 cells to inhibit cytokine secretion by Th1 cells (Fiorentino et al. 1989) highlighting IL-10's main role as an inflammatory cytokine. Since this first description, study of this cytokine has illuminated a variety of functions (Moore et al. 2001) revealing pleiotropic effects on different cell populations and with diverse suppressive roles during persistent viral infection (Ouyang et al. 2011; Wilson and Brooks 2011).

2 Establishing IL-10 as a Cause of Viral Persistence

2.1 IL-10 in LCMV

The first description of IL-10 as a major contributing factor to viral persistence came in 2005 from studies of LCMV (Brooks et al. 2006; Ejrnaes et al. 2006). The work stemmed from observations of the differential patterns of IL-10 expression during acute versus persistent infection. Serum IL-10 levels peak at equivalent levels in both ARM and CL-13 infected mice (Brooks et al. 2008b) and then quickly decrease by 2 and 3 days post-infection (Fig. 1). IL-10 levels in ARM continue to decrease over the next 12 days, while in Cl-13 infection these levels reverse to increase over the same period. The kinetics of IL-10 increase correlate with viremia (Brooks et al. 2008b; Wilson et al. 2012) and is concomitant with T cell exhaustion. Accordingly, blockade of this increase in IL-10 prevented T cell exhaustion, maintaining robust T cell responses, as measured by the number of anti-viral CD4 and CD8 T cells and their ability to produce IFN-γ, IL-2, and TNF-α. This augmented response resulted in early clearance of CL-13 infection (Brooks et al. 2006), establishing IL-10 as a major contributor to the decrease in virus-specific T cells and their function. Even after T cell exhaustion is established, blockade of IL-10 is able to rescue T cell activity and instigate early viral clearance Deletion of the *il10* gene has a similar effect, with *il10-/-* mice displaying greater numbers of anti-viral T cells as well as higher cytokine production compared to their wild-type counterparts (Brooks et al. 2006). These data established that IL-10 is necessary to both instill and maintain the immune suppressive state during persistent viral infection.

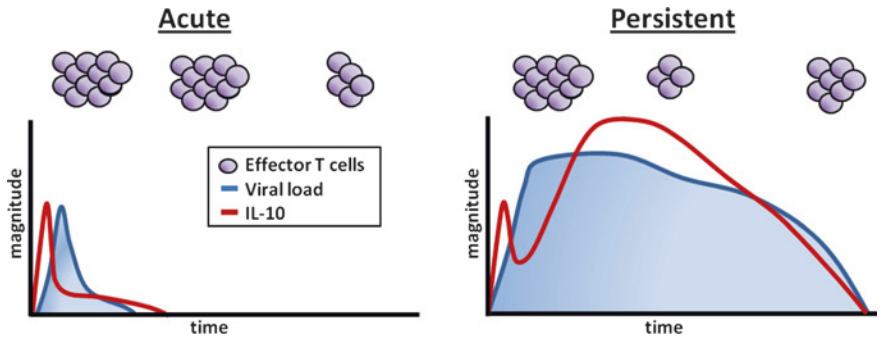

Fig. 1 IL-10 and T cell responses during acute versus persistent infection. IL-10 is initially by infection with both acute and persistent strains of LCMV. However, levels of IL-10 continue to increase in persistent viral infection, concomitantly with viral load. IL-10 supresses T cell responses and is a major contributor to T cell exhaustion in which T cells lose the capacity to secrete multiple cytokines and CTLs lose the capacity to lyse infected cells

Establishment of the role of IL-10 in LCMV persistence brought about renewed interest in the interactions of IL-10 during HIV, HCV, and HBV infections. Because it is difficult to perform tightly controlled experiments in humans, it has only been possible, thus far, to establish correlations between IL-10 and human viral infections. However, IL-10 is elicited during these infections as IL-10 correlates positively with viral load in HIV (Brockman et al. 2009; Orsilles et al. 2006), HBV (Peppa et al. 2010), and HCV (Akcam et al. 2012; Claassen et al. 2012; Reiser et al. 1997; Sofian et al. 2012).

2.2 IL-10 in HIV

The role of IL-10 during HIV infection is complex and multi-faceted. Although it is difficult to quantify the contribution of IL-10 to T cell exhaustion during HIV infection, *ex vivo* studies suggest that IL-10 is a major contributing factor. IL-10 expression positively correlates with HIV viremia (Brockman et al. 2009; Orsilles et al. 2006) and *ex vivo* blockade of IL-10 enhances activity of both CD4 and CD8 T cells isolated from HIV patients as measured by proliferation and expression of IFN-γ, TNF-α, and IL-2 (Bento et al. 2009; Brockman et al. 2009; Landay et al. 1996; Porichis et al. 2013; Yang et al. 2009). Notably, IL-10 blockade was effective for HIV patients with uncontrolled viremia, but not for those with highly controlled viremia (elite controllers or those on antiretroviral treatment) (Brockman et al. 2009), suggesting that HIV progression may be associated with IL-10-mediated immune suppression. However, this may be only during the pre-AIDS phase of infection, as IL-10 blockade was less successful at enhancing T cell responses in those with AIDS (<200 CD4 T cells/ul) (Landay et al. 1996).

In addition to modulating T cell function, IL-10 may negatively regulate the size of important immune cell populations. CD4 T cells from HIV-infected individuals

are highly sensitive to apoptosis (Clerici et al. 1994; Estaquier et al. 1995) and this sensitivity can be decreased by blocking IL-10, suggesting that IL-10 contributes to the depletion of this cell population. Not only does IL-10 control cell death in the T cell compartment, but also in the DC compartment. DCs produce IL-10 during HIV infection and this production targets them for lysis by NK cells (Alter et al. 2010). Hence, not only does IL-10 debilitate the anti-viral T cell response, but also enables the virus to directly target cells necessary to generate new anti-viral T cells.

The role of IL-10 in HIV extends beyond the suppression of immune cells. IL-10 has been observed to both induce and block viral replication depending on the cell type examined (Finnegan et al. 1996; Leghmari et al. 2008; Takeshita et al. 1995; Weissman et al. 1995). In infected macrophages/monocyte cell lines, exposure to IL-10 in the presence of TNFα induces viral replication (Finnegan et al. 1996; Weissman et al. 1995). Conversely, IL-10 was shown to block viral replication in *ex vivo* stimulated CD4 T cells (Bento et al. 2009). The variability of these observations may stem in part from the differential modulation of cellular receptors such as HIV co-receptors CCR5 and CXCR4, which are affected by IL-10 signaling (Kwon and Kaufmann 2010). Overall, it is clear that the role of IL-0 in HIV is not simple and is likely dependent on the cell-type.

2.3 IL-10 in HBV and HCV

The involvement of IL-10 in chronic HBV and HCV infections is controversial and has been difficult to study due to the shortage of small animal models. In chronic HBV-infected individuals, high serum IL-10 levels correlate with higher viral loads (Peppa et al. 2010). In the liver, the main site of HBV infection, IL-10 likely suppresses the activity of natural killer (NK) cells, which have a prominent population in this tissue (Maini and Peppa 2013). The suppression of NK activity, including their ability to produce IFN-γ, which is important to T cell activation (Maini and Peppa 2013), occurs in a manner that is inversely and temporally correlated with IL-10 levels (Dunn et al. 2009) and blockade of IL-10/IL-10R *ex vivo* can restore IFN-γ secretion by NK cells (Peppa et al. 2010). Thus, these data suggest that IL-10 regulates the behavior of NK cells in the liver.

Though whether IL-10 is a factor in suppression of anti-HCV responses is still under investigation, it is likely that there is some degree of contribution. IL-10 levels of HCV-infected patients are higher than those of uninfected individuals (Claassen et al. 2012; Reiser et al. 1997; Sofian et al. 2012). *Ex vivo* blockade of IL-10 is followed by improved function of exhausted HCV-specific T cells (Ludewig et al. 2012; Rigopoulou et al. 2005). Interestingly, long-term administration of IL-10 to individuals with HCV-related liver disease led to an increase in viral burden. The increase in viral titers was accompanied by the arrest of liver damage as demonstrated by a reduction in hepatic inflammation and fibrosis (Nelson et al. 2003), demonstrating that anti-HCV immunity is susceptible to control by HCV in the chronic phase. Further support for the role of IL-10 in chronic

HCV infection is derived from studies examining genetic polymorphisms that control the level of host IL-10 secretion. Although the data is highly variable, meta-analyses have demonstrated a correlation between susceptibility to chronic HCV and specific single nucleotide polymorphisms in the *il10* promoter, especially the IL-10–1082A/G polymorphism (Sun et al. 2013; Zhang et al. 2010). More research is necessary to establish the precise role of IL-10 in HBV and HCV infection and how this pathway can be manipulated to benefit the host.

3 Cellular Sources of IL-10 and Its Target(s)

A number of different lymphocyte populations are capable of producing IL-10 (Moore et al. 2001), however, the source(s) of IL-10 are likely dependent on the specific virus and the site, route, and cellular tropism of the particular infection as reflected by the differing profiles of IL-10 expression during different infection. For example, among acute viral infections, effector T cells in the lung secrete high levels of the cytokine during influenza infection (Sun et al. 2009), while splenic CD4 T cells are a major source of IL-10 during infection with west nile virus (Bai et al. 2009). During Cl-13 infection, one of the main producers of IL-10 during Cl-13 infection are the CD8α- subset of DCs (Ng and Oldstone 2012; Wilson et al. 2012). Comparatively, only minimal expression of IL-10 by DCs has been observed in ARM-infected mice (Brooks et al. 2006; Wilson et al. 2012). DC-specific deletion of *il10* results in a significant but not complete decrease in serum IL-10 levels (Ng and Oldstone 2012), suggesting that DCs are a major source of IL-10 but that other cell-types contribute as well (Ng and Oldstone 2012; Richter et al. 2013). A small percentage of B cells as well as CD4 T cells demonstrate IL-10 expression, while expression is negligable in CD8 T cells. Differing results have been observed with macrophages. However, it is interesting to note that antigen-presenting cells that express IL-10 also express high levels of other suppressive factors, including PD-L1 and indoleamine 2,3 dioxygenase (IDO) (Ng and Oldstone 2012; Wilson et al. 2012). Thus, when T cells interact with these cells, there is the delivery of multiple suppressive signals.

In relation to human infections, IL-10 is up-regulated by multiple cell types. During HIV infection, IL-10-producing cells include CD4 T cells, DCs, B cells, and natural killer (NK) cells (Brockman et al. 2009; Leghmari et al. 2008). Although this expression is likely due to host-programmed upregulation, HIV also may actively induce expression of IL-10. *In vitro* studies have demonstrated that monocytic cell lines and primary patient cells incubated with HIV proteins Tat, gp120, and nef upregulate expression of IL-10 (Blazevic et al. 1996; Borghi et al. 1995; Brigino et al. 1997; Contreras et al. 2004; Gee et al. 2007; Gupta et al. 2008; Masood et al. 1994; Schols and De Clercq 1996). In HBV infection, T cells and monocytes isolated from the blood of chronic HBV patients secrete IL-10 upon stimulation with HBV antigen (Hyodo et al. 2004) and increased levels of IL-10 are also elicited from the peripheral leukocytes of HCV-infected individuals

when exposed to the HCV core antigen (Barrett et al. 2008). While research has identified some sources of IL-10, further research is necessary to determine whether/how the identity of the IL-10 producing cell has impact on determining the specific downstream effects of IL-10.

Much has been elucidated regarding the effects of IL-10 in T cell function, yet the exact mechanism by which IL-10 elicits T cell exhaustion is not known as IL-10 can signal on multiple cell-types. Many lymphocytes express the receptor for IL-10 (IL-10R), including CD4 and CD8 T cells (Moore et al. 2001). Hence, there are several possibilities for the targets of IL-10 signaling. The first is signaling directly on T cells. In CL-13 infection, since DCs express IL-10, T cells that come into contact should be directly exposed to the cytokine. Surprisingly, specific deletion of IL-10R on T cells does not alleviate T cell exhaustion or lead to accelerated clearance of the virus (Ng and Oldstone, unpublished observation), suggesting that IL-10 signaling on T cells is not the route by which IL-10 elicits exhaustion. Comparatively, during acute infection with ARM, IL-10 signals directly on anti-viral CD4 T cells to limit the size of the effector and memory population (Brooks et al. 2010). *Ex vivo* blockade of IL-10R improves T cell function in cells from HIV- and HCV- infected patients (Brockman et al. 2009; Ludewig et al. 2012; Rigopoulou et al. 2005), however, these experiments have all been performed on PBMC, and thus, do not provide evidence on the target cell.

A second possibility for the mechanism of IL-10-mediated exhaustion is that IL-10 may suppress T cell function indirectly via antigen-presenting cells such as DCs. IL-10 signaling is known to inhibit maturation and expression of MHC I, MHC II, and stimulatory molecules CD80 and CD86 on monocytes and DCs (Chan et al. 2006; Koppelman et al. 1997) which in turn would affect their ability to fully activate T cells. DCs of CL-13 infected mice display a reduction in these molecules as well as a reduced capacity to activate T cells (Sevilla et al. 2000, 2004). However, the concept of IL-10 suppression of stimulatory molecules on DCs is not supported by current data as deletion of IL-10R on DCs does not alter their expression of stimulatory molecules during Cl-13 infection (Ng and Oldstone 2012). Thirdly, it is possible that IL-10 signaling on multiple cell-types is responsible for the observed exhaustion and that only by blocking signaling on all of these cells can T cell function be rescued. Indeed, cell-specific deletion of IL-10 signaling does not affect T cell exhaustion (unpublished observation), while IL-10R knockout mice and mice treated with anti-IL-10R antibody do not undergo T cell exhaustion and clear Cl-13 infection early (Brooks et al. 2006). Whether multiple cell types or an as yet-to-be determined single cell-type are the target(s) of IL-10 remains to be determined.

4 Triggers of IL-10 and T Cell Exhaustion

The precise molecular events that induce IL-10 and cause T cell exhaustion are still being examined. Data, thus far, suggests at least two factors. The first factor is viral antigen. In multiple infections, IL-10 expression and magnitude of expression is

correlated with viral load (Fig. 1) both longitudinally within an infected organism and across patient groups. Furthermore, prolonged exposure to high viral antigen load leads to T cell exhaustion (Fahey and Brooks 2010; Mueller and Ahmed 2009; Richter et al. 2012; Wherry 2011). LCMV studies examining CD8 T cell exhaustion have observed that increasing the number of antigen-presenting cells results in decreased T cell function, suggesting that the amount of antigen that the T cell encounters regulates functionality. The initiation of T cell exhaustion is not simply linked to the level of viremia, but to the amount and length of antigen presentation that is important in determining exhaustion. In combination with the amount of encountered antigen, the antigen-presenting cell may also play a role. Recognition of antigen on non-hematopoietic cells promotes T cell exhaustion. This is consistent with the observation that non-hematopoietic stromal cells in lymphoid tissue upregulate PD-L1 during Cl-13 infection, which prevents immunopathology (Mueller et al. 2007, 2010). Interestingly, these cells are implicated in regulation of peripheral tolerance and T cell activation.

The necessity of a high viral antigen load to trigger of T cell exhaustion emphasizes several points about virus-host interactions: (1) For a viral infection to become persistent, the virus must evade a functional T cell response long enough to replicate to levels that will trigger T cell exhaustion; (2) To prevent T cell exhaustion, the host must control viral replication to sufficiently low levels. The result of the virus-host interaction is likely determined very early on in infection and involves a family of cytokines until recently only perceived as anti-viral. Based on very recent work, the mechanism for how viral antigen triggers T cell exhaustion may involve the induction of type I interferons (IFN), such as IFNα and IFNβ. Two studies have elucidated a role for type I IFN in instituting viral persistence during LCMV infection (Teijaro et al. 2013; Wilson et al. 2013). Infection with both ARM and CL-13 initiates the production of IFNα in the first 24 h (Teijaro et al. 2013; Wilson et al. 2013). However, CL-13 infection produces significantly more IFNα and IFNβ, whereas ARM produces less IFNα and no measureable IFNβ, suggesting an association between persistent viruses and IFN-I signaling. Blockade of type I IFN significantly impacts a number of factors, including expression of negative immune regulators. At 1, 5, and 9 days post-infection, there is lower expression of IL-10 (Fig. 2) and PD-1 ligand despite significantly higher viral loads (Teijaro et al. 2013; Wilson et al. 2013). At 9 days post-infection when T cells are typically exhausted, type I IFN blockade results in significantly greater numbers of cytokine-producing anti-viral CD4 T cells despite significantly higher viral load and greater numbers of antigen presenting cells; CD8 T cells not only are initiated at a slower rate, but also exhibit a similar trend at later timepoints (Ng, Teijaro and Oldstone, unpublished observation). Surprisingly, these cells do not exhibit the characteristic signs of exhaustion despite prolonged exposure to high viral antigen loads. Ultimately, the contribution of these factors culminates in early clearance by around 40 versus 60 days post-infection. In essence, type I IFN is a master regulator, controlling expression of negative immune regulators and initiation of T cell exhaustion. Type I IFN blockade decouples the link between antigen exposure and T cell exhaustion suggesting that it is the ability of high viral load to elicit type I IFN that ultimately results in T cell exhaustion.

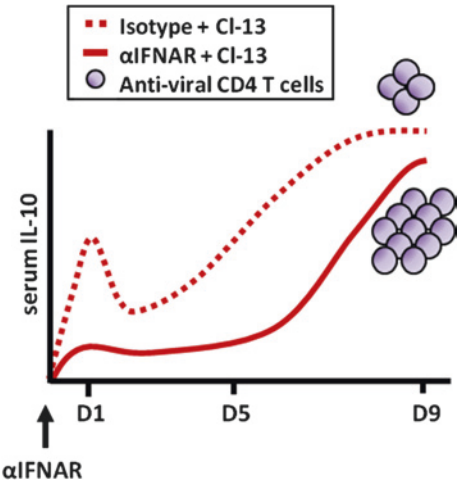

Fig. 2 Type I interferon (IFN) is a determinant of IL-10 expression and viral persistence. High expression levels of type I IFN predict high expression of IL-10. Early blockade of type I IFN signaling by an anti-IFN α/β receptor antibody results in the induction of significantly lower levels of serum IL-10 in the early phase of infection (day 1 and 5) and once persistence is established (day 9) compared to isotype control mice. This suggests that IL-10 expression is controlled by type I IFN. Concomitantly, treated mice exhibit greater numbers of anti-viral CD4 T cells. Treated mice clear Cl-13 infection at an accelerated rate in a CD4 T cell-dependent manner

5 Il-10 as a Therapeutic

Because IL-10 is a dominant suppressive pathway in the regulation of T cell exhaustion, it presents an interesting potential therapeutic target for persistent viral infections that are characterized by dampened T cell responses. Although the IL-10 pathway is an attractive target, manipulation of IL-10 must be balanced carefully to enhance anti-viral responses and with the need to minimize host tissue damage. T cell exhaustion severely hinders viral clearance; however, this strategy is likely a host-derived mechanism to prevent life-threatening immunopathology. Inhibition of the IL-10 pathway during LCMV not only enhances viral clearance, but also decreases survival. Importantly, the pleiotropic effect of IL-10 on different cell-types must also be considered as the function of various immune cells may be up- or down-regulated simultaneously. However, in proof of principle this strategy is viable. As discussed earlier, neutralization of IL-10 after the onset of T cell exhaustion, rescues CD4 and CD8 T cell responses and viral control. Human *in vivo* studies have yet to be performed, however, *in vitro* blockade of IL-10 is followed by improved function of exhausted T cells in HIV (Landay et al. 1996; Yang et al. 2009), HBV, and HCV. This treatment could be further improved upon by utilizing therapeutic vaccination. The combination of IL-10 blockade to relieve the immune suppressive environment with an LCMV-specific DNA vaccine to

stimulate immunity enhances both anti-LCMV CD4 and CD8 T cell responses leading to significant viral control that exceeds IL-10 blockade alone (Brooks et al. 2008b). This strategy is not only of interest for amplifying anti-viral responses, but also applicable for enhancing vaccine responses against other pathogens. HIV- and HCV-infected individuals have suboptimal immunological responses to vaccines such as influenza vaccination or HBV vaccination (De Sousa dos Santos et al. 2004; Malaspina et al. 2005; Moorman et al. 2011). Using blocking IL-10 signaling would likely improve the responses to vaccination resulting in better protection against secondary infection.

Another therapeutic consideration is the targeting of several inhibitory pathways to restore T cell function, since exhausted T cells express multiple inhibitory receptors. As discussed earlier, the PD-1 pathway is another dominant suppressive pathway that limits T cell function during persistent infections. PD-1 functions independently of the IL-10 pathway as evidenced by unchanged expression when the other pathway is manipulated. The simultaneous blockade of both pathways creates a synergistic effect that leads to faster clearance of persistent LCMV infection than the blockade of one pathway alone (Brooks et al. 2008a). Furthermore, blockade of either inhibitory receptor T-cell immunoglobulin domain and mucin domain 3 (Tim-3) or LAG-3, though ineffective alone (Blackburn et al. 2009; Jin et al. 2010; Leitner et al. 2013), have been shown to be effective at restoring T cell function when either are paired with PD-1 (Blackburn et al. 2009; Jin et al. 2010). Thus, it may be possible to utilize all four pathways to alleviate T cell exhaustion depending on the extent of hypofunctionality and potential immune pathology. Only further investigation of anti-IL-10 treatment will determine if it is a viable strategy to decrease T cell exhaustion in human chronic infections.

6 Conclusion

Collectively, the data presented in this review show that IL-10, though elicited early during most inflammatory events, is a prominent factor in persistent viral infections. In spite of the major advances in our understanding of IL-10 as a key suppressive pathway in persistent infection, there is still much left to be elucidated. First, it is important to understand how IL-10 is elicited from specific cellular subsets, because this likely determines the downstream effects of IL-10. Second, resolving the specific cellular mechanism by which IL-10 signals T cell exhaustion is crucial for attaining a complete understanding of this pathway and how it can be manipulated for therapeutic benefit. Lastly, the clinical contribution of IL-10 must be better quantitated to better define the potential impact of any manipulations of the IL-10 pathway, although this may prove difficult due to the relative lack of animal models in HIV, HBV, and HCV. Hopefully, these questions will be answered quickly to define the potential of this cytokine in the treatment of persistent viral infections.

References

Ahmed R, Salmi A, Butler LD, Chiller JM, Oldstone MB (1984) Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. Role in suppression of cytotoxic T lymphocyte response and viral persistence. J Exp Med 160:521–540

Akcam FZ, Tigli A, Kaya O, Ciris M, Vural H (2012) Cytokine levels and histopathology in chronic hepatitis B and chronic hepatitis C. J Interf Cytokine Res: Off J Int Soc Interf Cytokine Res 32:570–574

Alter G, Kavanagh D, Rihn S, Luteijn R, Brooks D, Oldstone M, van Lunzen J, Altfeld M (2010) IL-10 induces aberrant deletion of dendritic cells by natural killer cells in the context of HIV infection. J Clin Invest 120:1905–1913

Bai F, Town T, Qian F, Wang P, Kamanaka M, Connolly TM, Gate D, Montgomery RR, Flavell RA, Fikrig E (2009) IL-10 signaling blockade controls murine West Nile virus infection. PLoS Pathog 5:e1000610

Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439:682–687

Barrett L, Gallant M, Howley C, Bowmer MI, Hirsch G, Peltekian K, Grant M (2008) Enhanced IL-10 production in response to hepatitis C virus proteins by peripheral blood mononuclear cells from human immunodeficiency virus-monoinfected individuals. BMC Immunol 9:28

Bento CA, Hygino J, Andrade RM, Saramago CS, Silva RG, Silva AA, Linhares UC, Brindeiro R, Tanuri A, Rosenzwajg M et al (2009) IL-10-secreting T cells from HIV-infected pregnant women downregulate HIV-1 replication: effect enhanced by antiretroviral treatment. AIDS 23:9–18

Berger DP, Homann D, Oldstone MB (2000) Defining parameters for successful immunocytotherapy of persistent viral infection. Virology 266:257–263

Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ (2009) Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 10:29–37

Blazevic V, Heino M, Lagerstedt A, Ranki A, Krohn KJ (1996) Interleukin-10 gene expression induced by HIV-1 Tat and Rev in the cells of HIV-1 infected individuals. J Acquir Immune Defic Syndr Hum Retrovirol 13:208–214

Borghi P, Fantuzzi L, Varano B, Gessani S, Puddu P, Conti L, Capobianchi MR, Ameglio F, Belardelli F (1995) Induction of interleukin-10 by human immunodeficiency virus type 1 and its gp120 protein in human monocytes/macrophages. J Virol 69:1284–1287

Brigino E, Haraguchi S, Koutsonikolis A, Cianciolo GJ, Owens U, Good RA, Day NK (1997) Interleukin 10 is induced by recombinant HIV-1 Nef protein involving the calcium/calmodulin-dependent phosphodiesterase signal transduction pathway. Proc Natl Acad Sci U S A 94:3178–3182

Brockman MA, Kwon DS, Tighe DP, Pavlik DF, Rosato PC, Sela J, Porichis F, Le Gall S, Waring MT, Moss K et al (2009) IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. Blood 114:346–356

Brooks DG, Teyton L, Oldstone MB, McGavern DB (2005) Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. J Virol 79:10514–10527

Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB (2006) Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 12:1301–1309

Brooks DG, Ha SJ, Elsaesser H, Sharpe AH, Freeman GJ, Oldstone MB (2008a) IL-10 and PD-L1 operate through distinct pathways to suppress T-cell activity during persistent viral infection. Proc Natl Acad Sci U S A 105:20428–20433

Brooks DG, Lee AM, Elsaesser H, McGavern DB, Oldstone MB (2008b) IL-10 blockade facilitates DNA vaccine-induced T cell responses and enhances clearance of persistent virus infection. J Exp Med 205:533–541

Brooks DG, Walsh KB, Elsaesser H, Oldstone MB (2010) IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection. Proc Natl Acad Sci U S A 107:3018–3023

Chan A, Baird M, Mercer AA, Fleming SB (2006) Maturation and function of human dendritic cells are inhibited by orf virus-encoded interleukin-10. J Gen Virol 87:3177–3181

Claassen MA, de Knegt RJ, Turgut D, Groothuismink ZM, Janssen HL, Boonstra A (2012) Negative regulation of hepatitis C virus specific immunity is highly heterogeneous and modulated by pegylated interferon-alpha/ribavirin therapy. PLoS ONE 7:e49389

Clerici M, Sarin A, Coffman RL, Wynn TA, Blatt SP, Hendrix CW, Wolf SF, Shearer GM, Henkart PA (1994) Type 1/type 2 cytokine modulation of T-cell programmed cell death as a model for human immunodeficiency virus pathogenesis. Proc Natl Acad Sci U S A 91:11811–11815

Contreras X, Bennasser Y, Bahraoui E (2004) IL-10 production induced by HIV-1 Tat stimulation of human monocytes is dependent on the activation of PKC beta(II) and delta isozymes. Microbes Infect/Institut Pasteur 6:1182–1190

Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C et al (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443:350–354

De Sousa dos Santos S, Lopes MH, Simonsen V, Caiaffa Filho HH (2004) Haemophilus influenzae type b immunization in adults infected with the human immunodeficiency virus. AIDS Res Hum Retroviruses 20:493–496

Dunn C, Peppa D, Khanna P, Nebbia G, Jones M, Brendish N, Lascar RM, Brown D, Gilson RJ, Tedder RJ et al (2009) Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. Gastroenterology 137:1289–1300

Dutko FJ, Oldstone MB (1983) Genomic and biological variation among commonly used lymphocytic choriomeningitis virus strains. J Gen Virol 64(Pt 8):1689–1698

Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, von Herrath MG (2006) Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 203:2461–2472

Estaquier J, Idziorek T, Zou W, Emilie D, Farber CM, Bourez JM, Ameisen JC (1995) T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T cells from human immunodeficiency virus-infected persons. J Exp Med 182:1759–1767

Fahey LM, Brooks DG (2010) Opposing positive and negative regulation of T cell activity during viral persistence. Curr Opin Immunol 22:348–354

Finnegan A, Roebuck KA, Nakai BE, Gu DS, Rabbi MF, Song S, Landay AL (1996) IL-10 cooperates with TNF-alpha to activate HIV-1 from latently and acutely infected cells of monocyte/macrophage lineage. J Immunol 156:841–851

Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170:2081–2095

Gee K, Angel JB, Mishra S, Blahoianu MA, Kumar A (2007) IL-10 regulation by HIV-Tat in primary human monocytic cells: involvement of calmodulin/calmodulin-dependent protein kinase-activated p38 MAPK and Sp-1 and CREB-1 transcription factors. J Immunol 178:798–807

Gupta S, Boppana R, Mishra GC, Saha B, Mitra D (2008) HIV-1 Tat suppresses gp120-specific T cell response in IL-10-dependent manner. J Immunol 180:79–88

Hyodo N, Nakamura I, Imawari M (2004) Hepatitis B core antigen stimulates interleukin-10 secretion by both T cells and monocytes from peripheral blood of patients with chronic hepatitis B virus infection. Clin Exp Immunol 135:462–466

Jin HT, Anderson AC, Tan WG, West EE, Ha SJ, Araki K, Freeman GJ, Kuchroo VK, Ahmed R (2010) Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. Proc Natl Acad Sci U S A 107:14733–14738

Koppelman B, Neefjes JJ, de Vries JE, de Waal Malefyt R (1997) Interleukin-10 down-regulates MHC class II alphabeta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. Immunity 7:861–871

Kwon DS, Kaufmann DE (2010) Protective and detrimental roles of IL-10 in HIV pathogenesis. Eur Cytokine Netw 21:208–214

Landay AL, Clerici M, Hashemi F, Kessler H, Berzofsky JA, Shearer GM (1996) In vitro restoration of T cell immune function in human immunodeficiency virus-positive persons: effects of interleukin (IL)-12 and anti-IL-10. J Infect Dis 173:1085–1091

Leghmari K, Bennasser Y, Bahraoui E (2008) HIV-1 Tat protein induces IL-10 production in monocytes by classical and alternative NF-kappa B pathways. Eur J Cell Biol 87:947–962

Leitner J, Rieger A, Pickl WF, Zlabinger G, Grabmeier-Pfistershammer K, Steinberger P (2013) TIM-3 does not act as a receptor for Galectin-9. PLoS Pathog 9:e1003253

Ludewig B, Stein JV, Sharpe J, Cervantes-Barragan L, Thiel V, Bocharov G (2012) A global "imaging" view on systems approaches in immunology. Eur J Immunol 42:3116–3125

Maini MK, Peppa D (2013) NK cells: a double-edged sword in chronic hepatitis B virus infection. Front Immunol 4:57

Malaspina A, Moir S, Orsega SM, Vasquez J, Miller NJ, Donoghue ET, Kottilil S, Gezmu M, Follmann D, Vodeiko GM et al (2005) Compromised B cell responses to influenza vaccination in HIV-infected individuals. J Infect Dis 191:1442–1450

Masood R, Lunardi-Iskandar Y, Moudgil T, Zhang Y, Law RE, Huang CL, Puri RK, Levine AM, Gill PS (1994) IL-10 inhibits HIV-1 replication and is induced by tat. Biochem Biophy Res Commun 202:374–383

Matloubian M, Concepcion RJ, Ahmed R (1994) CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. J Virol 68:8056–8063

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Moorman JP, Zhang CL, Ni L, Ma CJ, Zhang Y, Wu XY, Thayer P, Islam TM, Borthwick T, Yao ZQ (2011) Impaired hepatitis B vaccine responses during chronic hepatitis C infection: involvement of the PD-1 pathway in regulating CD4(+) T cell responses. Vaccine 29:3169–3176

Mueller SN, Ahmed R (2009) High antigen levels are the cause of T cell exhaustion during chronic viral infection. Proc Natl Acad Sci U S A 106:8623–8628

Mueller SN, Matloubian M, Clemens DM, Sharpe AH, Freeman GJ, Gangappa S, Larsen CP, Ahmed R (2007) Viral targeting of fibroblastic reticular cells contributes to immunosuppression and persistence during chronic infection. Proc Natl Acad Sci U S A 104:15430–15435

Mueller SN, Vanguri VK, Ha SJ, West EE, Keir ME, Glickman JN, Sharpe AH, Ahmed R (2010) PD-L1 has distinct functions in hematopoietic and nonhematopoietic cells in regulating T cell responses during chronic infection in mice. J Clin Invest 120:2508–2515

Nakamoto N, Cho H, Shaked A, Olthoff K, Valiga ME, Kaminski M, Gostick E, Price DA, Freeman GJ, Wherry EJ et al (2009) Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. PLoS Pathog 5:e1000313

Nelson DR, Tu Z, Soldevila-Pico C, Abdelmalek M, Zhu H, Xu YL, Cabrera R, Liu C, Davis GL (2003) Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. Hepatology 38:859–868

Ng CT, Oldstone MB (2012) Infected CD8alpha-dendritic cells are the predominant source of IL-10 during establishment of persistent viral infection. Proc Natl Acad Sci U S A 109:14116–14121

Oldstone MB, Campbell KP (2011) Decoding arenavirus pathogenesis: essential roles for alpha-dystroglycan-virus interactions and the immune response. Virology 411:170–179

Oldstone MB, Salvato M, Tishon A, Lewicki H (1988) Virus-lymphocyte interactions. III. Biologic parameters of a virus variant that fails to generate CTL and establishes persistent infection in immunocompetent hosts. Virology 164:507–516

Orsilles MA, Pieri E, Cooke P, Caula C (2006) IL-2 and IL-10 serum levels in HIV-1-infected patients with or without active antiretroviral therapy. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica 114:55–60

Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG (2011) Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 29:71–109

Peng G, Li S, Wu W, Tan X, Chen Y, Chen Z (2008) PD-1 upregulation is associated with HBV-specific T cell dysfunction in chronic hepatitis B patients. Mol Immunol 45:963–970

Peppa D, Micco L, Javaid A, Kennedy PT, Schurich A, Dunn C, Pallant C, Ellis G, Khanna P, Dusheiko G et al (2010) Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathog 6:e1001227

Porichis F, Hart MG, Zupkosky J, Barblu L, Kwon DS, McMullen A, Brennan T, Ahmed R, Freeman GJ, Kavanagh DG et al (2013) Differential impact of PD-1 and/or IL-10 blockade on HIV-1-specific CD4 T cell and antigen-presenting cell functions. J Virol 88(5):2508–2518

Reiser M, Marousis CG, Nelson DR, Lauer G, Gonzalez-Peralta RP, Davis GL, Lau JY (1997) Serum interleukin 4 and interleukin 10 levels in patients with chronic hepatitis C virus infection. J Hepatol 26:471–478

Richter K, Brocker T, Oxenius A (2012) Antigen amount dictates CD8+ T-cell exhaustion during chronic viral infection irrespective of the type of antigen presenting cell. Eur J Immunol 42:2290–2304

Richter K, Perriard G, Behrendt R, Schwendener RA, Sexl V, Dunn R, Kamanaka M, Flavell RA, Roers A, Oxenius A (2013) Macrophage and T cell produced IL-10 promotes viral chronicity. PLoS Pathog 9:e1003735

Rigopoulou EI, Abbott WG, Haigh P, Naoumov NV (2005) Blocking of interleukin-10 receptor—a novel approach to stimulate T-helper cell type 1 responses to hepatitis C virus. Clin Immunol 117:57–64

Schols D, De Clercq E (1996) Human immunodeficiency virus type 1 gp120 induces anergy in human peripheral blood lymphocytes by inducing interleukin-10 production. J Virol 70:4953–4960

Sevilla N, Kunz S, Holz A, Lewicki H, Homann D, Yamada H, Campbell KP, de La Torre JC, Oldstone MB (2000) Immunosuppression and resultant viral persistence by specific viral targeting of dendritic cells. J Exp Med 192:1249–1260

Sevilla N, McGavern DB, Teng C, Kunz S, Oldstone MB (2004) Viral targeting of hematopoietic progenitors and inhibition of DC maturation as a dual strategy for immune subversion. J Clin Invest 113:737–745

Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ (2007) The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol 8:239–245

Sofian M, Aghakhani A, Farazi AA, Banifazl M, Eslamifar A, Rashidi N, Khadem Sadegh A, Ramezani A (2012) Serum profile of T helper 1 and T helper 2 cytokines in hepatitis C virus infected patients. Hepat Mon 12:e6156

Sun J, Madan R, Karp CL, Braciale TJ (2009) Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. Nat Med 15:277–284

Sun, X.R., Wu, J., Shi, K.Q., and Tang, K.F. (2013). Relationship between IL-10 gene −1082A/G and −592C/A polymorphisms and the risk of hepatitis C infection: a meta-analysis. J Viral Hepat n/a-n/a

Takeshita S, Breen EC, Ivashchenko M, Nishanian PG, Kishimoto T, Vredevoe DL, Martinez-Maza O (1995) Induction of IL-6 and IL-10 production by recombinant HIV-1 envelope glycoprotein 41 (gp41) in the THP-1 human monocytic cell line. Cell Immunol 165:234–242

Teijaro JR, Ng C, Lee AM, Sullivan BM, Sheehan KC, Welch M, Schreiber RD, de la Torre JC, Oldstone MB (2013) Persistent LCMV infection is controlled by blockade of type I interferon signaling. Science 340:207–211

Tishon A, Eddleston M, de la Torre JC, Oldstone MB (1993) Cytotoxic T lymphocytes cleanse viral gene products from individually infected neurons and lymphocytes in mice persistently infected with lymphocytic choriomeningitis virus. Virology 197:463–467

Tishon A, Lewicki H, Rall G, Von Herrath M, Oldstone MB (1995) An essential role for type 1 interferon-gamma in terminating persistent viral infection. Virology 212:244–250

Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, Boulassel MR, Delwart E, Sepulveda H, Balderas RS et al (2006) Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med 12:1198–1202

Tzeng HT, Tsai HF, Liao HJ, Lin YJ, Chen L, Chen PJ, Hsu PN (2012) PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model. PLoS ONE 7:e39179

Urbani S, Amadei B, Tola D, Massari M, Schivazappa S, Missale G, Ferrari C (2006) PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. J Virol 80:11398–11403

Weissman D, Poli G, Fauci AS (1995) IL-10 synergizes with multiple cytokines in enhancing HIV production in cells of monocytic lineage. J Acquir Immune Defic Syndr Hum Retrovirol 9:442–449

Wherry EJ (2011) T cell exhaustion. Nat Immunol 12:492–499

Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R (2003) Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol 77:4911–4927

Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, Kalia V, Subramaniam S, Blattman JN, Barber DL, Ahmed R (2007) Molecular signature of CD8+ T cell exhaustion during chronic viral infection. Immunity 27:670–684

Wilson EB, Brooks DG (2011) The role of IL-10 in regulating immunity to persistent viral infections. Curr Top Microbiol Immunol 350:39–65

Wilson EB, Kidani Y, Elsaesser H, Barnard J, Raff L, Karp CL, Bensinger S, Brooks DG (2012) Emergence of distinct multiarmed immunoregulatory antigen-presenting cells during persistent viral infection. Cell Host Microbe 11:481–491

Wilson EB, Yamada DH, Elsaesser H, Herskovitz J, Deng J, Cheng G, Aronow BJ, Karp CL, Brooks DG (2013) Blockade of chronic type I interferon signaling to control persistent LCMV infection. Science 340:202–207

Yang H, Guimaraes-Walker A, Hibbs S, Dong T, Stacey A, Borrow P, Hanke T, Davenport MP, McMichael A, Dorrell L (2009) Interleukin-10 responses to therapeutic vaccination during highly active antiretroviral therapy and after analytical therapy interruption. AIDS 23:2226–2230

Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R (1998) Viral immune evasion due to persistence of activated T cells without effector function. J Exp Med 188:2205–2213

Zhang LZ, Zhang TC, Pan FM, Zhang ZH, Li X (2010) Interleukin-10 gene polymorphisms in association with susceptibility to chronic hepatitis C virus infection: a meta-analysis study. Arch Virol 155:1839–1842

The Role of microRNAs in the Control and Mechanism of Action of IL-10

Susan R. Quinn and Luke A. O'Neill

Abstract Recent studies have shown an important interplay between Interleukin 10 (IL-10) and microRNAs. IL-10 can be directly post-transcriptionally regulated by several microRNA, including miR-106a, miR-4661, miR-98, miR-27, let7 and miR-1423p/5p. miRNA targeting of IL-10 has been suggested to play a role in autoimmune and inflammatory diseases such as SLE, reperfusion injury and asthma. Another miRNA, miR-21, has been shown to indirectly regulate IL-10 via downregulation of the IL-10 inhibitor PDCD4. The targeting of IL-10 in this way has been linked to host defence modulation by *Mycobacterium leprae*. Viral miRNAs, such as miR-K12-3 from Kaposi's sarcoma-associated herpesvirus (KSHV), can also decrease IL-10 to promote tumour development. Finally this interplay can operate in a feedback loop, with IL-10 capable of regulating microRNAs, upregulating those that can contribute to exerting the anti-inflammatory response, such as miR-187, and downregulating those that are highly pro-inflammatory, such as miR-155. Understanding the two-way regulation between miRNA and IL-10 is giving rise to new insights into this important cytokine.

Contents

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S. Fillatreau and A. O'Garra (eds.), *Interleukin-10 in Health and Disease*, Current Topics in Microbiology and Immunology 380, DOI: 10.1007/978-3-662-43492-5_7,

Interleukin-10 (IL-10) is an immunomodulatory cytokine, essential for negatively regulating and fine tuning inflammatory pathways and no factor can compensate for its loss (Murray 2006). It has been shown to be an essential and diverse orchestrator of the immune response but is primarily anti-inflammatory in many model systems (Asadullah et al. 2003). IL-10 is secreted from an abundance of different cell types including Th2 cells, regulatory T cells, B cells, macrophages, mast cells, eosinophils and dendritic cells. It is involved in many immune processes including antibody production, suppression of cytokine signalling, and inhibition of T cell activation. Dysregulation of IL-10 is likely to be important for various immunological diseases, such as rheumatoid arthritis, asthma and infectious disorders (Asadullah et al. 2003), therefore it is essential that IL-10 expression is tightly regulated.

Numerous levels of control of IL-10 have been found, including transcriptional control, involving silencing or enhancement of the promoter by transcription factors such as IRF3, Sp1 and Sp3 (Tone et al. 2000; Shinomiya et al. 2001) and post-transcriptional mechanisms of control. A key study has found that the 3'UTR of the IL-10 transcript is an important target for regulation (Powell et al. 2000). AU-rich elements in the 3'UTR of IL-10 lead to degradation of its mRNA, and these elements have been shown to be targeted by both RNA binding proteins (RBP) and microRNAs (miRNA) (Jing et al. 2005), resulting in mRNA instability which dictates their degradation. Here we discuss the targeting of IL-10 by microRNAs. We also discuss how IL-10 itself regulates miRNAs, this latter aspect potentially explains how IL-10 exerts its immunomodulatory effects, which remain poorly characterised in terms of mechanism. The interface between IL-10 and the world of miRNAs is revealing new insights into the control and mechanism of action of this important cytokine.

1 microRNAs

microRNAs were completely unknown just 20 years ago, and now a wealth of literature, research, clinical trials and biomarker studies are being carried out into their control and function. The first miRNA to be discovered was *lin-4* in *Caenorhabditis elegans,* a short RNA that is essential for negative regulation of the LIN14 gene in order for correct placental development (Lee et al. 1993; Wightman et al. 1993). The authors were surprised to discover this essential RNA coded for no protein. Instead it produced a 62-nucleotide (nt) long RNA hairpin, from which a 16 nt long fragment was excised, with antisense complementarity to the LIN-14 mRNA. An overview of miRNA biogenesis can be seen in Fig. 1. Further advances in this field caused researchers to believe this new phenomenon was restricted solely to *C. Elegans*, upon the subsequent discovery of let-7 which was shown to regulate developmental timing. It was not until 2001 that the breakthrough was made revealing miRNA conserved across all species of the metazoan kingdom, in three landmark papers (Lau et al. 2001; Lee and Ambros 2001; Lagos-Quintana et al. 2001). An explosion of interest developed in this field, as

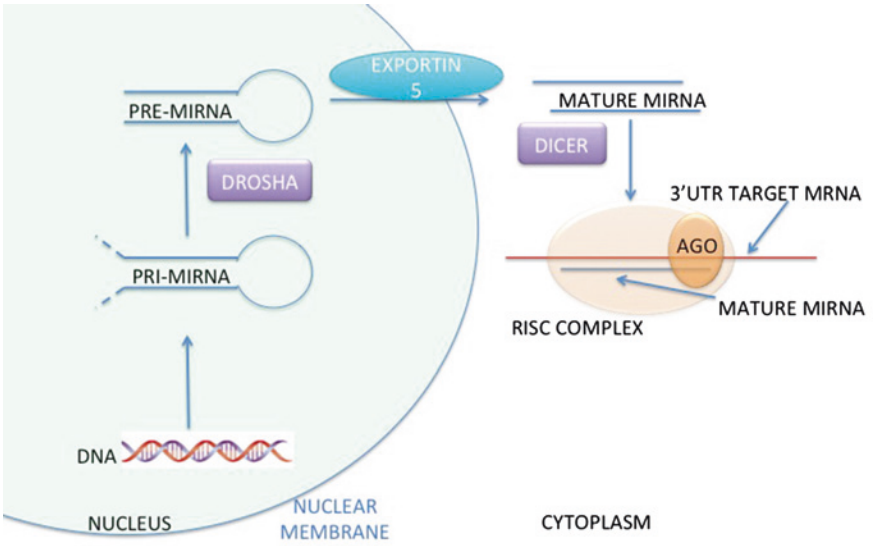

Fig. 1 The processing of miRNA. Pri-miRNAs are transcribed by RNA Polymerase II before post-transcriptionally cleaved by Drosha to the 65 base pre-miRNA form. Following exportation to the cytoplasm by Exportin-5, Dicer makes the final cleavage to the 21nt mature form. The mature form is loaded onto the RISC complex, and can now bind and degrade target mRNA

miRNA were discovered regulating all aspects of life, from apoptosis, to immunity, to aberrant expression in cancer (Schickel et al. 2008; Kent and Mendell 2006).

2 microRNA in the Immune Response

In terms of immunity, microRNAs were initially largely implicated in the differentiation of the immune system, consistent with other studies on miRNAs regulating development and differentiation in other systems. The earliest studies in this field revealed certain miRNAs restricted to the bone marrow, spleen and thymus, with miR-223 expression restricted to myeloid cells, and miR-181 expressed solely in B cells, where overexpression of miR-181 in progenitor cells led to a subsequent increase in B-cell production (Chen et al. 2004). Significant studies have emerged implicating various miRNA such as miR-155, miR-146a, miR-181a, and miR-223 in the development and differentiation of cells of the lymphoid and myeloid lineage (Lindsay 2008) and antibody switching (Thai et al. 2007). Pivotal studies by the Baltimore group (Taganov et al. 2006; O'Connell et al. 2007) assessed 200 miRNA for responsiveness to LPS, the Toll-like receptor 4 (TLR4) activator. Many miRNA were shown to be induced following TLR signalling. miR-155, along with miR-146a and

miR-132, are sharply upregulated in response to TLR4 and are also responsive to Poly I:C and various cytokines such as TNFα, ILIβ and IFNβ. An in vivo study subsequently investigated miRNA in lungs after exposure to LPS, and found twelve microRNA altered in response (Moschos et al. 2007). miR-146a was shown to affect TRAF6 and IRAK1 (Taganov et al. 2006), thereby having a negative feedback effect on TLR signalling. miR-155 was shown to have multiple targets including c-maf and TNF-α. In the case of c-maf miR-155 acted to limit the Th2 response, however, TNF-α mRNA was actually stabilised by miR-155. Both of the targets indicated a pro-inflammatory role of miR-155. miR-155 has subsequently been shown to target a number of inflammatory genes such as Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1) (O'Connell et al. 2009), a negative regulator of TLR4 signalling, thus allowing miR-155 to fine tune and feedback on the TLR4 induced immune response.

The targeting of IL-10 mRNA by miRNA, and the ability of IL-10 to modulate miRNAs was a highly likely prospect, given the overall role of miRNAs as 'fine-tuners' of immunity and inflammation.

3 Post-transcriptional Regulation of IL-10 by miRNA

A study by Sharma et al. (2009), predicted eight microRNAs have the potential to regulate IL-10 due to consensus seed sequence binding sites for these miRNA in the 3'UTR of IL-10, although examination of this regulation at a molecular level in their study only revealed one miRNA (miR-106a) having a significant ability to downregulate IL-10 in vitro, and the exact binding site on the IL-10 3'UTR was confirmed. Egr1 and Sp1 were implicated in the induction of miR-106a, and a negative correlation between IL-10 levels and Egr1-stimulated miR-106a levels was observed. This miRNA is part of a cluster that has been shown to be dysregulated in 46 % of human T cell leukemias, and the authors deduce that miR-106a may be modulating IL-10 expression that could promote leukemic cell survival. A subsequent study revealed that knock-down of miR-106a has the potential to alleviate symptoms in an asthma model, with the knockdown resulting in increased IL-10 levels in the lung, along with improved disease phenotype. There was decreased inflammation and hyper-responsiveness of airways, and decreased subepithelial fibrosis and goblet cell metaplasia in vivo (Sharma et al. 2012).

microRNA-466l has also been shown to regulate IL-10, but in this case has a positive effect. The binding of miR-466l to the 3'UTR of IL-10 results in a net increase in the half life of IL-10, by preventing Tristetraprolin binding. Tristetrapolin causes the degradation of the mRNA to which it binds. miR-466l will therefore increase the stability of the IL-10 mRNA (Ma et al. 2010) (Fig. 2).

In 2011 Liu et al. (2011) revealed a role for miR-98 in LPS-induced IL-10 production. miR-98 will bind to the IL-10 3'UTR and it was shown to be decreased

Fig. 2 Post Transcriptional control of IL-10 by miRNA. miR-4461 can stabilise *IL10* mRNA by competing with TTP for association with the AU-rich elements (AREs) in *IL10*. miR-106a, let7, miR-98 and miR-1423p/5p have been shown to directly target the 3′ untranslated region of *IL10*, leading to gene repression

upon LPS stimulation. miR-98 down-regulation by LPS may be essential for sufficient LPS-induced production of IL-10. The authors further showed inhibition of miR-98 expression is involved in diminishing induction of LPS tolerance, of which upregulation of IL-10 is a key hallmark.

4 IL-10 Post Transcriptional Regulation in Disease Phenotypes

Post-transciptional regulation of IL-10 by microRNA may be important in a number of diseases. The let-7 family of microRNAs can also directly regulate IL-10 (Swaminathan et al. 2012). Human Immunodeficiency Virus-1 (HIV-1) infected patients have significantly decreased levels of let-7 miRNAs, resulting in an increase in IL-10 production from CD4+ T cells. This could allow the virus to manipulate the host immune response, providing it with a significant survival advantage. Further studies have shown that let-7 targeting of IL-10 is also associated with the disease pathogenesis of a model of brain inflammation, Experimental Autoimmune Encephalomyelitis (EAE) (Guan et al. 2013), and that let-7c targeting IL-10 may additionally be associated with progression of Myasthenia gravis (MG), an autoimmune disease of neuromuscular junctions (Jiang et al. 2012).

In systemic lupus erythematosus (SLE), CD4+ T cells of patients with SLE had significantly downregulated levels of the microRNAs miR-142-3p and miR-142-5p compared to those of healthy controls, and this downregulation was accompanied by an inverse correlation of increased IL-10. Translation of IL-10 was shown to be directly inhibited by miR-142-3p/5p, and in the context of SLE, this caused reduced B cell hyperactivity and T-cell stimulation (Ding et al. 2012). The targeting of IL-10 by this range of miRNAs points to complexity in IL-10 regulation and is revealing an important insight into infectious and inflammatory disease.

5 Indirect Regulation of IL-10 by microRNA

Other miRNAs have been shown to impact on IL-10 production indirectly. miR-21 regulating PDCD4 is an example of this process, whereby LPS-induced miR-21 was found to target PDCD4, thus promoting IL-10 production (Sheedy et al. 2010) since PDCD4 is a negative regulator of IL-10. Mice deficient in PDCD4 were found to be protected from LPS lethality, which was likely due to increased IL-10 production in these mice. microRNAs were subsequently profiled in Systemic Lupus Erythematosus (SLE), and miR-21 found to be linked to disease activity and pathogenesis, and PDCD4 was suppressed in SLE as a result, again causing enhanced IL-10 production (Stagakis et al. 2011). Hsa-miR-21 has also been shown to indirectly upregulate IL-10 in leprosy. A 25 fold increase in miR-21 was reported in L-lep (progressive lepromatous) versus T-lep (self-limited tuberculoid) forms for disease (Liu et al. 2012). This led to a direct decrease in inflammatory mediators such as IL1-β, and an indirect enhancement of IL-10 induction, which led to the subsequent inhibition of CAMP and DEFB4a, two vitamin-D dependent antimicrobial peptides. This may partially contribute to the inhibition of antimicrobial gene expression and escape from the vitamin-D dependent antimicrobial pathway observed in L-lep. miR-27 has been shown to indirectly target the IL-10 pathway in an inflammatory state termed reperfusion injury (Yeh et al. 2012), resulting in increased apoptosis. In astrocytes, miR-181 has been shown to downregualte anti-inflammatory targets including IL-10, through targeting of MeCP2 and X-linked inhibitor of apoptosis, and an elevation of miR-181 has been observed in the brains of Alzheimers disease, possibly contributing to disease pathogenesis (Hutchison ER et al. 2013).

6 Viruses and microRNA: Implications for IL-10

A number of viruses have taken advantage of the global and subtle control miRNAs exert in the cell by encoding their own viral homologues of miRNAs. Kaposi's Sarcoma-associated herpesvirus (KSHV) encodes 17 mature microRNAs (Samols et al. 2005; Cai et al. 2005), and these, like their human homologues, have the ability to regulate cytokine production in the host. A study of KSHV-encoded microRNA revealed two viral microRNA, miR-K12-3 and miR-K12-7, activate transcription and secretion of IL-10 (Qin et al. 2010). These miRNA were found to be acting indirectly to regulate cytokine production by controlling key elements in the C/EBPβ p20 isoform 3'UTR. C/EBPβ p20 lacks a transactivation domain and is believed to function as a dominant-negative transcriptional repressor of activation. Therefore, as C/EBPβ p20 inhibits IL-10 transcription, this leads to an increase in IL-10 production, favouring an environment complimentary to KSHV-promoted tumour progression. Figure 3 illustrates this link between viral miRNA and IL-10.

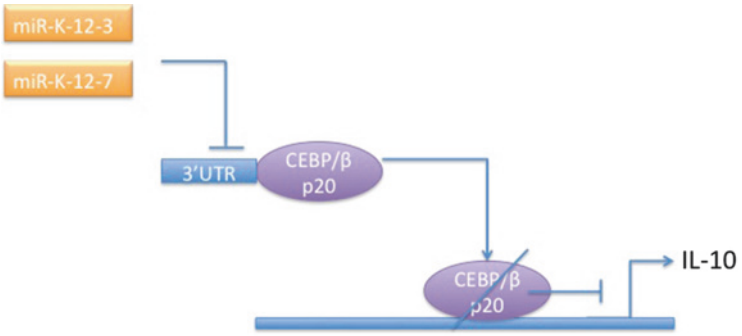

Fig. 3 Viral miRNA targets IL-10. miR-K-12-3/7 target and downregulate the 3′ UTR of CEBP/β p20, a negative regulator of IL-10, thus resulting in increased IL-10 production

Viruses have also been shown to regulate host miRNA. Human cytomegalovirus (HCMV) decreases miR-92a, which has been found to act indirectly to regulate IL-10. In the context of HCMV latency, altered miR-92a results in increased GATA-2 and thus increased cellular IL-10, which allows the virus to prolong survival by maintaining the latent viral genome (Poole et al. 2011). Secreted IL-10 has the ability to prolong the latent genome of the virus, which the authors speculate may be due to increased survival of the latently infected CD34+ population.

7 Regulation of microRNAs by IL-10

The interface between miRNAs and IL-10 is also evident in the effect on IL-10 on microRNAs, although little has been elucidated to date. The very first study implicating a role for IL-10 in miRNA regulation somewhat surprisingly did not uncover any IL-10 sensitive miRNAs. However, IL-10 was shown to potently downregulate the induction of miR-155 by LPS (McCoy et al. 2010). As described above, miR-155 is a pro-inflammatory microRNA. Mice deficient in miR-155 cannot generate protective immunity (Rodriguez et al. 2007), and are highly protected from immune related disorders such as EAE (O'Connell et al. 2010). IL-10 was found to directly downregulate this microRNA after LPS-mediated induction, and miR-155 expression doubled in IL-10 deficient cells. Through targeting of miR-155, IL-10 gained control of SHIP1, an important target for miR-155 which negatively regulates TLR4 signalling. SHIP1 levels were shown to be increased via the reduction in its repressor miR-155. The effect was shown to be specific, in that there was no inhibition in the induction of miR-146a or miR-21 in response. Since, as stated, both miR-146a (via targeting of IRAK and TNF-α) and miR-21 (via targeting of PDCD4 with subsequent boosting of IL-10) are anti-inflammatory, this specificity makes biological sense, as if they were to be targeted by IL-10, a pro-inflammatory effect would result. miR-155 targets a number

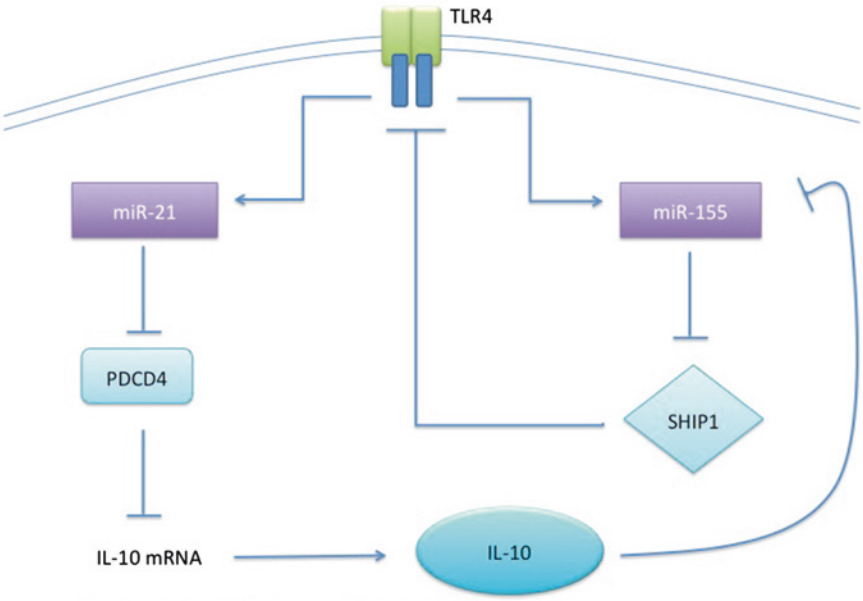

Fig. 4 IL-10 and microRNA's fine-tune TLR4 signalling. TLR4 induces miR-21, which through inhibition of PDCD4, can induce IL-10. IL-10 can then specifically inhibit miR-155, thus increasing its target SHIP1, which results in decreased TLR4 signalling

of genes involved in the immune response, such as BACH1, SOCS, IKBKE and FADD, so the targeting of this miRNA by IL-10 is likely to elucidate key mechanisms through which IL-10 exerts control in the cell, much of which remains to be uncovered. Figure 4 illustrates the control of miRNAs by IL-10, coupled with miR-21 controlling IL-10 through PDCD4. TLR4 activation increases the levels of miR-155 and miR-21 (O'Connell et al. 2007). miR-21 has been shown to target PDCD4, thus increasing IL-10, which can specifically inhibit miR-155. This then leads to an increase in SHIP1, and the subsequent inhibition of TLR4, revealing how miRNAs and IL-10 can act in a feedback loop to control TLR signalling. An additional study examining the effect of IL-10 on microRNA profiled IL-10 deficient mice for expression of 600 microRNA. 10 microRNA's were found to be upregulated in the IL-10 deficient mice (miR-19a, miR-21, miR-31, miR-101, miR-223, miR-326, miR-142-3p, miR-142-5p, miR-146a, and miR-155) (Schaefer et al. 2011). This was accompanied by inflammation of the colon in a model of inflammatory bowel disease in the IL-$10^{-/-}$ mice. This study further uncovered details of the IL-10 pathway. miR-223 can inhibit the 3'UTR of Roquin ubiquitin ligase, which regulates IL-17, thus revealing the mechanism by which IL-10 modulates IL-17 is through miR-223. Thus studies of microRNA can assist in revealing the complexity of pathways through which IL-10 operates.

IL-10 can additionally induce microRNAs which are anti-inflammatory, such as miR-198 (Rossato et al. 2012). miR-198 acts to suppress TNF-α, IL-6 and

IL-12p40, and thus IL-10 can manipulate microRNA in order to promote an anti-inflammatory environment in the cell. These results demonstrate an important role for miR-187 in the physiological regulation of IL-10–driven anti-inflammatory responses, and uncover another microRNA-mediated pathway controlling cytokine expression. Further studies in this area and the connection between IL-10 and microRNAs will continue to provide exciting new insights into the mechanistic details of the potent actions of IL-10 as in immunomodulatory cytokine.

8 Final Perspectives

Studies on the interface between IL-10 and microRNAs have revealed a key ability of miRNA, as global fine-tuners of gene expression, to modulate and control levels of IL-10 in the cell. The result of this control is altered IL-10 in order to regulate inflammation and promote host defence. Viral miRNAs can also control IL-10, to promote their own survival in the host. IL-10 can also alter levels of miRNAs, a notable example being miR-155, the targeting of which contributes to the anti-inflammatory mechanism of IL-10. These findings add a further level of complexity and control to the immune response, and the orchestration of successful resolution of inflammation. Further investigation of this control is likely to provide new insights into this important cytokine and will prove key to develop strategies to manipulate IL-10 in a clinical context.

References

Asadullah K, Sterry W, Volk HD (2003a) Interleukin-10 therapy–review of a new approach. Pharmacol Rev 55(2):241–269

Cai X et al (2005) Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci USA 102(15):5570–5575

Chen CZ et al (2004) microRNAs modulate hematopoietic lineage differentiation. Science 303(5654):83–86

Ding S et al (2012) Decreased microRNA-142-3p/5p expression causes CD4+ T cell activation and B cell hyperstimulation in systemic lupus erythematosus. Arthritis Rheum 64(9):2953–2963

Guan H et al (2013) MicroRNA let-7e is associated with the pathogenesis of experimental autoimmune encephalomyelitis. Eur J Immunol 43(1):104–114

Hutchison ER et al (2013) Evidence for miR-181 involvement in neuroinflammatory responses of astrocytes. Glia 61(7):1018–1028

Jiang L et al (2012) Altered let-7 expression in Myasthenia gravis and let-7c mediated regulation of IL-10 by directly targeting IL-10 in Jurkat cells. Int Immunopharmacol 14(2):217–223

Jing Q et al (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell 120(5):623–634

Kent OA, Mendell JT (2006) A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. Oncogene 25(46):6188–6196

Lagos-Quintana M et al (2001) Identification of novel genes coding for small expressed RNAs. Science 294(5543):853–858

Lau NC et al (2001) An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 294(5543):858–862
Lee RC, Ambros V (2001) An extensive class of small RNAs in Caenorhabditis elegans. Science 294(5543):862–864
Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854
Lindsay MA (2008) microRNAs and the immune response. Trends Immunol 29(7):343–351
Liu Y et al (2011) MicroRNA-98 negatively regulates IL-10 production and endotoxin tolerance in macrophages after LPS stimulation. FEBS Lett 585(12):1963–1968
Liu PT et al (2012) MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. Nat Med 18(2):267–273
Ma F et al (2010) MicroRNA-466l upregulates IL-10 expression in TLR-triggered macrophages by antagonizing RNA-binding protein tristetraprolin-mediated IL-10 mRNA degradation. J Immunol 184(11):6053–6059
McCoy CE et al (2010) IL-10 inhibits miR-155 induction by toll-like receptors. J Biol Chem 285(27):20492–20498
Moschos SA et al (2007) Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. BMC Genom 8:240
Murray PJ (2006) Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr Opin Pharmacol 6(4):379–386
O'Connell RM et al (2007) MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci USA 104(5):1604–1609
O'Connell RM et al (2009) Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci USA 106(17):7113–7118
O'Connell RM et al (2010) MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity 33(4):607–619
Poole E et al (2011) Virally induced changes in cellular microRNAs maintain latency of human cytomegalovirus in CD34(+) progenitors. J Gen Virol 92(Pt 7):1539–1549
Powell MJ et al (2000) Posttranscriptional regulation of IL-10 gene expression through sequences in the 3'-untranslated region. J Immunol 165(1):292–296
Qin Z et al (2010) Pivotal advance: Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded microRNA specifically induce IL-6 and IL-10 secretion by macrophages and monocytes. J Leukoc Biol 87(1):25–34
Rodriguez A et al (2007) Requirement of bic/microRNA-155 for normal immune function. Science 316(5824):608–611
Rossato M et al (2012) IL-10-induced microRNA-187 negatively regulates TNF-alpha, IL-6, and IL-12p40 production in TLR4-stimulated monocytes. Proc Natl Acad Sci USA 109(45):E3101–E3110
Samols MA et al (2005) Cloning and identification of a microRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus. J Virol 79(14):9301–9305
Schaefer JS et al (2011) Selective upregulation of microRNA expression in peripheral blood leukocytes in IL-10-/- mice precedes expression in the colon. J Immunol 187(11):5834–5841
Schickel R et al (2008) microRNAs: key players in the immune system, differentiation, tumorigenesis and cell death. Oncogene 27(45):5959–5974
Sharma A et al (2009) Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a. Proc Natl Acad Sci USA 106(14):5761–5766
Sharma A et al (2012) Antagonism of mmu-mir-106a attenuates asthma features in allergic murine model. J Appl Physiol 113(3):459–464
Sheedy FJ et al (2010) Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol 11(2):141–147
Shinomiya S et al (2001) Regulation of TNFalpha and interleukin-10 production by prostaglandins I(2) and E(2): studies with prostaglandin receptor-deficient mice and prostaglandin E-receptor subtype-selective synthetic agonists. Biochem Pharmacol 61(9):1153–1160

Stagakis E et al (2011) Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. Ann Rheum Dis 70(8):1496–1506

Swaminathan S et al (2012) Differential regulation of the Let-7 family of microRNAs in CD4+ T cells alters IL-10 expression. J Immunol 188(12):6238–6246

Taganov KD et al (2006) NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA 103(33):12481–12486

Thai TH et al (2007) Regulation of the germinal center response by microRNA-155. Science 316(5824):604–608

Tone M et al (2000) IL-10 gene expression is controlled by the transcription factors Sp1 and Sp3. J Immunol 165(1):286–291

Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 75(5):855–862

Yeh CH et al (2012) microRNA-27a regulates cardiomyocytic apoptosis during cardioplegia-induced cardiac arrest by targeting interleukin 10-related pathways. Shock 38(6):607–614

The Regulation of IL-10 Expression

Leona Gabryšová, Ashleigh Howes, Margarida Saraiva and Anne O'Garra

Abstract Interleukin (IL)-10 is an important immunoregulatory cytokine and an understanding of how IL-10 expression is controlled is critical in the design of immune intervention strategies. IL-10 is produced by almost all cell types within the innate (including macrophages, monocytes, dendritic cells (DCs), mast cells, neutrophils, eosinophils and natural killer cells) and adaptive (including $CD4^+$ T cells, $CD8^+$ T cells and B cells) immune systems. The mechanisms of IL-10 regulation operate at several stages including chromatin remodelling at the *Il10* locus, transcriptional regulation of *Il10* expression and post-transcriptional regulation of *Il10* mRNA. In addition, whereas some aspects of *Il10* gene regulation are conserved between different immune cell types, several are cell type- or stimulus-specific. Here, we outline the complexity of IL-10 production by discussing what is known about its regulation in macrophages, monocytes, DCs and $CD4^+$ T helper cells.

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Contents

List of Abbreviations

AHR	Aryl hydrocarbon receptor
AP-1	Activator protein 1
APC	Antigen presenting cell
ARE	AU rich element
ATF	Activating transcription factor 1
AUF-1	ARE/poly(U) binding degradation factor 1
BATF	Basic leucine zipper transcription factor ATF-like transcription factor
BCL-6	B-cell lymphoma 6
BLIMP-1	PR domain zinc finger protein 1
BM	Bone marrow
C/EBP	CCAAT/enhancer binding protein
cAMP	Cyclic adenosine monophosphate
CBP	CREB-binding protein
CD40L	CD40 ligand
ChIP-Seq	Chromatin immunoprecipitation-sequencing
CNS	Conserved non-coding sequences
CREB	cAMP response element-binding protein
CRTC3	CREB-regulated transcription coactivator 3
DC	Dendritic cell
DLL	Delta-like Notch ligands
DRE	Distal regulatory element
DUSP1	Dual specificity phosphatase-1
E4BP4	E4 promoter-binding protein 4
ERK	Extracellular signal-regulated kinase
E. coli	*Escherichia coli*
ETS-1	E26 transformation-specific 1
GATA3	GATA binding protein 3
GM-CSF	Granulocyte-monocyte colony stimulating factor
GSK3	Glycogen synthase kinase 3
HAT	Histone acetyl transferase
HDAC	Histone deacetylase

HMT	Histone methyl transferase
HSS	DNaseI hypersensitive sites
ICOS	Inducible T cell costimulator
IFN	Interferon
IL	Interleukin
IRF	Interferon regulatory factor
JDP	Jun dimerising protein
LPS	Lipopolysaccharide
MAP kinase	Mitogen-activated protein kinase
MARE	C-MAF responsive element
mDC	Myeloid dendritic cell
MHC	Major histocompatibility complex
miRNA	MicroRNA
MSK1/2	Mitogen- and stress-activated protein kinases 1/2
mTOR	Mammalian target of rapamycin
M. tuberculosis	*Mycobacterium tuberculosis*
MyD88	Myeloid differentiation factor 88
NF-κB	Nuclear factor-κB
NFAT	Nuclear factor of activated T cells
PBX1	Pre-B cell leukaemia homeobox 1
pDC	Plasmocytoid dendritic cell
PDCD4	Programmed cell death 4
PGE2	Prostaglandin E2
PI(3)K	Phosphatidylinositol 3 kinase
PKA	Protein kinase A
PKR	Protein kinase R
PREP1	PBX-regulating protein 1
PRR	Pattern recognition receptor
RORγt	RAR-related orphan receptor gamma t
SIK2	Salt-inducible kinase 2
Sp1/3	Specific protein 1/3
STAT	Signal transducer and activator of transcription
SWI/SNF	Switching-defective-sucrose non-fermenting
SYK	Spleen tyrosine kinase
TBET	T-box transcription factor
TCR	T cell receptor
Tfh	T follicular helper cell
TGF	Transforming growth factor
Th	T helper cell
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TPL-2	Tumour progression locus 2
TRIF	TIR-domain-containing adapter-inducing interferon-β
TSS	Transcription start site
TTP	Tristetraprolin
UTR	Untranslated region

1 Introduction

The immune system has evolved to protect the host from a wide range of potentially pathogenic microorganisms. However, immune responses also have the potential to cause damage to the host if not adequately regulated. Interleukin-10 (IL-10) is an anti-inflammatory cytokine with a crucial role in preventing inflammatory and autoimmune pathologies by limiting the immune response to pathogens and microbial flora. However, the production of IL-10 can also inappropriately restrict protective immune responses and, in this context, IL-10 can contribute to chronic infection (Moore et al. 2001). Thus, IL-10 is an important immunoregulatory cytokine and an understanding of how IL-10 expression is regulated is critical in the design of immune intervention strategies.

IL-10 is produced by cells within both the innate (including macrophages, monocytes, dendritic cells (DCs), mast cells, neutrophils, eosinophils and natural killer cells) and adaptive (including $CD4^+$ T cells, $CD8^+$ T cells and B cells) immune systems (Saraiva and O'Garra 2010). Cells within the innate immune system require stimulation by pathogen-derived products via pattern recognition receptors (PRR) to induce IL-10 production (Saraiva and O'Garra 2010). T cells recognise microbial peptides presented in the context of major histocompatibility complexes (MHC) by antigen presenting cells (APC) including macrophages and DCs through the interaction with their T cell receptor (TCR). In contrast to the innate cells that can immediately produce IL-10 in response to environmental stimuli, naïve $CD4^+$ T cells first need to differentiate into the different T helper (Th) cell subsets, including Th1, Th2 and Th17 cells (Zhu et al. 2010), in order to produce IL-10 (Saraiva and O'Garra 2010).

The mechanisms of IL-10 regulation operate at several stages including chromatin remodelling at the *Il10* locus, transcriptional regulation of *Il10* expression, and post-transcriptional regulation of *Il10* mRNA. In addition, whereas some aspects of *Il10* gene regulation are conserved between different immune cell types, several may be cell type- or stimulus-specific. Here, we outline the complexity of IL-10 regulation by discussing what is known about its regulation in macrophages, monocytes, DCs and $CD4^+$ Th cells.

2 Concepts of Transcriptional Gene Regulation

Within chromosomes, DNA is wrapped around octamers of core histones to form nucleosomes, the basic units of eukaryotic chromatin. Multiple nucleosomes are arranged in a linear array along the DNA referred to as 'beads on a string' (accessible euchromatin) that can be further compacted into 30 nm fibres (inaccessible heterochromatin) upon the addition of linker histones (Jiang and Pugh 2009) (Fig. 1). Factors that influence the accessibility of chromatin include DNA methylation, nucleosome remodelling and covalent histone modifications

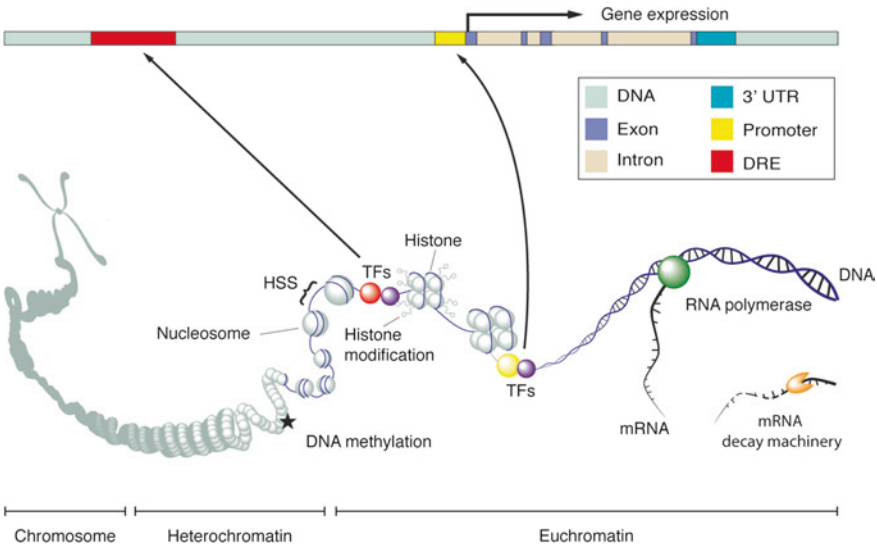


Fig. 1 *Concepts of gene regulation*. Mechanisms governing gene expression operate at several levels including chromatin accessibility, active regulation of transcription as well as post-transcriptional regulation. Within chromosomes, DNA is wrapped around octamers of core histones forming nucleosomes, the basic units of eukaryotic chromatin. Multiple nucleosomes are then arranged along the DNA as 'beads on a string' (accessible euchromatin). The compaction of chromatin into 30 nm fibres is achieved upon the addition of linker histones to nucleosomes giving rise to inaccessible, transcriptionally inactive heterochromatin. Depicted are features governing the accessibility of chromatin including DNA methylation, histone modifications or DNase HSS. Open chromatin allows for transcription factor binding to gene regulatory elements such as DRE or promoters and initiation of gene expression by RNA polymerase. Once transcribed, mRNA can further be subject to post-transcriptional regulation at the 3'UTR by AU rich (ARE)-binding proteins that increase or decrease mRNA stability by preventing or aiding its degradation by RNA decay machinery, respectively

such as acetylation or methylation (Jenuwein and Allis 2001) (Fig. 1). Histone modifications, usually referred to as the histone code, have been reported to be associated with different functional elements such as promoters or distal regulatory elements (DRE), also known as enhancers or insulators, and are key to gene regulation (Lenhard et al. 2012) (Fig. 1). Histone modifying enzymes such as histone acetyl transferases (HAT) and deacetylases (HDAC) or histone methyl transferases (HMT) alter histone 'tails' to form repressive or active marks, remodelling the chromatin from high density transcriptionally inactive heterochromatin to low density transcriptionally active euchromatin and vice versa thus governing gene expression (Jenuwein and Allis 2001). Changes in the chromatin landscape can be monitored by DNaseI treatment that marks nucleosome-poor regions, indicative of 'open' chromatin, known as hypersensitive sites (HSS), where transcription factors can interact with the exposed regulatory DNA sequences (Fig. 1).

3 Structure of the IL-10 Gene Locus

The genes encoding both human and murine IL-10 are located within the *Il10* gene family cluster on Chromosome 1 of the respective genomes (Kim et al. 1992). The *Il10* gene is flanked upstream by other members of the IL-10 family of cytokines *Il19*, *Il20* and *Il24*, and downstream by the *Mapkapk2* (Mitogen–activated protein (MAP) kinase-activated protein kinase 2) gene (Fig. 2a). The *Il10* gene itself is comprised of 5 exons and 4 introns followed by a 3' untranslated region (UTR) spanning around 5 kb in total on the forward strand (Fig. 2b and c). The *Il10* transcription start site (TSS) is relatively well defined, with proximal downstream TATA and CCA[A/G in mice]T elements within the core promoter (Fig. 2d) characteristic of a 'sharp' or 'focused' promoter that is thought to be associated with tissue-specific transcription often in an environmentally responsive manner (Lenhard et al. 2012). There is a high degree of homology between the human and mouse *Il10* gene loci with a number of conserved non-coding sequences (CNS, named according to their distance in kb from the *Il10* TSS) shown to be present using VISTA-Point software (Frazer et al. 2004) (Fig. 2b and c). This is indicative of an evolutionary constraint most likely resulting from functional relevance of these regions, some of which have been shown to be functionally characterised as enhancers of *Il10* gene expression (Li et al. 2012; Lee et al. 2009; Ahyi et al. 2009; Jones and Flavell 2005; Wang et al. 2005).

Changes in chromatin structure at the *Il10* gene locus are fundamental to the regulation of its expression. Several studies in a variety of cells including both macrophages and Th cells have investigated the pattern of DNaseI HSSs at the *Il10* gene locus. In bone marrow (BM)-derived macrophages, five HSS sites have been identified at positions −4.5, −2, −0.12 kb upstream and +1.65 and +2.98 kb downstream of the *Il10* TSS following their stimulation with PRR ligands including lipopolysaccharide (LPS), CpG and zymosan A (Saraiva et al. 2005) (Fig. 2c). The HSS −4.5 kb was also present in BM-derived DCs when stimulated with the same ligands (Saraiva et al. 2005) (Fig. 2c). Unstimulated macrophages also showed some degree of sensitivity to DNaseI digestion at these sites suggestive of poised chromatin conformation in the resting state. This may be in line with the immediate expression of the *Il10* gene in these cells upon exposure to appropriate stimuli. In contrast, the *Il10* gene locus in naïve T cells is in a closed, transcriptionally inactive conformation with only one HSS −8.8 kb upstream of the *Il10* TSS (Jones and Flavell 2005) (Fig. 2b). Substantial remodelling of the *Il10* gene locus occurs upon differentiation into Th1 and Th2 cells, as demonstrated by the increased number of HSS sites detected, most of which were found to be present in both Th1 and Th2 cells (Jones and Flavell 2005; Wang et al. 2005; Saraiva et al. 2005; Im et al. 2004) (Fig. 2b and c). A few HSS sites were more prominent in Th2 cells including HSS −30.4, −29.8, −21, −17.5 and −0.12 kb downstream and +6.45 kb upstream of the *Il10* gene TSS (Jones and Flavell 2005) (Fig. 2b and c). These differences may account for the enhanced production of IL-10 in Th2 cells when compared to Th1 cells, although this is yet to be investigated. In contrast to Th1 and Th2 cells, little is known about the chromatin conformation at the *Il10*

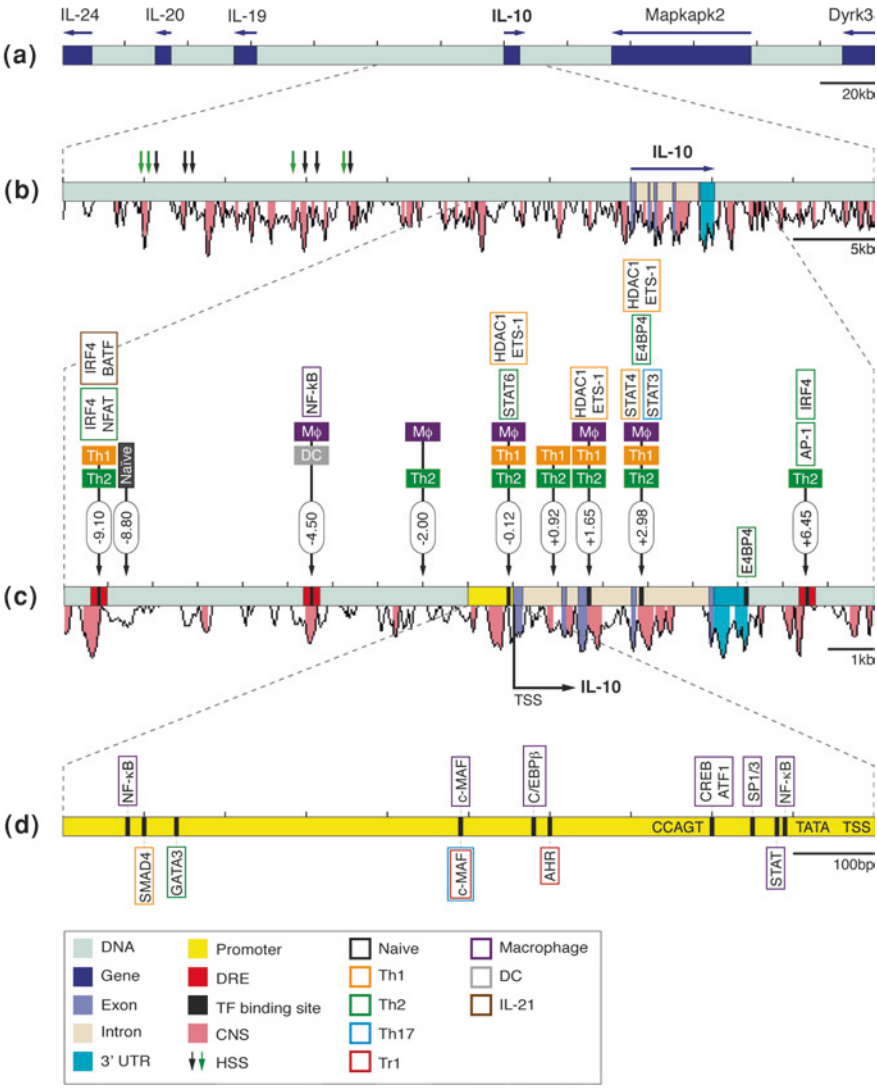


Fig. 2 *A diagram of mouse chromosome 1 showing the Il10 gene locus*. **a** *Il10* gene and its neighbours, *horizontal arrows* (*blue*) depict orientation of gene expression. **b** Detail of DNA surrounding the *Il10* gene locus, *vertical arrows* denote HSS sites present equally in Th1 and Th2 cells (*black*) or more prominently in Th2 cells (*green*) aligned against Vista plot of the murine sequence *versus* human homology, *pink peaks* denote CNS. **c** *Il10* gene locus and its regulatory elements again aligned against Vista plot of the murine sequence *versus* human homology, *pink peaks* denote CNS, *purple peaks* denote exons and *turquoise* peak denotes 3′UTR. *Arrows* denote HSS sites with their relative position to the *Il10* TSS indicated, and the cell type in which they have been detected depicted (macrophage, DC, Th1 and Th2), note that HSS −0.12 and +6.45 kb have been shown to be more prominent in Th2 over Th1 cells. The binding sites of transcription factors that have been shown to regulate *Il10* gene expression in the different cell types (indicated by different colour borders) are also shown. **d** *Il10* gene proximal promoter, depicts further binding sites of transcription factors regulating *Il10* gene expression

gene locus in Th17 cells. Interestingly, many of the HSS studied were common between macrophages, DCs and Th cells, apart from HSS −4.5 kb which was macrophage and DC-specific (Saraiva et al. 2005) (Fig. 2c). Moreover, in most instances, the DNaseI HSS detected correspond with the computationally obtained CNS sites (Fig. 2b and c), further supporting a regulatory role for these CNS.

Additional evidence informing on the accessibility of the *Il10* gene locus comes from studies on histone modifications as markers of permissive *versus* repressive chromatin. In IL-10-producing macrophages, the presence of hyperacetylation of histone H4 (active mark) was detected at the −4.5 and −1.2 kb HSS regions (Saraiva et al. 2005). In Th1 and Th2 cells, the presence of H3K4me3 (active mark) and absence of H3K27me3 (repressive mark) was shown across the *Il10* gene locus by chromatin immunoprecipitation-sequencing (ChIP-Seq) (Wei et al. 2010). These histone marks indicate a transcriptionally competent state of the *Il10* gene locus, although their presence does not always correlate with active gene expression. The possible candidates initiating chromatin remodelling at the *Il10* gene locus will be discussed in some detail later.

4 Molecular Signals and Transcriptional Regulation of IL-10 Expression

4.1 The Regulation of IL-10 in Macrophages and Dendritic Cells

Almost all cells within the innate immune system, with the exception of plasmacytoid DCs (pDC) (Boonstra et al. 2006; Kaiser et al. 2009; Ito et al. 2006), have been shown to produce IL-10. These include macrophages, monocytes and myeloid (or conventional) DCs (mDC), which largely require stimulation via PRRs to induce IL-10 production (Saraiva and O'Garra 2010). Additionally, co-stimulation of DCs with a PRR ligand and CD40 ligand (CD40L) or immune-complexes can enhance the production of IL-10 (Gerber and Mosser 2001; Edwards et al. 2002). Importantly, other signals, such as those received from autocrine or paracrine cytokines, can also modulate IL-10 production (Chang et al. 2007a; Hu et al. 2006; Staples et al. 2007; Cao et al. 2005). Thus, the ultimate level of IL-10 produced by macrophages or mDCs is determined by the integration of several molecular signals (Fig. 3a).

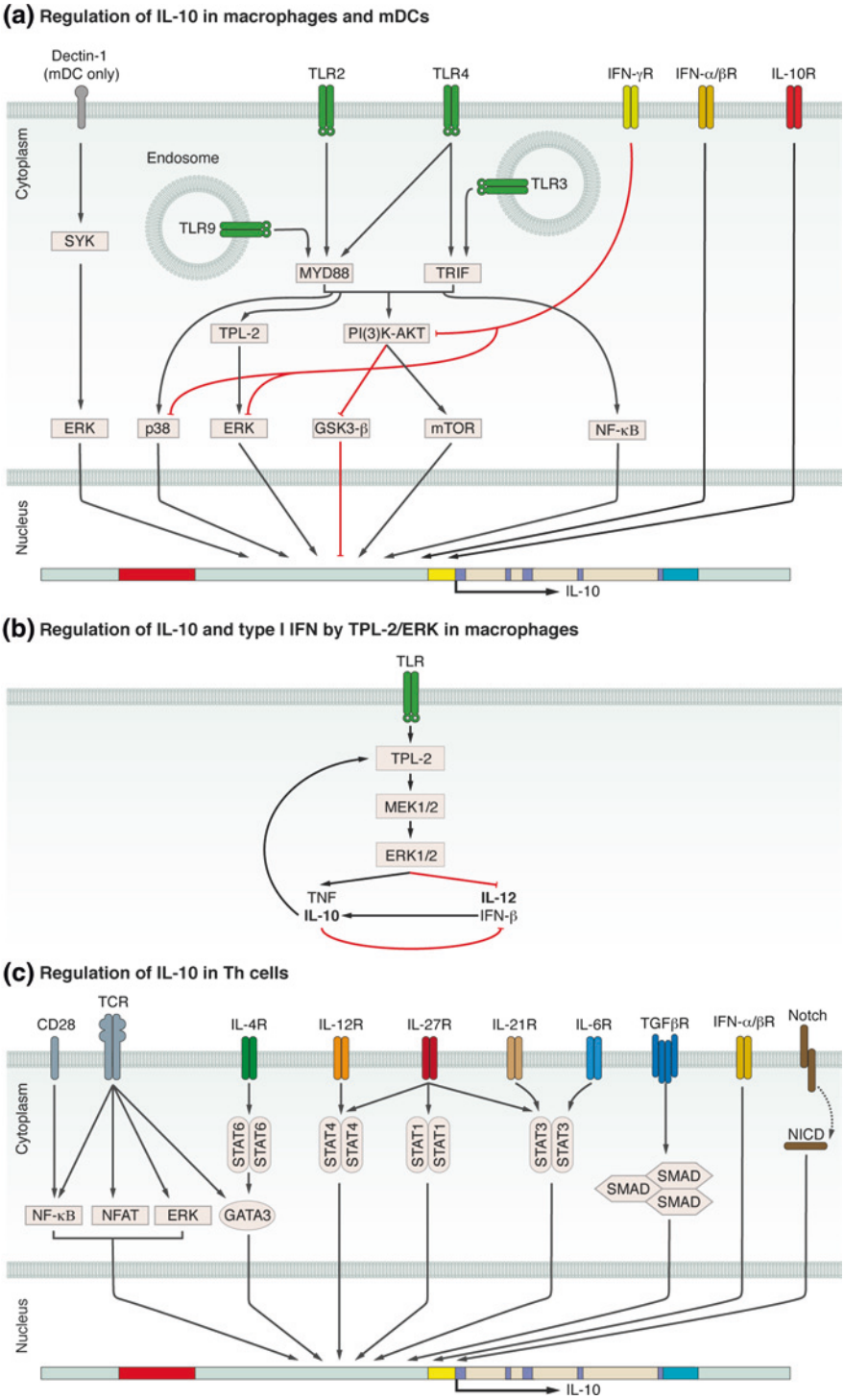
4.1.1 The Regulation of IL-10 Downstream of Pattern Recognition Receptors

Amongst the PRRs, the Toll-like receptor (TLR) family is the best studied. TLRs can be expressed at the plasma membrane or endosomally and recognise a range of ligands including bacterial lipopeptides (TLR 2), viral RNAs (TLRs 3 and 7), LPS

found in the cell wall of Gram-negative bacteria (TLR 4), flagellin (TLR 5), and CpG DNA (TLR 9) (Kawai and Akira 2010). It has been shown that TLR expression may vary amongst macrophage, monocyte and DC subsets (Kadowaki et al. 2001; Jarrossay et al. 2001). For example, in humans, TLR 7 and TLR 9 are more highly expressed on pDCs than monocytes or other DC subsets (Kadowaki et al. 2001; Jarrossay et al. 2001). In contrast, human monocytes have been shown to more highly express TLR 2 and TLR 4 (Kadowaki et al. 2001). Of note, these differences may not be conserved in the murine system where it has been shown that the majority of TLR mRNAs (with the exception of TLRs 3, 5 and 7) are similarly expressed in DC subsets (Edwards et al. 2003). The ligation of several TLRs including 2, 4, 5, 7 and 9 has been shown to induce the production of IL-10 in human and murine macrophages and mDCs (Boonstra et al. 2006; Kaiser et al. 2009; Agrawal et al. 2003; Hacker et al. 2006; Chi et al. 2006), although, macrophages and mDCs may not have the same capacity to produce IL-10. For example, TLR 3 activation has been shown to induce IL-10 production in macrophages but not mDC (Boonstra et al. 2006), and TLR 9 ligation induces IL-10 production more readily in murine macrophages than mDC, and not at all in pDC, despite all of these cell types expressing TLR 9 (Boonstra et al. 2006; Kaiser et al. 2009). Importantly however, in mDCs, the co-ligation of TLR 2 and Dectin-1, a C-type Lectin receptor which recognises fungal β-glucans (Reid et al. 2009), has been shown to enhance the level of IL-10 production relative to TLR2 or Dectin-1 stimulation alone (Dennehy et al. 2009). This demonstrates that PRRs are able to cooperate in their induction of IL-10, a concept which may have relevance in the context of infection when several PRR ligands are likely to be present at the same time.

Downstream of TLR ligation, adaptor proteins are essential for signal transduction. The adaptor myeloid differentiation factor 88 (MyD88) is required for signalling downstream of all TLRs with the exception of TLR 3 which utilises the adaptor TIR-domain-containing adapter-inducing interferon-β (TRIF). TLR 4 is the only TLR that mediates signal transduction through both MyD88 and TRIF (Kawai and Akira 2010) and indeed both adaptors are required for optimal IL-10 production downstream of this receptor (Boonstra et al. 2006). TLR signalling through these adaptor proteins leads to the activation of several signalling pathways including MAP kinase pathways, the phosphatidylinositol 3 kinase (PI(3)K)-AKT pathway, the nuclear factor (NF)-κB pathway and the activation of interferon regulatory factors (IRF). These downstream pathways collectively regulate the production of TLR-induced cytokines, including IL-10 (Kawai and Akira 2010) (Fig. 3a).

Several studies have shown that the activation of the MAP kinases extracellular signal-regulated kinase (ERK) 1 and ERK 2 (here referred to collectively as ERK) is critical for the production of IL-10 in macrophages and mDCs downstream of TLRs 2, 4 and 9 (Kaiser et al. 2009; Banerjee et al. 2006; Dillon et al. 2004; Yi et al. 2002), and in *Mycobacterium tuberculosis (M. tuberculosis)* infected macrophages and monocytes (McNab et al. 2013). In keeping with this, tumour progression locus 2 (TPL-2), the MAP 3-kinase upstream of TLR-induced ERK activation (Dumitru et al. 2000), which is itself regulated by the NF-κB family member p105 (Gantke et al. 2011),


(a) Regulation of IL-10 in macrophages and mDCs
Dectin-1 (mDC only)
TLR2
TLR4
IFN-γR
IFN-α/βR
IL-10R
Cytoplasm
Endosome
TLR3
TLR9
SYK
MYD88
TRIF
TPL-2
PI(3)K-AKT
ERK
p38
ERK
GSK3-β
mTOR
NF-κB
Nucleus
IL-10
(b) Regulation of IL-10 and type I IFN by TPL-2/ERK in macrophages
TLR
TPL-2
MEK1/2
ERK1/2
TNF
IL-10
IL-12
IFN-β
(c) Regulation of IL-10 in Th cells
CD28
TCR
IL-4R
IL-12R
IL-27R
IL-21R
IL-6R
TGFβR
IFN-α/βR
Notch
Cytoplasm
STAT6
STAT6
STAT4
STAT4
STAT1
STAT1
STAT3
STAT3
NICD
SMAD
SMAD
SMAD
NF-κB
NFAT
ERK
GATA3
Nucleus
IL-10

◀ **Fig. 3** *Signals that induce IL-10 expression in representative cells of the innate (macrophages and DCs) and adaptive (Th cells) immune system*. **a** The expression of *Il10* is induced by signals downstream of PRRs, such as TLRs, in macrophages and mDCs. Downstream of TLR ligation the recruitment of the adaptor molecules MYD88 and TRIF are critical for the activation of subsequent signalling pathways. Of these, ERK and p38 MAP kinases, the PI(3)K-AKT pathway and the NF-κB pathway have been shown to have roles in the induction of *Il10* gene expression. Importantly, several of these pathways also mediate the expression of proinflammatory cytokines. Non-TLR PRRs can also induce IL-10 production. An example of this is Dectin-1, which is expressed mainly on DCs and induces IL-10 production via a SYK and ERK-dependent pathway. *Il10* expression is further modulated by signals induced by surrounding cytokines such as type I IFN and IL-10 itself which promote IL-10 production, and IFN-γ which inhibits IL-10 production through the inhibition of MAP kinase and PI(3)K-AKT pathways. **b** The TPL-2/ERK pathway induces IL-10 production which positively feeds back to upregulate TPL-2. In contrast, the TPL-2/ERK pathway negatively regulates IFN-β production, itself a positive regulator of IL-10. The negative regulation of IFN-β by this pathway is both dependent and independent of IL-10 production. This complex molecular network also includes the regulation of proinflammatory cytokines e.g. TNF and IL-12, which are positively and negatively regulated by the TPL-2/ERK pathway, respectively. **c** The expression of IL-10 in $CD4^+$ T cells is initiated by signalling downstream of the TCR and costimulatory molecules such as CD28 and involves the MAP kinase ERK, the calcineurin-NFAT and the NF-κB pathways, amongst others. It is further modulated by signals downstream of cytokine receptors via STAT or SMAD proteins that are also critical for the differentiation of naïve $CD4^+$ cells into the different Th cell subsets. At least in Th2 cells, IL-10 production is further regulated by the lineage specific master regulator transcription factor GATA3 induced mainly downstream of the STAT6 signalling but also of TCR. In addition to the TCR and cytokine signals, signalling through Notch ligands induced on macrophages and DCs by pathogen-derived signals has an impact on the differentiation of $CD4^+$ Th cells as well as their IL-10 production

is also required for IL-10 production in TLR stimulated macrophages and mDCs, and in *M. tuberculosis* infected macrophages and monocytes (Kaiser et al. 2009; Banerjee et al. 2006; McNab et al. 2013) (Fig. 3a and b). In these cell types, the TPL-2/ERK pathway also negatively regulates type I interferon (IFN) (Kaiser et al. 2009; McNab et al. 2013) which, as discussed in more detail later, is a positive regulator, of IL-10 production (Chang et al,. 2007a; McNab et al. 2013; Aman et al. 1996; Pattison et al. 2012; Mayer-Barber et al. 2011) (Fig. 3b). However, despite the relatively higher level of type I IFN produced in *Tpl2*$^{-/-}$ macrophages, mDCs and monocytes, IL-10 production remains reduced compared to wild-type cells (Kaiser et al. 2009; McNab et al. 2013). Together, this demonstrates a central role for the TPL-2/ERK pathway in the regulation of IL-10 that cannot be compensated for by elevated autocrine type I IFN production. Further supporting an important role for ERK in the regulation of IL-10, differential ERK activation has been correlated with the different levels of IL-10 produced by macrophages, mDCs and pDCs (Kaiser et al. 2009) and ERK activation promotes IL-10 production in mDC downstream of the non-TLR PRR Dectin-1 (Slack et al. 2007; Dillon et al. 2006).

Downstream of MAP kinase signalling transcription factors including activator protein 1 (AP-1), a heterodimeric transcription factor composed of dimers of the Fos, Jun, Jun dimerising protein (JDP) and activating transcription factor (ATF) family members, are activated (Karin et al. 1997). In TLR stimulated macrophages and mDCs, the AP-1 member c-FOS has been associated with the ERK-dependent positive regulation of IL-10 (Kaiser et al. 2009; Hu et al. 2006; Agrawal et al.

2003; Dillon et al. 2004). In contrast to TLR-induced IL-10 production however, Dectin-1-dependent IL-10 induction requires the upstream kinase spleen tyrosine kinase (SYK) and is not dependent on c-FOS (Slack et al. 2007; Dillon et al. 2006; Rogers et al. 2005) (Fig. 3a). In addition to the activation of AP-1, in macrophages co-stimulated with a TLR 4 ligand and immune-complexes, ERK activation has been shown to promote the phosphorylation of histone H3 at sites across the *Il10* promoter (Lucas et al. 2005; Zhang et al. 2006). This histone modification enhanced the accessibility of the *Il10* promoter, providing a permissive chromatin structure for the binding of transcription factors such as the constitutively expressed specific protein 1 (Sp1) (Lucas et al. 2005; Zhang et al. 2006), shown to bind within the proximal *Il10* promoter at a site critical for transactivation of the *Il10* gene (Brightbill et al. 2000) (Fig. 2d). Of note, in addition to Sp1, Sp3 has been reported to bind at the *Il10* promoter and transactivate the *Il10* gene in the Drosophila SL2 cell line (Tone et al. 2000). The concept of chromatin remodelling at the *Il10* locus prior to *Il10* gene expression is in keeping with the dependence of *Il10* gene expression on the activity of SWI/SNF (switching-defective-sucrose non-fermenting, also known as BRG1 associated factors in mammals) nucleosome remodelling complex which enhances accessibility of certain genetic loci in response to TLR stimulation (Ramirez-Carrozzi et al. 2009).

Similarly to ERK, the MAP kinase p38 has been shown to positively regulate IL-10 production in TLR stimulated macrophages, monocytes and mDCs (Hu et al. 2006; Chi et al. 2006; Yi et al. 2002; Foey et al. 1998; Jarnicki et al. 2008; Kim et al. 2005) (Fig. 3a). In a TLR 4 stimulated human monocytic cell line, p38 was proposed to induce Sp1 binding to an alternative upstream Sp1 binding site which was important for the transactivation of the *Il10* gene in this system (Ma et al. 2001). Also downstream of p38, pre-B cell leukaemia homeobox 1 (PBX1) and PBX-regulating protein 1 (PREP1) have been shown to promote IL-10 production in response to apoptotic cells (Chung et al. 2007).

ERK and p38 may also cooperate in their regulation of IL-10 production, demonstrated by the finding that both ERK and p38 contribute to the activation of mitogen- and stress-activated protein kinases MSK1 and MSK2, which in turn promote IL-10 production in TLR 4 stimulated macrophages (Ananieva et al. 2008). Downstream of MSK1 and MSK2, the transcription factors cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) and ATF1 were found to bind the *Il10* promoter (Fig. 2d) (Ananieva et al. 2008). CREB and ATF1 binding at the *Il10* gene locus and transactivation of the *Il10* promoter has also been demonstrated in a cAMP-treated human monocytic cell line (Platzer et al. 1999). CREB has further been shown to induce IL-10 production in response to Zymosan, a yeast-derived combined TLR 2/Dectin-1 stimulus (Alvarez et al. 2009).

The third MAP kinase, JNK, has also been proposed to positively regulate IL-10 production in TLR stimulated macrophages and monocytes (Hu et al. 2006; Chi et al. 2006; Chakrabarti et al. 2008). However, the downstream mechanisms of this are unclear and detailed studies of the role of JNK in the regulation of IL-10 have until recently been complicated by the lack of specific inhibitors for this kinase.

TLR-induced signalling through the PI(3)K-AKT pathway also contributes to IL-10 production in macrophages, monocytes and mDCs (Martin et al. 2003; Ohtani et al. 2008; Weichhart et al. 2008) (Fig. 3a). In TLR 4 stimulated mDCs and *Leishmania* infected macrophages, this has been shown to be mediated at least in part through the inhibition of glycogen synthase kinase 3 (GSK3)-β (Ohtani et al. 2008; Nandan et al. 2012), a constitutively active kinase which inhibits the production of IL-10 by antagonising the DNA binding activity of CREB (Martin et al. 2005). It has also been proposed that the PI(3)K-AKT pathway activates ERK (Martin et al. 2003) and this may promote IL-10. The PI(3)K-AKT pathway additionally leads to the downstream activation of mammalian target of rapamycin (mTOR) and this also promotes the production of IL-10 (Ohtani et al. 2008; Weichhart et al. 2008) (Fig. 3a). Recently it has been shown that the MAP kinase p38 can also activate mTOR, providing an additional mechanism whereby p38 activation promotes the production of IL-10 (Katholnig et al. 2013). mTOR may further regulate IL-10 production through the activation of signal transducers and activators of transcription (STAT) 3 (Weichhart et al. 2008), reported to bind at a STAT consensus site within the proximal *Il10* promoter, important for transactivation of the *Il10* gene (Benkhart et al. 2000) (Fig. 2d). Of note however, STAT3 activation also occurs downstream of the IL-10 receptor (Murray 2006) and in human monocyte-derived macrophages STAT3 has been implicated in the feed-forward loop whereby IL-10 positively regulates its own production (Staples et al. 2007). Thus, whether STAT3 regulates IL-10 downstream of PRR stimulation via mTOR, or downstream of autocrine IL-10 signalling, or both, is unclear.

NF-κB is also activated in response to TLR ligation (Fig. 3a) and the finding that IKK2 deficient macrophages, which have impaired NF-κB activation, show reduced production of IL-10 (Kanters et al. 2003) implicated NF-κB in the regulation of IL-10. Although the interpretation of this result is difficult as IKK2 is also upstream of TPL-2 activation (Gantke et al. 2011), later studies have continued to support a role for NF-κB in the regulation of IL-10. For example, an NF-κB binding site is present at HSS −4.5 kb upstream of the *Il10* TSS and recruits the NF-κB subunit p65 in TLR 4 stimulated macrophages (Saraiva et al. 2005) (Fig. 2c). Further, p105 (encoded by *Nfkb1*) has been shown to regulate IL-10 production through both NF-κB-dependent and TPL-2/ERK-dependent pathways (Banerjee et al. 2006). NF-κB p50 homodimer recruitment to an NF-κB site proximal to the *Il10* TSS has also been shown to promote IL-10 production in TLR 4 stimulated macrophages (Fig. 2d) and the complexing of these p50 homodimers with the coactivator CREB-binding protein (CBP) further enhances *Il10* promoter activity (Cao et al. 2006). Finally, p65 binding at an additional site in the *Il10* promoter (Fig. 2d) has been reported to upregulate IL-10 in dsRNA-stimulated macrophages and this was dependent on protein kinase R (PKR) (Chakrabarti et al. 2008).

Several other transcription factors have been implicated in the regulation of *Il10* gene expression in PRR stimulated macrophages and/or mDCs. The transcription factor c-MAF has been reported to bind at the *Il10* promoter (Fig. 2d) and enhance IL-10 production in LPS stimulated macrophages, although this mechanism of IL-10 regulation may be most relevant in the context of IL-4 which increases the recruitment of c-MAF to the *Il10* promoter (Cao et al. 2005). Several CCAAT/enhancer

binding protein (C/EBP) sites have been identified within the *Il10* promoter (Brenner et al. 2003). In a human monocyte cell line, binding of C/EBPα and β to a C/EBP site proximal to the TATA box was important for basal *Il10* gene expression and transactivation of the *Il10* gene in response to cAMP (Brenner et al. 2003). Roles for C/EBPβ and C/EBPδ in cooperation with Sp1, have also been identified in the expression of *Il10* in a TLR 4 stimulated murine macrophage cell line (Liu et al. 2003). C/EBPβ has further been shown to mediate *Il10* expression downstream of the A_{2A} adenosine receptor in *Escherichia coli (E. coli)* infected macrophages (Csoka et al. 2007). Of note, the synergistic induction of IL-10 in adenosine/*E. coli* infected macrophages was dependent on p38 activation (Csoka et al. 2007). The ligand-activated transcription factor aryl hydrocarbon receptor (AHR) is additionally reported to be required for maximal production of IL-10 in TLR 4 but not TLR 9 stimulated macrophages, suggesting a stimulus-specific requirement for AHR in the regulation of IL-10 (Kimura et al. 2009).

4.1.2 Negative Regulators of IL-10 Production

Negative mechanisms of IL-10 regulation in PRR stimulated macrophages and monocytes have also been identified, in keeping with the important biological functions of IL-10 in various scenarios, and the need for tight control of its expression. For example, in a TLR 4 stimulated macrophage cell line, the chromatin modifying activity of HDAC11 has been shown to inhibit IL-10 production, possibly by impairing the transition of repressed to active chromatin at the *Il10* locus and restricting the binding of Sp1, STAT3 and PolII to the proximal *Il10* promoter (Villagra et al. 2009). Recently, IRF5, the expression of which is induced downstream of TLR ligation, has been identified as a negative regulator of IL-10 in human GM-CSF differentiated monocytes (Krausgruber et al. 2011). This study also showed recruitment of IRF5 to the IL-10 promoter, suggesting a direct mechanism of transcriptional inhibition (Krausgruber et al. 2011). IL-10 is additionally negatively regulated at the post-transcriptional level, discussed in detail later.

4.1.3 The Modulation of IL-10 Production by Autocrine and Paracrine Factors

Signalling induced by environmental cytokines can modulate the level of IL-10 produced by PRR stimulated macrophages or mDCs (Fig. 3a). A key example of this is the effect of IL-10 on itself. In addition to the already mentioned activation of STAT3 which can enhance IL-10 production (Staples et al. 2007), IL-10 signalling has been shown to upregulate the expression of *Tpl2* (Lang et al. 2002) (Fig. 3b) which also positively regulates IL-10 (Kaiser et al. 2009; Banerjee et al. 2006; McNab et al. 2013). In contrast, IL-10 can induce the expression of dual specificity phosphatase-1 (DUSP1), which negatively regulates p38 activation, a positive regulator of IL-10 production (Hammer et al. 2005).

Type I IFN has been shown to enhance the production of IL-10 from TLR 4 stimulated murine macrophages and human monocytes (Chang et al. 2007a; Aman et al. 1996; Pattison et al. 2012). Type I IFN also promotes IL-10 production in *M. tuberculosis* infected macrophages (McNab et al. 2013; Mayer-Barber et al. 2011). Reported mechanisms of type I IFN enhancement of IL-10 production in PRR stimulated cells include the recruitment of IRF1 and STAT3 to the *Il10* promoter in a human B cell line (Ziegler-Heitbrock et al. 2003), the activation of STAT1 in murine macrophages (Guarda et al. 2011) and the activation of the PI(3)K-AKT pathway followed by the subsequent inactivation of GSK3-β in human DCs (Wang et al. 2010). It has also been reported that IL-27 is required for the maximal induction of IL-10 by type I IFN in TLR 4 stimulated murine macrophages (Iyer et al. 2010), however another study reported that resting murine macrophages are unresponsive to IL-27 and that IL-27 had an inhibitory effect on IL-10 production in human monocytes via STAT1 (Kalliolias and Ivashkiv 2008). Thus, the role of IL-27 and STAT1 in the regulation of IL-10 remains incompletely understood. In contrast to type I IFN, in TLR 2 activated human macrophages, IFN-γ has been shown to reduce the production of IL-10 through the inhibition of MAP kinases and enhancement of GSK3-β activity (Hu et al. 2006). This led to the suppression of the transcription factors AP-1 and CREB, which both positively regulate IL-10 (Hu et al. 2006).

The enhancement of IL-10 by the lipid-derived molecule prostaglandin E2 (PGE2) in TLR 4-activated macrophages has also been reported (Strassmann et al. 1994), illustrating that factors other than cytokines can modulate the production of PRR-induced IL-10 production. A model for the mechanism of PGE2-mediated enhancement of IL-10 has been proposed in which PGE2 treatment activates protein kinase A (PKA) to phosphorylate salt-inducible kinase 2 (SIK2) (Clark et al. 2012; MacKenzie et al. 2013). This inhibits the ability of SIK2 to phosphorylate CREB-regulated transcription coactivator 3 (CRTC3), permitting CRTC3 to translocate to the nucleus and act as a coactivator of CREB, ultimately enhancing IL-10 production (Clark et al. 2012; MacKenzie et al. 2013).

Thus, PRR activation induces IL-10 production in macrophages and mDCs with further modulation of IL-10 levels by other environmental cues. However, there are still many incompletely understood aspects of IL-10 regulation in macrophages, monocytes and DCs. For example, how signalling pathways from multiple PRRs, or PRRs in combination with signals from environmental factors such as cytokines are integrated to either positively or negatively regulate *Il10* expression is not clear. Further, it is not fully understood if the ability of certain PRRs (e.g. TLR 3 and TLR 9) to more efficiently induce the production of IL-10 in macrophages compared DCs (Boonstra et al. 2006; Kaiser et al. 2009) is a consequence of cell type-specific signal transduction pathways, or if it is also contributed to by a differing chromatin state of the *Il10* locus in the distinct immune cell types. In addition, most molecular studies investigating the regulation of *Il10* have been conducted in TLR 4 stimulated cells. Thus, the continuation of studies delineating the common and distinct factors required for *Il10* expression downstream of other TLR and non-TLR PRRs will enhance our understanding of IL-10 regulation in various immune scenarios.

4.2 Transcriptional Regulation of IL-10 in CD4$^+$ T Helper Cells

Within the adaptive immune system, the regulation of *Il10* gene expression has been most extensively characterised in CD4$^+$ Th cells. In contrast to macrophages and DCs that can instantly produce IL-10 in response to microbial products, naïve CD4$^+$ T cells first need to differentiate into the different effector Th cell subsets in order to produce IL-10 (Saraiva and O'Garra 2010). The different Th cells subsets include Th1, Th2 and Th17 cells, each of which expresses a unique combination of cytokines that promote their specific effector functions (Zhu et al. 2010). The distinct Th cell fates are determined by the STAT family of transcription factors signalling downstream of cytokines present in the environment during T cell activation and are ultimately controlled by the subsequent induction of lineage specific master regulators. Th1 differentiation is driven predominantly by IL-12 signalling through STAT4 (Murphy et al. 2000) and the expression of the master regulator T-box transcription factor (TBET) that induces the production of the Th1 hallmark cytokine IFN-γ (Szabo et al. 2000). Th2 differentiation requires IL-4 signalling through STAT6 and the expression of the master regulator GATA binding protein 3 (GATA3) (Zheng and Flavell 1997) that induces the production of Th2 hallmark cytokines IL-4, IL-5 and IL-13 (Mowen and Glimcher 2004). Th17 differentiation is dependent on transforming growth factor (TGF)-β and IL-6, the latter of which signals through STAT3, and the expression of the master regulator RAR-related orphan receptor gamma t (RORγt) that induces the production of the Th17 hallmark cytokine IL-17 (Veldhoen et al. 2006; Ivanov et al. 2006). Interestingly, despite the differences in their developmental requirements outlined above, IL-10 has been shown to be produced by all of the different Th cell subsets (Saraiva and O'Garra 2010). In fact, the production of IL-10 by Th cells is tightly interlinked with each of their differentiation programmes. For example, strong TCR signalling, together with IL-12 and STAT4 are required for IL-10 production in Th1 cells (Saraiva et al. 2009), while IL-4 activation of STAT6 signalling and GATA3 are essential for IL-10 production in Th2 cells (Wei et al. 2010; Saraiva et al. 2009; Chang et al. 2007b; Shoemaker et al. 2006; Zhu et al. 2004), and TFG-β, IL-6 and STAT3 have been shown to drive the production of IL-10 in Th17 cells (Stumhofer et al. 2007; Xu et al. 2009). Additionally, type I IFN has been shown to promote IL-10 production by human CD4$^+$ T cells (Aman et al. 1996; Corre et al. 2013; Levings et al. 2001), although the downstream mediators of type I IFN and its effects on the different Th cell subsets are not well defined (Fig. 3c). Thus, the discrete signals required for IL-10 induction are tightly linked with those involved in directing the differentiation of each unique Th cell subset.

The current model of how Th cell identity is established involves a layered architecture of transcription factors induced downstream of the TCR and cytokine signalling pathways. The TCR signals are thought to induce pioneer factors able to bind closed chromatin that are, together with specific STATs, involved in the establishment of permissive epigenetic patterns across a broad

range of Th cell genes in differentiating $CD4^+$ T cells (Vahedi et al. 2013). In contrast, gene targets of the master regulator transcription factors are thought to be more limited to a set of key genes crucial for lineage specificity, be it those involved in lineage reinforcement or in the limitation of an alternative differentiation programme (Vahedi et al. 2013). Since the production of IL-10 is so closely intertwined with Th lineage specification, it is likely that this model would also apply to the regulation of IL-10, whereby layers of transcription factors, induced downstream of TCR and cytokine signalling or other environmental cues, act together to promote *Il10* gene expression (Fig. 3c). However, the specific signalling cascades and transcription factor complexes that determine IL-10 production by the different Th cell subsets are still being defined.

4.2.1 The Regulation of IL-10 by T Cell Receptor Signalling

Following TCR activation, numerous signalling pathways are initiated leading to the induction, nuclear translocation and/or activation of downstream transcription factors including AP-1, nuclear factor of activated T cells (NFAT) and NF-κB (Brownlie and Zamoyska 2013) (Fig. 3c).

Similar to macrophages and DCs, the activation of the MAP kinase ERK has been shown to be a common positive regulator of IL-10 in the different Th cell subsets (Saraiva et al. 2009). Of note, in contrast to TPL-2 driven ERK activation downstream of TLRs in macrophages and DCs (Dumitru et al. 2000), in T cells ERK is activated via Ras downstream of the TCR (Dong et al. 2002). As already mentioned, the most prominent family of transcription factors induced downstream of ERK is AP-1. Binding of AP-1, comprised of JunB and to a lesser extent c-Jun (Wang et al. 2005), at the HSS +6.45 kb in the *Il10* gene locus in Th2, but not Th1 cells has been demonstrated to enhance IL-10 expression using luciferase reporter assays (Jones and Flavell 2005; Wang et al. 2005) (Fig. 2c). Furthermore, retroviral expression of c-Jun and JunB in activated naïve $CD4^+$ T cells showed a five-fold increase in IL-10 and to a lesser extent IFN-γ production, while expression of a dominant negative c-Jun protein decreased the amount of IL-10 produced, thus confirming the role of Jun family proteins in IL-10 regulation (Wang et al. 2005). More recently, the basic leucine zipper ATF-like transcription factor (BATF), another AP-1 family member, and the transcription factor IRF4 have been shown to cooperatively bind at CNS-9, in Th2, Th9 and Th17 cells as well as $CD4^+$ T cells stimulated in the presence of IL-21 (Li et al. 2012). BATF has further been demonstrated to positively regulate IL-10 expression in Th2 cells, although its effect is only revealed in the absence of BATF3 (Tussiwand et al. 2012). Of note, the production of IL-4 in the Th2 driven $Batf^{-/-}Batf3^{-/-}$ $CD4^+$ T cells was also significantly affected, indicative of a more general defect in the Th2 differentiation pathway as opposed to just IL-10 production (Murphy et al. 2013). In keeping, with this $Irf4^{-/-}$ $CD4^+$ T cells stimulated in the presence of IL-21 failed to express IL-10 mRNA when compared to the wild type T cells, although the effect on other cytokines was not assessed (Li et al. 2012). Mutating either the IRF

or AP-1 motif within CNS-9 resulted in a diminished luciferase reporter activity in IL-21 stimulated $CD4^+$ T cells consistent with functional cooperation between these factors in *Il10* gene regulation (Li et al. 2012) (Fig. 2c). Furthermore in Th2 cells, IRF4 alone has been shown to positively regulate IL-10 by binding to the *Il10* promoter and at CNS+6.45 (Ahyi et al. 2009) (Fig. 2c). Of note, similarly to the effect of BATF on Th2 differentiation, IRF4 also increased the production of IL-4 in these cells by binding and transactivating the *Il4* promoter, showing that IL-4 and IL-10 can be co-regulated in Th2 cells, suggesting a more general effect on Th2 cell differentiation, accompanied by IL-10 production (Ahyi et al. 2009).

Upon TCR activation, NFAT1 translocates from the cytoplasm into the nucleus where it is known to interact with AP-1 and other transcriptional partners to promote cytokine gene transcription (Macian 2005). NFAT1 has been shown to bind regions in the *Il10* promoter in Th2 and in intron 4 of the *Il10* gene locus in Th1 cell lines (Im et al. 2004). In a later study, NFAT1 binding further upstream at CNS-9 together with IRF4 has been shown to synergistically enhance IL-10 expression in Th2 cells using luciferase reporter assays (Lee et al. 2009) (Fig. 2c). Furthermore, blocking of NFAT signalling with a cyclosporine A inhibitor also decreased IL-10 mRNA expression in differentiated Th2 as well as Th1 cells, although it is not known what effect it had on the hallmark cytokine production and therefore could represent a generalised effect on Th cell differentiation (Lee et al. 2009).

NF-κB activation downstream of the TCR has been reported to play a role in the production of Th1 (Gerondakis et al. 2006), Th2 (Das et al. 2001) and Th17 hallmark cytokines (Chen et al. 2011). Although NF-κB has been shown to regulate IL-10 production in macrophages as described above, little is known about its role in IL-10 regulation in Th cells other than the reported binding of NF-κB complexes at the *Il10* gene locus in a human T lymphoma line (Mori and Prager 1997).

Thus, even though $CD4^+$ T cell activation through the TCR is crucial for Th cell differentiation, the roles of factors downstream of the TCR in the regulation of IL-10 are only partially characterised and warrant further investigation.

4.2.2 The Regulation IL-10 by Cytokine Signalling

In contrast to what is known about TCR signals promoting IL-10 production, there is a large body of evidence supporting a role for STAT and SMAD proteins downstream of cytokine receptors in the regulation of IL-10 expression in Th cells, although again this may be tightly linked to the differentiation of each Th cell subset. STAT1 downstream of IL-27 (Stumhofer et al. 2007; Fitzgerald et al. 2007; Batten et al. 2008), STAT3 downstream of IL-27, IL-21 or IL-6 (Stumhofer et al. 2007; Xu et al. 2009; Spolski et al. 2009), STAT4 downstream of IL-12 (Saraiva et al. 2009) and STAT6 downstream of IL-4 (Saraiva et al. 2009) have all been linked to IL-10 production in Th cells (Fig. 3c). Furthermore, IL-10 could potentially also enhance its own production in T cells through the activation of STAT3, as previously shown in macrophages (Staples et al. 2007). However, it is important

to note these functional studies use STAT deficient T cells that are not responsive to the cytokines and thus unable to differentiate into each discrete Th cell subset. Direct binding of the various STATs to the *Il10* locus has recently been demonstrated for STAT4 in intron 4 in Th1 cells and STAT6 in the promoter region in Th2 cells (Fig. 2c), where it is thought to facilitate the acquisition of the activating H3K4me3 and loss of the repressive H3K27me3 histone marks, and thus increasing the accessibility of the *Il10* gene locus to transcription factors (Wei et al. 2010). In Th17 cells, STAT3 has been shown to bind in an intron 4 in the *Il10* gene locus (Li et al. 2012) (Fig. 2c), although its role in the remodelling of the *Il10* gene locus is yet to be determined.

SMADs downstream of TGF-β signalling have been shown to regulate IL-10 production in Th cells, albeit at the expense of inhibiting Th1 and Th2, but not Th17 differentiation (Saraiva et al. 2009). In particular, SMAD4 has been shown bind and transactivate the *Il10* gene promoter in Th1 but not in Th2 cells (Kitani et al. 2003) (Fig. 2d), while SMAD3 together with GATA3 has been shown to positively regulate IL-10 production in response to TGF-β in a Th2 cell line (Blokzijl et al. 2002).

4.2.3 The Role of T Helper Cell Master Regulators in IL-10 Regulation

Given the role of master regulators in the differentiation of Th cell subsets, to which the production of IL-10 is tightly coupled, it is possible that IL-10 production in Th cells is further regulated by the lineage specific master regulator transcription factors induced mainly downstream of the STAT signalling but also of TCR. Recent genome-wide studies of Th cells following the binding of p300, a marker of active enhancers, in wild type *versus* STAT deficient Th cells transfected with lineage specific master regulators, have identified a small subset of enhancers that are regulated directly by GATA3 (Vahedi et al. 2012) and also but to a lesser extent by TBET (Vahedi et al. 2012) and RORγt (Ciofani et al. 2012). Namely, overexpression of GATA3 in STAT6 deficient Th2 cells resulted in the recovery of half of the Th2-specific STAT6-dependent p300 binding sites including many in the *Il4* gene locus (Vahedi et al. 2012). Similarly, overexpression of Tbet in STAT4 deficient Th1 cells resulted in the recovery of only about a quarter of the Th1-specific STAT4-dependent p300 binding sites including many in the *Ifng* gene locus (Vahedi et al. 2012). The fact that GATA3 binding can precede enhancer activation in developing thymocytes (Zhang et al. 2012) does suggest that it may function as a pioneer-like factor at certain genetic loci. For example, in Th2 cells, GATA3 has been shown to drive the expression of IL-4 by both mediating the remodelling of the *Il4* gene locus (Fields et al. 2002; Lee et al. 2000) and transactivation of its promoter (Zheng and Flavell 1997). GATA3 also binds and facilitates remodelling of the *Il10* gene locus, but it does not transactivate the *Il10* promoter (Chang et al. 2007b; Shoemaker et al. 2006) (Fig. 2d). It remains to be determined whether GATA3 acts as a true pioneer-like transcription factor or whether its action is aided by other factors in order for it to mediate the

remodelling of the *Il10* gene locus. Furthermore, the fact that GATA3 alone is not able to transactivate the *Il10* promoter and is not required for IL-10 production in differentiated Th2 cells (Zhu et al. 2004) illustrates the need for additional factors that promote *Il10* gene expression in differentiated Th2 cells. Importantly, GATA3 is only expressed to a high level in Th2 cells, implying that other mechanisms are likely to regulate IL-10 production in the different Th cell subsets that express no or low levels of GATA3. Since both TBET and RORγt can, similarly to GATA3, regulate a subset of genes independently of the STAT proteins, it would be interesting to know whether they can also alter the accessibility of the *Il10* gene locus in addition to the hallmark cytokines in Th1 and Th17 cells.

4.2.4 The Role of Other Transcription Factors in IL-10 Regulation

Although originally described as a Th2-specific transcription factor (Ho et al. 1996), c-MAF expression is detectable in all the different Th cells subsets, where its expression has been shown to correlate with the levels of IL-10 (Saraiva et al. 2009). In Th2 cells, c-MAF is thought to direct the expression of IL-4 by binding to a c-MAF responsive element (MARE) in the proximal promoter of the *Il4* gene (Ho et al. 1996), the action of which has further been shown to be augmented by IRF4/NFAT1 (Rengarajan et al. 2002) and AP-1 (JunB) (Li et al. 1999). However, c-MAF alone is thought not to be able to transactivate the *Il10* gene promoter (Kim et al. 1999). It is yet to be determined whether c-MAF in combination with other factors such as NFAT or AP-1, similarly to their role in the regulation of IL-4, can also regulate IL-10 production, both of which have been shown to bind the *Il10* gene locus as discussed above. The expression of c-MAF in Th1 cells is driven by IL-12, however, the role of c-MAF in regulating IL-10 is only correlative whereby the expression of c-MAF decreases together with IL-10 upon treatment with a MEK inhibitor that abrogates ERK activation (Saraiva et al. 2009). Furthermore, it is unclear whether ERK activation is induced downstream of the TCR via Ras, or of IL-12R via TPL-2 (Saraiva et al. 2009; Watford et al. 2008), or both. In the so called 'Tr1' cells, $CD4^+$ T cells that co-secrete IFN-γ and IL-10 differentiated in vitro in the presence of IL-27 or IL-27 and TGF-β (Pot et al. 2009), c-MAF together with AHR have been implicated to play a role in the positive regulation of IL-10 production (Apetoh et al. 2010). As such, knocking down c-MAF or AHR in 'Tr1' cells has been shown to correlate with a decrease in *Il10* mRNA expression and both have been demonstrated to bind and synergistically transactivate the *Il10* gene promoter (Apetoh et al. 2010) (Fig. 2d). The induction of c-MAF in the 'Tr1' setting is thought to be further regulated by IL-21 and possibly by inducible T cell costimulator (ICOS) since both IL-21 and ICOS deficient T cells show reduced IL-10 production and c-MAF expression (Pot et al. 2011). Of note, both IL-27 (Owaki et al. 2006) and IL-21 (Fuqua et al. 2008) are thought to also activate ERK. Downstream of TGF-β signalling c-MAF has been shown to play a role in the differentiation programme of Th17 cells (Rutz et al. 2011), where it is primarily thought to act as a repressor of proinflammatory gene

expression, e.g. of *Rora*, *Runx1*, *Il1r1*, *Ccr6* and *Tnf* but where it can also act as an inducer of genes involved in the attenuation of inflammation, including of *Il10* (Ciofani et al. 2012). c-MAF has been further shown to positively regulate IL-10 (and possibly IL-17) in Th17 cells, but to have a negative effect on IL-22 production, as demonstrated using retroviral transduction and siRNA knock-down assays (Rutz et al. 2011). In Th17 cells, c-MAF has additionally been shown to bind and transactivate the *Il10* gene promoter in a luciferase reporter assay (Xu et al. 2009) (Fig. 2d). Taken together, these findings indicate that c-MAF could act as a common transcriptional regulator of IL-10 in different immune cell types, as suggested by both the Th cell and macrophage studies described above. However, at least in Th2 cells, it is unclear whether c-maf is involved in differentiation rather than IL-10 production *per se*.

The transcriptional repressor E4 promoter-binding protein 4 (E4BP4) encoded by the *Nfil3* gene has recently been shown to play a role in positively regulating the production of IL-10 in Th1 and Th2 cells independently of their hallmark cytokines IFN-γ and IL-4, respectively (Motomura et al. 2011) (Fig. 2c). E4BP4 has been shown to bind to intron 4 and to the 3' UTR at the *Il10* gene locus in Th2 cells and, owing to the decrease in histone acetylation in Th2 cells from *Nfil3*$^{-/-}$ mice, E4BP4 is thought to regulate the permissive status of the *Il10* gene locus (Motomura et al. 2011). Specifically, E4BP4 has been shown to increase *Il10* gene expression in a GATA3-independent manner, as demonstrated by retroviral transduction studies in *Nfil3*$^{-/-}$ and *Gata3* conditional knockout cells (Motomura et al. 2011). Thus, E4BP4 could be a dominant factor responsible for epigenetic regulation controlling accessibility of the *Il10* gene locus in Th1 cells, akin to GATA3 in Th2 cells. Although it is not yet known which signalling pathways lead to the induction of *Nfil3* expression in T cells, in macrophages *Nfil3* is an IL-10 responsive gene (Smith et al. 2011). Of note, overexpression of E4BP4 does not allow Th17 differentiation (Ciofani et al. 2012), implying that other mechanisms must operate to allow IL-10 production in Th17 cells. Moreover, *Nfil3*$^{-/-}$ mice develop mild diarrhoea reminiscent of colitis observed in *Il10*$^{-/-}$ animals (Motomura et al. 2011). It is not clear, however, whether this is due to the possible lack of IL-10 in T cells or myeloid cells, a Th cell imbalance, or all of these.

PR domain zinc finger protein 1 (BLIMP-1) encoded by the *Prdm1* gene is a transcriptional repressor induced downstream of the TCR that has also been shown to positively regulate IL-10 production in CD4$^+$ T cells, as demonstrated by decreased IL-10 production in *Prdm1* $^{fl/fl}$ *Lck Cre*$^-$ CD4$^+$ T cells, which in vivo is accompanied by pathology and increased inflammation (Martins et al. 2006). However, in addition to the observed decrease in IL-10 production, *Prdm1* $^{fl/fl}$ *Lck Cre*$^-$ CD4$^+$ T cells produce increased amounts of IL-2 and IFN-γ, while their IL-4 production is not affected and the effect on IL-17 production is unknown (Martins et al. 2006). BLIMP-1 downstream of IL-27 has further been demonstrated to mediate IL-10 induction in CD4$^+$ (Iwasaki et al. 2013) and CD8$^+$ T cells (Sun et al. 2011). IL-10 production by CD4$^+$ Treg cells is also regulated by BLIMP-1, primarily by its synergistic effect with IRF4 on histone acetylation at

the *Il10* gene promoter (Cretney et al. 2011). However, the molecular mechanism of how BLIMP-1 regulates IL-10 production in Th cells is not known. Hence, the severe colitis that is observed in *Prdm1* $^{fl/fl}$ *Lck Cre* mice may be due to the collective effect of defective thymic output, decreased Treg cell IL-10 production and/or excessive effector T cell function (Martins et al. 2006).

Of note, BLIMP-1 expression is regulated by B-cell lymphoma 6 (BCL-6), itself a transcriptional repressor typically involved in the establishment of T follicular helper (Tfh) cell identity (Yu et al. 2009; Johnston et al. 2009; Nurieva et al. 2009). Similarly to what is known about BCL-6 regulation in B cells, the action of BCL-6 in T cells is in turn suppressed by BLIMP-1 (Johnston et al. 2009). BCL-6 expression is driven by IL-6 and IL-21 and it functions mainly by inhibiting the expression and/or function of TBET, GATA3 and RORγt master regulator transcription factors thus suppressing the Th1, Th2 and Th17 differentiation programmes, respectively, while concurrently inducing the expression Tfh cell-related genes (Liu et al. 2013; Kassiotis and O'Garra 2009). In view of the reciprocal regulation of BLIMP-1 and BCL-6, it is possible that the role of BLIMP-1 in IL-10 regulation is indirect via the repression of BCL-6 and subsequent upregulation of GATA3 (Kusam et al. 2003).

4.2.5 The Regulation of IL-10 by Notch Signalling

In addition to the TCR and cytokine signals, signalling via Notch following interaction with ligands induced on macrophages and DCs by pathogen-derived signals have been shown to have an impact on the differentiation of $CD4^+$ Th cells as well as their production of IL-10 (Fig. 3c). In particular, the engagement of Delta-like Notch ligands (DLL) has been shown to favour the development of Th1 cells, while ligation of Jagged preferentially leads to Th2 cell differentiation (Radtke et al. 2013). DLL-1 and DLL-4 Notch ligands have further been shown to induce IL-10 but have little effect on IFN-γ production in Th1 cells (Rutz et al. 2008). Of interest, Jagged1, typically associated with inducing IL-4 production via Notch, has paradoxically been identified as the ligand of CD46 (Le Friec et al. 2012) driving the production of IL-10 in human T cells that co-secrete IFN-γ (Kemper et al. 2003). However, the exact molecular mechanisms of IL-10 regulation by Notch signalling are not yet known and warrant further investigation.

4.2.6 Negative Regulators of IL-10 Production

Similarly to macrophages and DCs, negative mechanisms of IL-10 regulation have also been identified in Th cells.

E26 transformation-specific 1 (ETS-1) is a member of the ETS family of transcription factors, the expression of which is high in resting T cells but decreases following their activation (Romano-Spica et al. 1995). Of note, ETS-1 can be further regulated at a post-transcriptional level by ERK (Hollenhorst 2012), although

it is not known whether this is the case in T cells. ETS-1 has been shown to negatively regulate IL-10 production in Th1 and Th2 cells, as demonstrated by enhanced IL-10 in *Ets1*$^{-/-}$ CD4^{+} T cells stimulated under Th1 and Th2 skewing conditions, though the effect of ETS-1 deficiency on the hallmark cytokines has not been investigated (Lee et al. 2012). Of note, ETS-1 has previously been shown to positively regulate IFN-γ together with TBET (Grenningloh et al. 2005). ETS-1 has been to shown to downregulate the expression of IL-10 in Th1 cells via its interaction and recruitment of HDAC1 to HSS −0.12, +1.65 and +2.98 kb at the *Il10* gene locus, thus maintaining its relatively closed conformation (Lee et al. 2012) (Fig. 2c).

PU.1, another member of the ETS family, is expressed specifically in Th2 cells where it inhibits the production of Th2 hallmark cytokines including IL-10 by interfering with DNA binding of the master regulator GATA3, although a direct effect PU.1 on the *Il10* gene locus has not been reported (Chang et al. 2005). In addition to GATA3, PU.1 also complexes with IRF4, a transcription factor correlating with high levels of IL-10 expression in Th2 cells, thus preventing the ability of IRF4 to bind and to transactivate the *Il10* gene promoter (Ahyi et al. 2009).

In summary, the above studies indicate that the activity of transcription factors downstream of the TCR, cytokine signalling and other environmental cues regulate the production of IL-10 at multiple levels ranging from increasing the accessibility of the *Il10* gene locus to cooperative binding of transcription factors. This allows the assembly of transcription factor complexes at discrete sites along the *Il10* gene locus, which likely act as platforms to integrate TCR and cytokine-driven signals allowing the transcription of the *Il10* gene in a context-specific manner. However, the components of the transcription factor complexes and their temporal recruitment to the *Il10* gene locus during the differentiation of the various Th cell subsets remain unclear and require further investigation. However, the tight relationship between Th cell differentiation versus IL-10 production poses a challenge in the future studies in this field. Furthermore, it will be important to determine what are the common or cell type-specific requirements for IL-10 production in the Th cell subsets. Ultimately, these studies will need to be recapitulated in human cells, as most of the data to date has been generated using mouse as a model organism.

5 Post-Transcriptional Regulation of IL-10

Post-transcriptional control of cytokine production is a key step for the quantitative regulation of cytokines produced by cells of the immune system (Anderson 2008; Ivanov and Anderson 2013) (Fig. 1). This layer of regulation is critical to ensure that the rapid transcription of cytokines in response to an initial stimulus, such as microbial infection, is also rapidly turned off to avoid excessive proinflammatory cytokine production likely resulting in tissue damage, or the development of chronic infection in the case of an anti-inflammatory cytokine such

as IL-10. One of the first pieces of evidence of post-transcriptional regulation of *Il10* expression came from studies of the 3′UTR of the *Il10* mRNA, which showed the existence of AU rich elements (AREs), capable of mediating mRNA decay in a lymphocyte cell line (Powell et al. 2000). Another study, however, concluded that the same destabilising effect of the 3′UTR of *Il10* mRNA was not observed in RAW 264.7 macrophages (Nemeth et al. 2005). AREs are found in many cytokine 3'UTRs and mark short-lived mRNA, as they recruit various ARE-binding proteins that increase or decrease mRNA stability (Anderson 2008). The ARE-binding protein that was identified in a genome-wide analysis to target *Il10* mRNA is tristetraprolin (TTP) (Stoecklin et al. 2008). TTP also promotes the rapid decay of other cytokine mRNAs, including that of tumour necrosis factor (TNF) (Carballo et al. 1998), IL-6 (Neininger et al. 2002) and granulocyte-monocyte colony stimulating factor (GM-CSF) (Carballo et al. 2000), by initiating the assembly of RNA decay machinery thus causing the elimination of bound mRNAs (Franks and Lykke-Andersen 2007; Kedersha et al. 2005). In line with a role for TTP in regulating *Il10* mRNA stability, the half-life of *Il10* mRNA in TLR 4 stimulated macrophages was increased in the absence of this molecule (Stoecklin et al. 2008; Gaba et al. 2012). Consequently, in the absence of TTP the amount of IL-10 produced by TLR 4 stimulated macrophages was found to be higher (Gaba et al. 2012). More importantly, higher IL-10 levels were also described in vivo in the serum of LPS-treated mice where TTP was ablated from myeloid cells (Kratochvill et al. 2011). However, such differences in IL-10 production were not found in TLR 4 stimulated TTP deficient DCs (Bros et al. 2010), potentially due to increased p38 activity in these cells (Yu et al. 2004). Indeed, TTP is negatively regulated by the MAP kinase p38 (Stoecklin et al. 2004; Brook et al. 2006; Hitti et al. 2006; Tudor et al. 2009), which although needed for TTP expression, also restrains the mRNA destabilising activity of TTP (Sandler and Stoecklin 2008). Although the impact of p38 control of TTP-mediated mRNA destabilisation varies with target mRNAs, in the case of IL-10, the inhibition of p38 activation allows for elevated TTP activity and thus enhanced TTP-mediated mRNA decay (Tudor et al. 2009). In a recent study, the activity of p38 during the inflammatory response was shown to qualitatively and temporally control TTP activity, thus leading to a timely induction of cytokine mRNA decay (Kratochvill et al. 2011). This temporal control of TTP by p38 is influenced by different mechanisms including by TRIF signalling, as shown by comparing the activity of p38 activity and the *Il10* mRNA decay in macrophages stimulated through TLR 2 or TLR 4 (Teixeira-Coelho et al., in press). Although less studied than TTP, the RNA-binding protein ARE/poly(U) binding degradation factor 1 (AUF1) is also known to bind to the 3'UTR of the *Il10* mRNA. Diminished levels of AUF1 correlated with increased half-life of the *Il10* mRNA in a melanoma cell line (Brewer et al. 2003). More recently, the translocation of AUF1 from the nucleus to the cytosol of TLR 4 stimulated macrophages was found to be regulated by DUSP1 (Chaudhry et al. 2011). Consequently, in the absence of DUSP1, both *Il10* mRNA stability and IL-10 secretion were increased (Yu et al. 2011).

IL-10 has been shown itself to regulate the stability of cytokine mRNAs, including its own, in TLR 4 stimulated blood monocytes (Brown et al. 1996). More recently, this effect of IL-10 was found to be dependent on TTP. In addition to being a target of TTP, IL-10 is itself an activator of TTP, by increasing TTP expression and reducing late p38 activity (Gaba et al. 2012; Schaljo et al. 2009). Thus, in situations when IL-10 is being produced, TTP is activated and a post-transcriptional programme for cytokine gene regulation is initiated, mediated by IL-10 induction of mRNA decay. It is possible that this mechanism results in limiting IL-10 translation, thus helping in guaranteeing the appropriate balance of the immune response.

Additionally to the 3′UTRs that control the mRNA stability and decay rates, 5′UTR regions are also linked to post-transcriptional gene regulation by mediating the binding rate of mRNA to the ribosomal 40S subunit, thus controlling the mRNA translation (Pichon et al. 2012). Two alternative variants of the 5′UTR sequences were found in *Il10* mRNA synthesised after TLR 4 stimulation of blood cells, suggesting that modulation of IL-10 protein translation may also be implicated in the network of post-transcriptional mechanisms regulating IL-10 (Forte et al. 2009).

MicroRNAs (miRNA) have also been shown to contribute to the post-transcriptional mechanisms regulating TLR signalling and subsequent cytokine production, including that of IL-10 (O'Neill et al. 2011). For example, hsa-miRNA-106a binds to the 3'UTR of the *Il10* mRNA and negatively regulates its expression in different cell lines (Sharma et al. 2009). Also, miRNA-466L was shown to upregulate TLR-induced IL-10 expression in macrophages by antagonising TTP-mediated *Il10* mRNA degradation and extending the half-life of *Il10* mRNA (Ma et al. 2010). MiRNA-21 induced upon TLR 4 stimulation targeted the effector molecule programmed cell death 4 (PDCD4) and promoted IL-10 production (Sheedy et al. 2010).

Thus, a growing body of evidence clearly demonstrates that IL-10 production by immune cells is controlled at the post-transcriptional level along with many other cytokines. Since the majority of the studies have been performed in TLR-activated macrophages, it will be interesting to study these potential mechanisms in other immune cells. For example, in $CD4^+$ T cells, p38 was found to be involved in regulating the stability of IL-4 and IL-5 mRNAs (Dodeller et al. 2005), but such studies are lacking in relation to IL-10.

6 Concluding Remarks

IL-10 mediates the fine balance between immunosuppression and immunopathology in a number of settings. Therefore, a thorough understanding of the molecular mechanisms that regulate the expression of this cytokine is crucial for the design of immune intervention strategies. The subject of IL-10 regulation is complicated by the fact that IL-10 can be expressed by almost all cells within both the innate and adaptive immune systems, despite the fact that each cell type responds to different stimuli. This complexity is further compounded by the numerous positive

and negative regulatory networks which integrate to determine the final level of IL-10 production.

In macrophages and DCs, although several mechanisms have been identified which regulate the production of IL-10, most studies focus on single PRR stimuli, whereas pathogens likely activate multiple PRRs. As such, how the signals from different PRRs cooperate to modulate IL-10 production versus the production of different proinflammatory cytokines remain only partially understood. Additionally, the production of IL-10 in response to damage-associated molecular patterns (DAMP) has to date not been investigated. These studies would provide a better understanding of the regulation of IL-10 in the context of infection and sterile inflammation. In addition, the mechanisms controlling the distinct levels of IL-10 produced by the different myeloid populations, for example, the relatively higher level of IL-10 production by macrophages compared to DCs, are poorly defined. Fully investigating whether the signalling pathways induced by the same TLRs are fundamentally different in macrophages, DCs and myeloid populations from distinct anatomical locations, or if the chromatin state of the *Il10* locus is different between these cell types would greatly enhance our understanding of how IL-10 is regulated.

In T cells, several signalling pathways and downstream transcription factors regulating IL-10 have been identified in the different Th cell subsets. However, the ultimate challenge lies in distinguishing whether these factors are truly a requirement for IL-10 expression as opposed to a requirement for Th cell differentiation, or both. Furthermore, many of the studies to date concentrate on a single Th cell subset. A concurrent analysis of the all the subsets would allow us to determine whether there are common or Th cell type-specific factors for IL-10 production in the different Th cell subsets. Lastly, owing to the heterogeneity within each of the Th cell populations with respect to hallmark cytokines as well as IL-10 production, it would be important to investigate these separately in order to obtain more meaningful results.

Therefore, for all cell types, including those currently less studied but known to produce IL-10, such as mast cells, B cells or regulatory T cells, the future challenge is to decipher the complex IL-10 regulatory networks including the temporal recruitment of transcription factors and their hierarchies of binding to the *Il10* gene locus. The use of genome-wide, high-throughput, high resolution and bioinformatic approaches will complement traditional molecular and biochemical methods and be key to gaining a better understanding of IL-10 regulation in the different immune cell types. Finally, it will be important to identify the immune scenarios in which these various factors regulate IL-10 production in vivo in order to use this knowledge in the fine-tuning of immune intervention strategies.

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References

Agrawal S et al (2003) Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. J Immunol 171(10):4984–4989

Ahyi AN et al (2009) IFN regulatory factor 4 regulates the expression of a subset of Th2 cytokines. J Immunol 183(3):1598–1606

Alvarez Y et al (2009) The induction of IL-10 by zymosan in dendritic cells depends on CREB activation by the coactivators CREB-binding protein and TORC2 and autocrine PGE2. J Immunol 183(2):1471–1479

Aman MJ et al (1996) Interferon-alpha stimulates production of interleukin-10 in activated CD4+ T cells and monocytes. Blood 87(11):4731–4736

Ananieva O et al (2008) The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling. Nat Immunol 9(9):1028–1036

Anderson P (2008) Post-transcriptional control of cytokine production. Nat Immunol 9(4):353–359

Apetoh L et al (2010) The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol 11(9):854–861

Banerjee A et al (2006) Diverse Toll-like receptors utilize Tpl2 to activate extracellular signal-regulated kinase (ERK) in hemopoietic cells. Proc Natl Acad Sci USA 103(9):3274–3279

Batten M et al (2008) Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. J Immunol 180(5):2752–2756

Benkhart EM et al (2000) Role of Stat3 in lipopolysaccharide-induced IL-10 gene expression. J Immunol 165(3):1612–1617

Blokzijl A, ten Dijke P, Ibanez CF (2002) Physical and functional interaction between GATA-3 and Smad3 allows TGF-beta regulation of GATA target genes. Curr Biol 12(1):35–45

Boonstra A et al (2006) Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88- and TRIF-dependent TLR signals, and TLR-independent signals. J Immunol 177(11):7551–7558

Brenner S et al (2003) cAMP-induced Interleukin-10 promoter activation depends on CCAAT/enhancer-binding protein expression and monocytic differentiation. J Biol Chem 278(8):5597–5604

Brewer G et al (2003) Increased interleukin-10 mRNA stability in melanoma cells is associated with decreased levels of A + U-rich element binding factor AUF1. J Interferon Cytokine Res 23(10):553–564

Brightbill HD et al (2000) A prominent role for Sp1 during lipopolysaccharide-mediated induction of the IL-10 promoter in macrophages. J Immunol 164(4):1940–1951

Brook M et al (2006) Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. Mol Cell Biol 26(6):2408–2418

Bros M et al (2010) The RNA binding protein tristetraprolin influences the activation state of murine dendritic cells. Mol Immunol 47(5):1161–1170

Brown CY et al (1996) Differential regulation of the stability of cytokine mRNAs in lipopolysaccharide-activated blood monocytes in response to interleukin-10. J Biol Chem 271(33):20108–20112

Brownlie RJ, Zamoyska R (2013) T cell receptor signalling networks: branched, diversified and bounded. Nat Rev Immunol 13(4):257–269

Cao S et al (2006) NF-kappaB1 (p50) homodimers differentially regulate pro- and anti-inflammatory cytokines in macrophages. J Biol Chem 281(36):26041–26050

Cao S et al (2005) The protooncogene c-Maf is an essential transcription factor for IL-10 gene expression in macrophages. J Immunol 174(6):3484–3492

Carballo E, Lai WS, Blackshear PJ (2000) Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood 95(6):1891–1899

Carballo E, Lai WS, Blackshear PJ (1998) Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science 281(5379):1001–1005
Chakrabarti A et al (2008) Protein kinase R-dependent regulation of interleukin-10 in response to double-stranded RNA. J Biol Chem 283(37):25132–25139
Chang EY et al (2007a) Cutting edge: involvement of the type I IFN production and signaling pathway in lipopolysaccharide-induced IL-10 production. J Immunol 178(11):6705–6709
Chang HC et al (2005) PU.1 expression delineates heterogeneity in primary Th2 cells. Immunity 22(6):693–703
Chang HD et al (2007b) Expression of IL-10 in Th memory lymphocytes is conditional on IL-12 or IL-4, unless the IL-10 gene is imprinted by GATA-3. Eur J Immunol 37(3):807–817
Chaudhry A et al (2011) Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity 34(4):566–578
Chen G et al (2011) The NF-kappaB transcription factor c-Rel is required for Th17 effector cell development in experimental autoimmune encephalomyelitis. J Immunol 187(9):4483–4491
Chi H et al (2006) Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. Proc Natl Acad Sci USA 103(7):2274–2279
Chung EY et al (2007) Interleukin-10 expression in macrophages during phagocytosis of apoptotic cells is mediated by homeodomain proteins Pbx1 and Prep-1. Immunity 27(6):952–964
Ciofani M et al (2012) A validated regulatory network for Th17 cell specification. Cell 151(2):289–303
Clark K et al (2012) Phosphorylation of CRTC3 by the salt-inducible kinases controls the interconversion of classically activated and regulatory macrophages. Proc Natl Acad Sci USA 109(42):16986–16991
Corre B et al (2013) Type I interferon potentiates T-cell receptor mediated induction of IL-10-producing CD4(+) T cells. Eur J Immunol 43(10):2730–2740
Cretney E et al (2011) The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. Nat Immunol 12(4):304–311
Csoka B et al (2007) A2A adenosine receptors and C/EBPbeta are crucially required for IL-10 production by macrophages exposed to Escherichia coli. Blood 110(7):2685–2695
Das J et al (2001) A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. Nat Immunol 2(1):45–50
Dennehy KM et al (2009) Reciprocal regulation of IL-23 and IL-12 following co-activation of Dectin-1 and TLR signaling pathways. Eur J Immunol 39(5):1379–1386
Dillon S et al (2004) A Toll-like receptor 2 ligand stimulates Th2 responses in vivo, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. J Immunol 172(8):4733–4743
Dillon S et al (2006) Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. J Clin Invest 116(4):916–928
Dodeller F et al (2005) The p38 mitogen-activated protein kinase regulates effector functions of primary human CD4 T cells. Eur J Immunol 35(12):3631–3642
Dong C, Davis RJ, Flavell RA (2002) MAP kinases in the immune response. Annu Rev Immunol 20:55–72
Dumitru CD et al (2000) TNF-alpha induction by LPS is regulated posttranscriptionally via a Tpl2/ERK-dependent pathway. Cell 103(7):1071–1083
Edwards AD et al (2002) Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. J Immunol 169(7):3652–3660
Edwards AD et al (2003) Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines. Eur J Immunol 33(4):827–833
Fields PE, Kim ST, Flavell RA (2002) Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. J Immunol 169(2):647–650

Fitzgerald DC et al (2007) Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat Immunol 8(12):1372–1379
Foey AD et al (1998) Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF-alpha: role of the p38 and p42/44 mitogen-activated protein kinases. J Immunol 160(2):920–928
Forte GI et al (2009) Characterization of two alternative Interleukin(IL)-10 5'UTR mRNA sequences, induced by lipopolysaccharide (LPS) stimulation of peripheral blood mononuclear cells. Mol Immunol 46(11–12):2161–2166
Franks TM, Lykke-Andersen J (2007) TTP and BRF proteins nucleate processing body formation to silence mRNAs with AU-rich elements. Genes Dev 21(6):719–735
Frazer KA et al. (2004) VISTA: computational tools for comparative genomics. Nucleic Acids Res 32(Web Server issue):W273-9
Fuqua CF et al (2008) Involvement of ERK-1/2 in IL-21-induced cytokine production in leukemia cells and human monocytes. Cytokine 44(1):101–107
Gaba A et al (2012) Cutting edge: IL-10-mediated tristetraprolin induction is part of a feedback loop that controls macrophage STAT3 activation and cytokine production. J Immunol 189(5):2089–2093
Gantke T, Sriskantharajah S, Ley SC (2011) Regulation and function of TPL-2, an IkappaB kinase-regulated MAP kinase kinase kinase. Cell Res 21(1):131–145
Gerber JS, Mosser DM (2001) Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. J Immunol 166(11):6861–6868
Gerondakis S et al (2006) Unravelling the complexities of the NF-kappaB signalling pathway using mouse knockout and transgenic models. Oncogene 25(51):6781–6799
Grenningloh R, Kang BY, Ho IC (2005) Ets-1, a functional cofactor of T-bet, is essential for Th1 inflammatory responses. J Exp Med 201(4):615–626
Guarda G et al (2011) Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 34(2):213–223
Hacker H et al (2006) Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature 439(7073):204–207
Hammer M et al (2005) Control of dual-specificity phosphatase-1 expression in activated macrophages by IL-10. Eur J Immunol 35(10):2991–3001
Hitti E et al (2006) Mitogen-activated protein kinase-activated protein kinase 2 regulates tumor necrosis factor mRNA stability and translation mainly by altering tristetraprolin expression, stability, and binding to adenine/uridine-rich element. Mol Cell Biol 26(6):2399–2407
Ho IC et al (1996) The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. Cell 85(7):973–983
Hollenhorst PC (2012) RAS/ERK pathway transcriptional regulation through ETS/AP-1 binding sites. Small GTPases 3(3):154–158
Hu X et al (2006) IFN-gamma suppresses IL-10 production and synergizes with TLR2 by regulating GSK3 and CREB/AP-1 proteins. Immunity 24(5):563–574
Im SH et al (2004) Chromatin-level regulation of the IL10 gene in T cells. J Biol Chem 279(45):46818–46825
Ito T et al (2006) Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells. Blood 107(6):2423–2431
Ivanov II et al (2006) The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126(6):1121–1133
Ivanov P, Anderson P (2013) Post-transcriptional regulatory networks in immunity. Immunol Rev 253(1):253–272
Iwasaki Y et al (2013) Egr-2 transcription factor is required for Blimp-1 mediated IL-10 production in IL-27 stimulated CD4(+) T cells. Eur J Immunol 43(4):1063–1073
Iyer SS, Ghaffari AA, Cheng G (2010) Lipopolysaccharide-mediated IL-10 transcriptional regulation requires sequential induction of type I IFNs and IL-27 in macrophages. J Immunol 185(11):6599–6607

Jarnicki AG et al (2008) Attenuating regulatory T cell induction by TLR agonists through inhibition of p38 MAPK signaling in dendritic cells enhances their efficacy as vaccine adjuvants and cancer immunotherapeutics. J Immunol 180(6):3797–3806
Jarrossay D et al (2001) Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. Eur J Immunol 31(11):3388–3393
Jenuwein T, Allis CD (2001) Translating the histone code. Science 293(5532):1074–1080
Jiang C, Pugh BF (2009) Nucleosome positioning and gene regulation: advances through genomics. Nat Rev Genet 10(3):161–172
Johnston RJ et al (2009) Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science 325(5943):1006–1010
Jones EA, Flavell RA (2005) Distal enhancer elements transcribe intergenic RNA in the IL-10 family gene cluster. J Immunol 175(11):7437–7446
Kadowaki N et al (2001) Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. J Exp Med 194(6):863–869
Kaiser F et al (2009) TPL-2 negatively regulates interferon-beta production in macrophages and myeloid dendritic cells. J Exp Med 206(9):1863–1871
Kalliolias GD, Ivashkiv LB (2008) IL-27 activates human monocytes via STAT1 and suppresses IL-10 production but the inflammatory functions of IL-27 are abrogated by TLRs and p38. J Immunol 180(9):6325–6333
Kanters E et al (2003) Inhibition of NF-kappaB activation in macrophages increases atherosclerosis in LDL receptor-deficient mice. J Clin Invest 112(8):1176–1185
Karin M, Liu Z, Zandi E (1997) AP-1 function and regulation. Curr Opin Cell Biol 9(2):240–246
Kassiotis G, O'Garra A (2009) Establishing the follicular helper identity. Immunity 31(3):450–452
Katholnig K et al (2013) p38alpha Senses Environmental Stress To Control Innate Immune Responses via Mechanistic Target of Rapamycin. J Immunol 190(4):1519–1527
Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11(5):373–384
Kedersha N et al (2005) Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J Cell Biol 169(6):871–884
Kemper C et al (2003) Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature 421(6921):388–392
Kim JI et al (1999) The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity 10(6):745–751
Kim JM et al (1992) Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. J Immunol 148(11):3618–3623
Kim L et al (2005) p38 MAPK autophosphorylation drives macrophage IL-12 production during intracellular infection. J Immunol 174(7):4178–4184
Kimura A et al (2009) Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. J Exp Med 206(9):2027–2035
Kitani A et al (2003) Transforming growth factor (TGF)-beta1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. J Exp Med 198(8):1179–1188
Kratochvill F et al (2011) Tristetraprolin-driven regulatory circuit controls quality and timing of mRNA decay in inflammation. Mol Syst Biol 7:560
Krausgruber T et al (2011) IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. Nat Immunol 12(3):231–238
Kusam S et al (2003) Inhibition of Th2 differentiation and GATA-3 expression by BCL-6. J Immunol 170(5):2435–2441
Lang R et al (2002) Shaping gene expression in activated and resting primary macrophages by IL-10. J Immunol 169(5):2253–2263
Le Friec G et al (2012) The CD46-Jagged1 interaction is critical for human TH1 immunity. Nat Immunol 13(12):1213–1221

Lee CG et al (2009) A distal cis-regulatory element, CNS-9, controls NFAT1 and IRF4-mediated IL-10 gene activation in T helper cells. Mol Immunol 46(4):613–621
Lee CG et al (2012) Interaction of Ets-1 with HDAC1 represses IL-10 expression in Th1 cells. J Immunol 188(5):2244–2253
Lee HJ et al (2000) GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. J Exp Med 192(1):105–115
Lenhard B, Sandelin A, Carninci P (2012) Metazoan promoters: emerging characteristics and insights into transcriptional regulation. Nat Rev Genet 13(4):233–245
Levings MK et al (2001) IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. J Immunol 166(9):5530–5539
Li B et al (1999) Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. EMBO J 18(2):420–432
Li P et al (2012) BATF-JUN is critical for IRF4-mediated transcription in T cells. Nature 490(7421):543–546
Liu X, Nurieva RI, Dong C (2013) Transcriptional regulation of follicular T-helper (Tfh) cells. Immunol Rev 252(1):139–145
Liu YW et al (2003) Functional cooperation of simian virus 40 promoter factor 1 and CCAAT/enhancer-binding protein beta and delta in lipopolysaccharide-induced gene activation of IL-10 in mouse macrophages. J Immunol 171(2):821–828
Lucas M et al (2005) ERK activation following macrophage FcgammaR ligation leads to chromatin modifications at the IL-10 locus. J Immunol 175(1):469–477
Ma F et al (2010) MicroRNA-466l upregulates IL-10 expression in TLR-triggered macrophages by antagonizing RNA-binding protein tristetraprolin-mediated IL-10 mRNA degradation. J Immunol 184(11):6053–6059
Ma W et al (2001) The p38 mitogen-activated kinase pathway regulates the human interleukin-10 promoter via the activation of Sp1 transcription factor in lipopolysaccharide-stimulated human macrophages. J Biol Chem 276(17):13664–13674
Macian F (2005) NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol 5(6):472–484
MacKenzie KF et al (2013) PGE(2) induces macrophage IL-10 production and a regulatory-like phenotype via a protein kinase A-SIK-CRTC3 pathway. J Immunol 190(2):565–577
Martin M et al (2003) Role of the phosphatidylinositol 3 kinase-Akt pathway in the regulation of IL-10 and IL-12 by Porphyromonas gingivalis lipopolysaccharide. J Immunol 171(2):717–725
Martin M et al (2005) Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. Nat Immunol 6(8):777–784
Martins GA et al (2006) Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat Immunol 7(5):457–465
Mayer-Barber KD et al (2011) Innate and adaptive interferons suppress IL-1alpha and IL-1beta production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. Immunity 35(6):1023–1034
McNab FW et al (2013) TPL-2-ERK1/2 signaling promotes host resistance against intracellular bacterial infection by negative regulation of type I IFN production. J Immunol 191(4):1732–1743
Moore KW et al (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765
Mori N, Prager D (1997) Activation of the interleukin-10 gene in the human T lymphoma line HuT 78: identification and characterization of NF-kappa B binding sites in the regulatory region of the interleukin-10 gene. Eur J Haematol 59(3):162–170
Motomura Y et al (2011) The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in CD4+ T cells. Nat Immunol 12(5):450–459
Mowen KA, Glimcher LH (2004) Signaling pathways in Th2 development. Immunol Rev 202:203–222
Murphy KM et al (2000) Signaling and transcription in T helper development. Annu Rev Immunol 18:451–494

Murphy TL, Tussiwand R, Murphy KM (2013) Specificity through cooperation: BATF-IRF interactions control immune-regulatory networks. Nat Rev Immunol 13(7):499–509
Murray PJ (2006) Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr Opin Pharmacol 6(4):379–386
Nandan D et al (2012) Myeloid cell IL-10 production in response to leishmania involves inactivation of glycogen synthase kinase-3beta downstream of phosphatidylinositol-3 kinase. J Immunol 188(1):367–378
Neininger A et al (2002) MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. J Biol Chem 277(5):3065–3068
Nemeth ZH et al (2005) Adenosine augments IL-10 production by macrophages through an A2B receptor-mediated posttranscriptional mechanism. J Immunol 175(12):8260–8270
Nurieva RI et al (2009) Bcl6 mediates the development of T follicular helper cells. Science 325(5943):1001–1005
Ohtani M et al (2008) Mammalian target of rapamycin and glycogen synthase kinase 3 differentially regulate lipopolysaccharide-induced interleukin-12 production in dendritic cells. Blood 112(3):635–643
O'Neill LA, Sheedy FJ, McCoy CE (2011) MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nat Rev Immunol 11(3):163–175
Owaki T et al (2006) IL-27 induces Th1 differentiation via p38 MAPK/T-bet- and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways. J Immunol 177(11):7579–7587
Pattison MJ, Mackenzie KF, Arthur JS (2012) Inhibition of JAKs in Macrophages Increases Lipopolysaccharide-Induced Cytokine Production by Blocking IL-10-Mediated Feedback. J Immunol 189(6):2784–2792
Pichon X et al (2012) RNA binding protein/RNA element interactions and the control of translation. Curr Protein Pept Sci 13(4):294–304
Platzer C et al (1999) Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells. Eur J Immunol 29(10):3098–3104
Pot C et al (2009) Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. J Immunol 183(2):797–801
Pot C et al (2011) Induction of regulatory Tr1 cells and inhibition of T(H)17 cells by IL-27. Semin Immunol 23(6):438–445
Powell MJ et al (2000) Posttranscriptional regulation of IL-10 gene expression through sequences in the 3'-untranslated region. J Immunol 165(1):292–296
Radtke F, MacDonald HR, Tacchini-Cottier F (2013) Regulation of innate and adaptive immunity by Notch. Nat Rev Immunol 13(6):427–437
Ramirez-Carrozzi VR et al (2009) A unifying model for the selective regulation of inducible transcription by CpG islands and nucleosome remodeling. Cell 138(1):114–128
Reid DM, Gow NA, Brown GD (2009) Pattern recognition: recent insights from Dectin-1. Curr Opin Immunol 21(1):30–37
Rengarajan J et al (2002) Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. J Exp Med 195(8):1003–1012
Rogers NC et al (2005) Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity 22(4):507–517
Romano-Spica V et al (1995) Role of ETS1 in IL-2 gene expression. J Immunol 154(6):2724–2732
Rutz S et al (2008) Notch regulates IL-10 production by T helper 1 cells. Proc Natl Acad Sci USA 105(9):3497–3502
Rutz S et al (2011) Transcription factor c-Maf mediates the TGF-beta-dependent suppression of IL-22 production in T(H)17 cells. Nature Immunol 12:1238–1245
Sandler H, Stoecklin G (2008) Control of mRNA decay by phosphorylation of tristetraprolin. Biochem Soc Trans 36(Pt 3):491–496

Saraiva M, O'Garra A (2010) The regulation of IL-10 production by immune cells. Nat Rev Immunol 10(3):170–181
Saraiva M et al (2005) Identification of a macrophage-specific chromatin signature in the IL-10 locus. J Immunol 175(2):1041–1046
Saraiva M et al (2009) Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. Immunity 31(2):209–219
Schaljo B et al (2009) Tristetraprolin is required for full anti-inflammatory response of murine macrophages to IL-10. J Immunol 183(2):1197–1206
Sharma A et al (2009) Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a. Proc Natl Acad Sci USA 106(14):5761–5766
Sheedy FJ et al (2010) Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nature Immunology 11(2):141–147
Shoemaker J, Saraiva M, O'Garra A (2006) GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. J Immunol 176(6):3470–3479
Slack EC et al (2007) Syk-dependent ERK activation regulates IL-2 and IL-10 production by DC stimulated with zymosan. Eur J Immunol 37(6):1600–1612
Smith AM et al (2011) A distal enhancer in Il12b is the target of transcriptional repression by the STAT3 pathway and requires the basic leucine zipper (B-ZIP) protein NFIL3. J Biol Chem 286(26):23582–23590
Spolski R et al (2009) IL-21 mediates suppressive effects via its induction of IL-10. J Immunol 182(5):2859–2867
Staples KJ et al (2007) IL-10 induces IL-10 in primary human monocyte-derived macrophages via the transcription factor Stat3. J Immunol 178(8):4779–4785
Stoecklin G et al (2008) Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. J Biol Chem 283(17):11689–11699
Stoecklin G et al (2004) MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. EMBO J 23(6):1313–1324
Strassmann G et al (1994) Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. J Exp Med 180(6):2365–2370
Stumhofer JS et al (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8(12):1363–1371
Sun J et al (2011) CD4+ T cell help and innate-derived IL-27 induce Blimp-1-dependent IL-10 production by antiviral CTLs. Nat Immunol 12(4):327–334
Szabo SJ et al (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100(6):655–669
Tone M et al (2000) IL-10 gene expression is controlled by the transcription factors Sp1 and Sp3. J Immunol 165(1):286–291
Tudor C et al (2009) The p38 MAPK pathway inhibits tristetraprolin-directed decay of interleukin-10 and pro-inflammatory mediator mRNAs in murine macrophages. FEBS Lett 583(12):1933–1938
Tussiwand R et al (2012) Compensatory dendritic cell development mediated by BATF-IRF interactions. Nature 490(7421):502–507
Vahedi G et al (2013) Helper T-cell identity and evolution of differential transcriptomes and epigenomes. Immunol Rev 252(1):24–40
Vahedi G et al (2012) STATs shape the active enhancer landscape of T cell populations. Cell 151(5):981–993
Veldhoen M et al (2006) TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 24(2):179–189
Villagra A et al (2009) The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol 10(1):92–100
Wang H et al (2010) The Role of Glycogen Synthase Kinase 3 in Regulating IFN-{beta}-Mediated IL-10 Production. J Immunol 186(2):675–684

Wang ZY et al (2005) Regulation of IL-10 gene expression in Th2 cells by Jun proteins. J Immunol 174(4):2098–2105

Watford WT et al (2008) Tpl2 kinase regulates T cell interferon-gamma production and host resistance to Toxoplasma gondii. J Exp Med 205(12):2803–2812

Wei L et al (2010) Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation. Immunity 32(6):840–851

Weichhart T et al (2008) The TSC-mTOR signaling pathway regulates the innate inflammatory response. Immunity 29(4):565–577

Xu J et al (2009) c-Maf regulates IL-10 expression during Th17 polarization. J Immunol 182(10):6226–6236

Yi AK et al (2002) Role of mitogen-activated protein kinases in CpG DNA-mediated IL-10 and IL-12 production: central role of extracellular signal-regulated kinase in the negative feedback loop of the CpG DNA-mediated Th1 response. J Immunol 168(9):4711–4720

Yu D et al (2009) The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity 31(3):457–468

Yu H et al (2011) MKP-1 regulates cytokine mRNA stability through selectively modulation subcellular translocation of AUF1. Cytokine 56(2):245–255

Yu Q et al (2004) The role of the p38 mitogen-activated protein kinase, extracellular signal-regulated kinase, and phosphoinositide-3-OH kinase signal transduction pathways in CD40 ligand-induced dendritic cell activation and expansion of virus-specific CD8+ T cell memory responses. J Immunol 172(10):6047–6056

Zhang JA et al (2012) Dynamic transformations of genome-wide epigenetic marking and transcriptional control establish T cell identity. Cell 149(2):467–482

Zhang X, Edwards JP, Mosser DM (2006) Dynamic and transient remodeling of the macrophage IL-10 promoter during transcription. J Immunol 177(2):1282–1288

Zheng W, Flavell RA (1997) The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 89(4):587–596

Zhu J et al (2004) Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol 5(11):1157–1165

Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol 28:445–489

Ziegler-Heitbrock L et al (2003) IFN-alpha induces the human IL-10 gene by recruiting both IFN regulatory factor 1 and Stat3. J Immunol 171(1):285–290

The Molecular Basis of IL-10 Function: from Receptor Structure to the Onset of Signaling

Mark R. Walter

Abstract Assembly of the cell surface IL-10 receptor complex is the first step in initiating IL-10 signaling pathways that regulate intestinal inflammation, viral persistence and even tumor surveillance. The discovery of IL-10 homologs in the genomes of herpes viruses suggests IL-10 signaling pathways can be manipulated at the level of the receptor complex. This chapter will describe our current molecular understanding of IL-10 receptor assembly based on crystal structures and biochemical analyses of cellular and viral IL-10 receptor complexes.

Contents

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1 Introduction

Interleukin-10 (IL-10) is the founding member of the IL-10 cytokine family, which includes IL-19, IL-20, IL-22, IL-24, and IL-26 (Fickenscher et al. 2002; Kotenko 2002; Pestka et al. 2004; Trivella et al. 2010; Zdanov 2010). With the interferons (type I, IFNα/β; type II, IFNγ; and type III, IFNλ or IL28/IL29), the IL-10 family forms the class 2 cytokine family, where membership is based on conserved cysteine positions in their receptor sequences (Bazan 1990; Ho et al. 1993; Walter 2004). IL-10 is a unique class 2 cytokine because it potently inhibits the production of proinflammatory cytokines such as IFNγ, tumor necrosis factor α (TNFα), IL-1β and IL-6 in several cell types and prevents dendritic cell (DC) maturation in part by inhibiting the expression of IL-12 (Chang et al. 2004; Moore et al. 2001). IL-10 also inhibits the expression of MHC and costimulatory molecules important for cell-mediated immunity (Moore et al. 2001). However, IL-10 also exhibits immunostimulatory activities that include the ability to stimulate IFNγ production in CD8+ T cells activated with anti-CD3/anti-CD28, or other cytokine cocktails (Mumm et al. 2011; Santin et al. 2000). IL-10 is also a potent growth and differentiation factor for B-cells, mast cells and thymocytes (Moore et al. 2001; Rousset et al. 1992; Thompson-Snipes et al. 1991).

IL-10 cellular responses require specific recognition and assembly of a heterodimeric cell surface complex comprised of IL-10R1 and IL-10R2 chains (Fig. 1) (Kotenko et al. 1997). IL-10R1 is an ~80,000 kDa protein with an extracellular ligand binding domain (ECD) of 227 residues, a transmembrane helix of 21 residues, and an intracellular domain (ICD) of 322 amino acids (Liu et al. 1994). The ECD of IL-10R2 is about the same length as IL-10R1, consisting of 201 residues (Lutfalla et al. 1993). However, the ICD of IL-10R2 consists of only 83 residues. The IL-10R1 ECD forms specific high-affinity interactions (K_D = 50–200 pM) with IL-10, while IL-10R2 is a low affinity (~mM) shared receptor that participates in receptor complexes with other class 2 cytokine family members (Donnelly et al. 2004; Tan et al. 1993). Thus, in addition to pairing with IL-10R1 to form the IL-10 signaling complex, IL-10R2 forms IL22R1/IL-10R2 (Kotenko et al. 2001; Xie et al. 2000), IL-20R1/IL-10R2 (Hor et al. 2004; Sheikh et al. 2004), and IL28R1/IL-10R2 (Kotenko et al. 2003; Sheppard et al. 2003) heterodimers that are activated by IL-22, IL-26, and IL-28a,b/IL29, respectively.

While the IL-10R1 and IL-10R2 ECDs bind IL-10, the ICDs are constitutively associated with JAK1 and TYK2 kinases, respectively (Finbloom and Winestock 1995; Ho et al. 1995). IL-10 receptor binding activates JAK1 and TYK2, which phosphorylate themselves and IL-10R1 ICD tyrosines $Y446^{IL-10R1}$ and $Y496^{IL10R1}$. IL-10R1 phosphotyrosines provide docking sites that predominantly recruit and activate, via additional phosphorylation, the transcription factor STAT3

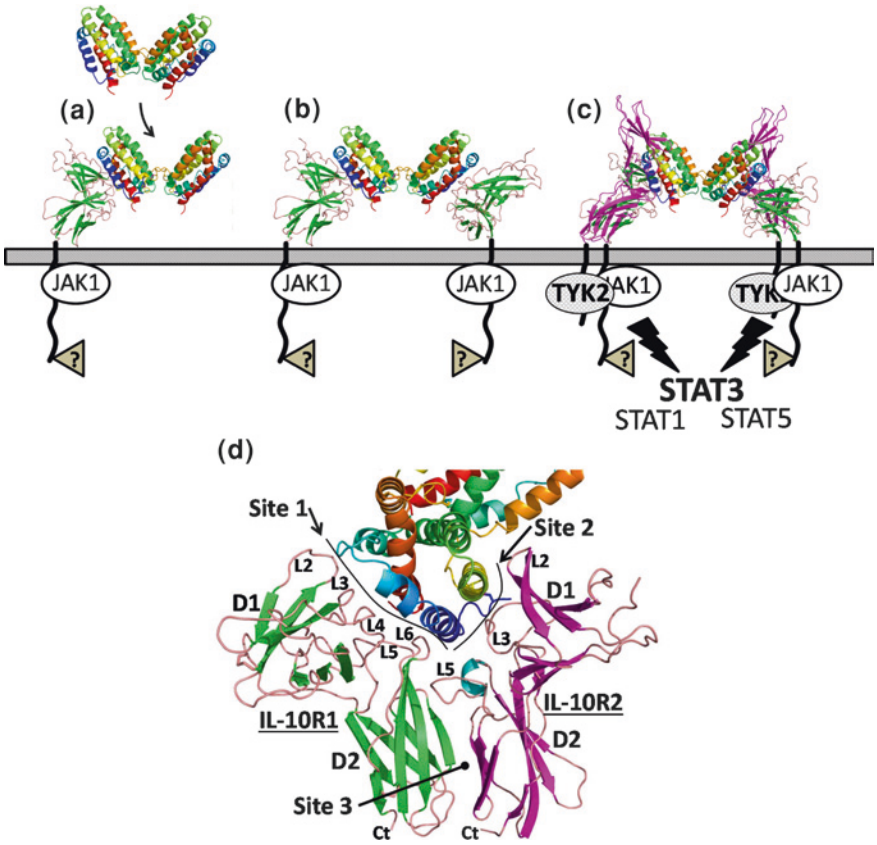

Fig. 1 Sequential assembly of the IL-10/IL-10R1/IL-10R2 Signaling Complex. **a** 1:1 IL-10/IL-10R1 complex. **b** 1:2 IL10/IL-10R1 complex. **c** 1:2:2 IL-10/IL-10R1/IL-10R2 complex. As noted in the text, an engineered monomeric cIL-10 can induce cell signaling (Josephson et al. 2000). Thus, IL-10R2 can engage the 1:1 IL-10/IL-10R1 complex. The intracellular receptor domain and JAK/TYK kinases are shown schematically. The *triangle* represents the docking site/s for unknown proteins required for TNF-α inhibition (Riley et al. 1999). **d** Enlarged image of one cIL-10 subunit bound to IL-10R1 and IL-10R2 in the ternary signaling complex, which denotes the location of the three binding interfaces that regulate complex formation

although STAT1 and STAT5 can also be activated (Donnelly et al. 1999; Finbloom and Winestock 1995; Weber-Nordt et al. 1996; Wehinger et al. 1996). The STAT3 docking sites in IL-10R1 appear to be sufficient to induce IL-10-mediated proliferative responses (Riley et al. 1999). In contrast, inhibition of TNFα requires STAT3 and additional intracellular molecules that recognize serines located in the C-terminal 30 residues of IL-10R1 (Riley et al. 1999; Takeda et al. 1999). A docking site for one or more E3 ligases has also been found in the IL-10R1 ICD that promotes ubiquitination and destruction of the receptor (Jiang et al. 2011; Wei et al. 2006). Removal of this region of IL-10R1 increased the potency of IL-10 on cells by 10 to 100 fold (Ho et al. 1995). Thus, the IL-10R1 IDC engages multiple

proteins/protein complexes that regulate its expression level and diverse biological activities. In contrast, the only known function of the IL-10R2 IDC is to provide a docking site for TYK2. Thus, all "IL-10" specific cellular functions appear to reside in the IL-10R1 chain, while IL-10R2 provides a generic activation signal that can be used to activate signaling pathways associated with the four different R1 chains (e.g., IL-10R1, IL-20R1, IL-22R1, and IL-28R1).

The diverse biology of IL-10 observed in cell culture studies is also observed in animal models and in humans. In particular, disruption of the IL-10 signaling pathway results in severe inflammatory disease, which was first observed in IL-10 knockout (KO) mice that spontaneously developed inflammatory bowel disease (IBD) (Kuhn et al. 1993). The IBD phenotype was dependent on T-cells and gut bacteria, suggesting IL-10 controls immune responses to commensal intestinal pathogens (Rennick and Fort 2000; Sellon et al. 1998). Consistent with the studies in animals, mutations in IL-10, IL-10R1, and IL-10R2 that disrupt IL-10 signaling have been identified in infants/children, who suffer from early onset IBD (Engelhardt et al. 2013; Glocker et al. 2009; Grundtner et al. 2009).

The possibility that IL-10 signaling could promote viral infection was established by identifying an open reading frame in the Epstein Barr virus (EBV) genome that encoded an IL-10 homolog (ebvIL-10) (Moore et al. 1990). Analysis of another persistent herpes virus, cytomegalovirus (CMV), revealed that it also harbored an IL-10 homolog (cmvIL-10) in its genome (Kotenko et al. 2000; Lockridge et al. 2000). Subsequent studies have established that ebvIL-10 and cmvIL-10 exhibit distinct receptor binding and biological activity profiles that may promote viral persistence (Ding et al. 2000; Ding et al. 2001; Liu et al. 1997; Raftery et al. 2004; Rousset et al. 1992; Yoon et al. 2005). In contrast to EBV and CMV, other persistent human pathogens, including HIV, upregulate cellular IL-10, which contributes to T-cell exhaustion (Blackburn and Wherry 2007; Duell et al. 2012; Wilson and Brooks 2011). Remarkably, antibody mediated disruption of IL-10 signaling has been shown to enhance T cell responses and eliminate persistent viral infection in animal models. (Brooks et al. 2006; Ejrnaes et al. 2006). Thus, strategies to efficiently block IL-10 signaling may have therapeutic potential against persistent viral infections.

While these studies are consistent with the dogma that IL-10 is an immunosuppressive cytokine, IL-10 clearly has potent immunostimulatory activities, which are observed in studies in animals and humans. Strikingly, tumor immune surveillance was shown to be weakened in IL-10 KO mice, whereas transgenic overexpression of IL-10 protected mice from carcinogenesis (Mumm et al. 2011). Furthermore, injection of PEGylated IL-10 into Her2 transgenic mice led to tumor rejection that was dependent on activated CD8+ T cells that expressed IFNγ and granzyme B. (Mumm et al. 2011). Consistent with the animal studies, the administration of lipopolysaccharide (LPS) and IL-10 to humans reduced serum TNFα levels, but also elevated IFNγ and granzyme B (Lauw et al. 2000).

These brief examples demonstrate that IL-10 receptor signaling plays an essential role in inflammation, viral pathogenesis, and cancer. To understand the complex nature of IL-10 activation, this review will describe the results of structural and biochemical studies to define molecular mechanisms that control IL-10 receptor assembly and subsequent cellular responses.

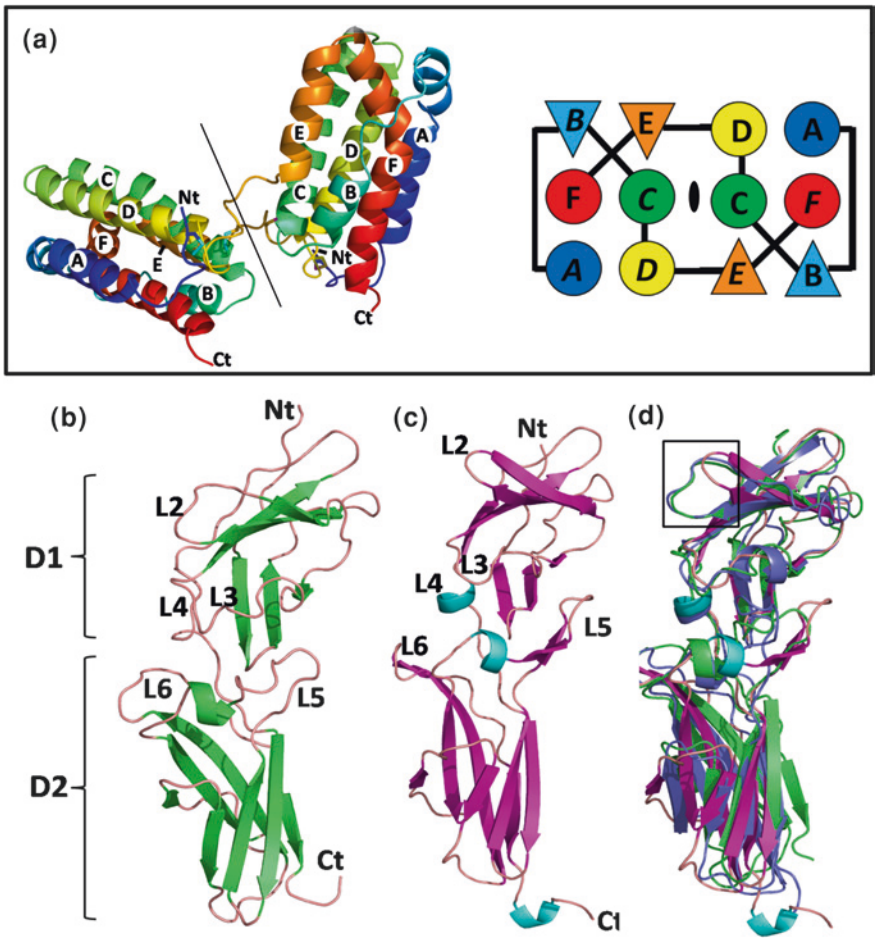

Fig. 2 Structure of cIL-10 and IL-10 Receptors. **a** Ribbon and schematic diagrams showing the structure and topology of the IL-10 domain swapped dimer. Overall structures of IL-10R1 (**b**), IL-10R2 (**c**), and a superposition of the receptor chains (**d**). Structural differences in the L2 receptor loops are highlighted in the *box*

2 Structures of IL-10 and IL-10 Receptor Complexes

2.1 Cellular IL10 (cIL-10)

Cellular human IL-10 (cIL-10) is a 160 amino acid polypeptide that adopts a domain-swapped dimer with its subunits oriented at ~90° with respect to one another (Fig. 2a) (Walter and Nagabhushan 1995; Zdanov et al. 1995; Zdanov et al. 1996). Helices A, B, C, and D, from one peptide chain assemble with helices E and F, from the twofold related chain, to form two helical bundles containing six α-helices. The helices are connected by three tight turns (BC, CD, and EF loops)

and two longer 12-residue loops (AB and DE loops) located at either ends of the bundles. Two intra-chain disulfide bonds link the N-terminus, and helix C, to the DE loop in each subunit. Thus, the disulfide bonds stabilize the assembly of helices A–D, but do not cross-link the two domain-swapped chains of the dimer.

The subunit structure of cIL-10 is similar to crystal structures of other IL-10 family cytokines (IL-19, IL-20, IL-22, IL-28, and IL-29), and based on sequence similarity, similar to IL-24 and IL-26 (Chang et al. 2003; Gad et al. 2009; Logsdon et al. 2012; Miknis et al. 2010; Nagem et al. 2002; Xu et al. 2005). However, with the possible exception of IL-26, cIL-10 is the only IL-10 family member that adopts a domain-swapped dimeric structure. To address the necessity of cIL-10 dimer formation to induce IL-10 signaling, a monomeric cIL-10 (cIL-10M1) was engineered by inserting a 6 amino acid linker peptide into the DE loop (Josephson et al. 2000). Despite its monomeric structure, cIL-10M1 was able to induce short-term cellular proliferation, albeit with ~8–18 fold lower activity than the cIL-10 dimer. However, maximal proliferative responses, equivalent to cIL-10, could be achieved with higher concentrations of cIL-10M1. These studies suggest a 1:1 cIL-10M1/IL-10R1 interaction is sufficient to engage the IL-10R2 chain and induce cIL-10 cellular responses.

2.2 cIL-10/IL-10R1 Complex

The structure of the cIL-10/IL-10R1 revealed two IL-10R1 chains bind the twofold related surfaces of IL-10, comprised of helix A, the AB loop and helix F (the site 1 interface, Fig. 3), to form a 1:2 cIL-10/IL-10R1 binary complex (Fig. 1b). The IL-10R1 ECD consists of two β-sandwich domains (D1 and D2) oriented at ~90° to one another (Fig. 2b). IL-10R1 contacts IL-10 through five loops (L2-L6), which are located on the convex surface of the receptor, at the junction of the D1 and D2 domains (Fig. 3b). IL-10R1 recognizes IL-10 in a "vertical" orientation, meaning the helical bundle axis of each subunit is parallel to the long axis of IL-10R1. To form these interactions, each IL-10R1 in the complex must rotate ~60° (counter-clockwise) away from a vector perpendicular to the plane of the cell membrane (Fig. 4a). In this orientation, the C-terminal ends of the two receptors are separated by ~110 Å and incapable of activating a JAK mediated signaling cascade. The IL-10/IL-10R1 recognition paradigm is a unique feature of class 2 cytokines, relative to the class 1 cytokine family typified by growth hormone, IL-2 and IL6, where the helical bundle axis is recognized in a horizontal orientation, relative to the long axis of their respective receptors (de Vos et al. 1992; Josephson et al. 2001; Wang et al. 2009).

2.3 Viral IL-10/IL-10R1 Complexes

The viral IL-10 homologs ebvIL-10 and cmvIL-10 share 83 and 27 % sequence identity with cIL-10, respectively (Hsu et al. 1990; Kotenko et al. 2000; Lockridge et al. 2000). As suggested by their high-sequence identity, the overall ebvIL-10/

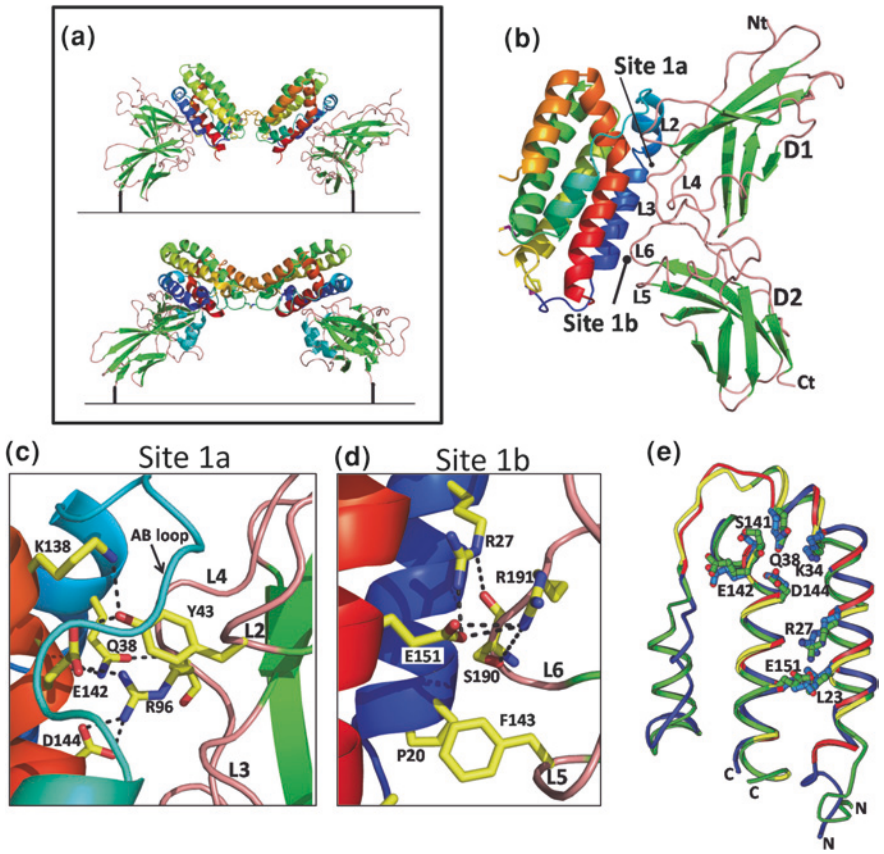

Fig. 3 Structure of the IL-10/IL-10R1 Binary Complex. **a** Comparison of the binary cIL-10/IL-10R1 (*top*) and cmvIL-10/IL-10R1 complexes (*bottom*). **b** Enlarged region of the cIL-10/IL-10R1 complex showing the site 1 interface. **c** Key molecular contacts in the site 1a interface enlarged from Fig. 3b. Amino acid sidechains described in the text are shown with carbon atoms and bonds colored *yellow*, oxygen atoms *red*, and nitrogen atoms *blue*. The amino acids are labeled using single letter amino acid codes (e.g. Glu = E). **d** Important contacts in the site 1b interface, enlarged from Fig. 3b. Amino acid side chains described in the text are colored and labeled as described in Fig. 3c. **e** Backbone superposition of site 1 (helices A, B and F) from cIL-10 (*green*) and cmvIL-10 (*blue*). cIL-10 and cmvIL-10 residues that donate sidechains to the site 1 interface are colored on the protein backbone *yellow* and *red*, respectively. The sidechains of amino acids conserved in the cIL-10 and cmvIL-10 site 1 interface are shown with carbon atoms and bonds colored *green* for cIL-10 and *blue* for cmvIL-10. For all sidechains shown, oxygen atoms are *red* and nitrogen atoms are *blue*

IL-10R1 structure is almost identical to the cIL-10/IL-10R1 complex (Yoon et al. 2005). In contrast, the cmvIL-10/IL-10R1 complex adopts a significantly different structure from cIL-10/IL-10R1 (Jones et al. 2002). The largest differences between cIL-10/IL-10R1 and cmvIL-10/IL-10R1 complexes occur in the subunit orientations of cmvIL-10 and cIL-10, whereas the structures of IL-10R1 and the site 1 binding interfaces are very similar (Fig. 3a). Like cIL-10 and ebvIL-10, cmvIL-10

folds as a domain swapped dimer. However, the two peptide chains in cmvIL-10 are cross linked by an interchain disulfide bond that is not found in cIL-10 or ebvIL-10 (Jones et al. 2002; Yoon et al. 2005; Zdanov et al. 1997). As a result of structural changes within the dimer interface, the subunits of cmvIL-10 are oriented at an angle of 130°, compared to 90° for cIL-10. The change in subunit orientation causes the IL-10R1 chains, in the cmvIL-10/IL-10R1 complex, to rotate counter clockwise an additional ~25° relative to their position in the cIL-10/IL-10R1 complex (Fig. 3a). Despite the change in orientations of the IL-10R1s in the cmvIL-10/IL-10R1 complex, the C-terminal ends of the IL-10R1 folds chains are separated by 105 Å, which is essentially identical to their separation in the cIL-10/IL-10R1 complex (110 Å). Conservation of the twofold relationship and similar spacing of the receptor C-termini suggests these features are important for optimal IL-10 signal transduction.

The distinct orientation of the IL-10R1s in the cmvIL-10/IL-10R1 complex (Fig. 3a, e.g., almost parallel with the cell membrane) could alter the gene expression profile of cmvIL-10 relative to cIL-10. In support of this hypothesis, structural analyses of erythropoietin (EPO), and EPO peptide mimetics, bound to EPO receptor (EPOR), revealed agonist or antagonist activity was correlated, not with dimerization of the receptor chains, but the orientation of EPOR in the different complexes (Livnah et al. 1998). Despite these observations, no significant differences in biological activity have been found between cmvIL-10 and cIL-10. Interestingly, cIL-10 and ebvIL-10, which share identical complex structures, have markedly different biological activities (Ding et al. 2001; Liu et al. 1997; Rousset et al. 1992; Yoon et al. 2012). In the case of ebvIL-10, the functional differences between ebvIL-10 and cIL-10 are largely due to ebvIL-10's reduced binding affinity for IL-10R1 and not large structural changes in the receptor complex (Ding et al. 2001; Liu et al. 1997; Yoon et al. 2005).

2.4 cIL-10/IL-10R1 Site 1 Interface

Residues in the cIL-10/IL-10R1 site 1 interface are mostly polar (~70 %) and cluster into two structurally distinct interaction surfaces, site 1a and site 1b (Josephson et al. 2001). Site 1a is centered on the bend in helix F and includes the AB loop while site 1b is located near the N-terminus of helix A and the C-terminus of helix F (Fig. 3b). Receptor binding loops L2-L4 from the IL-10R1 D1 domain interact exclusively with site 1a, while loops L5 and L6 from the D2 domain interact with site 1b.

Site 1a accounts for approximately 67 % of the total buried surface area (~1000 $Å^2$) in the site 1 interface (Fig. 3c). IL-10R1 residues Tyr-43, Arg-76, and Arg-96 make extensive interactions in the interface. The phenolic group of Tyr-43 buries the most surface area of any IL-10R1 residue into a shallow cavity created by helix F and the AB loop. The base of the cavity is formed by cIL-10 residues Glu-142 and Lys-138 that each form hydrogen bonds to the OH of Tyr-43. Adjacent to Tyr-43, the N of Gly-$44^{IL-10R1}$ forms a hydrogen bond with the carbonyl oxygen of AB loop residue Asp-44^{IL-10}. An extensive hydrogen bond/

salt bridge network is also formed between Arg-96^{IL10R1} and Asp-144^{IL-10}, Gln-38^{IL-10}, and the carbonyl oxygen of Ser-141^{IL-10}.

Site 1b is centered on the ion pair formed between Arg-27^{IL-10} and Glu-151^{IL-10} located on helices A and F, respectively (Fig. 3d). Glu-151^{IL-10} forms a series of hydrogen bonds with Ser-190$^{IL-10R1}$ and Arg-191$^{IL-10R1}$ located on the L6 loop. Additional interactions are formed between the carbonyl oxygens of Ser-190$^{IL-10R1}$ and Arg-191$^{IL-10R1}$ with the Nε atoms of Arg-27^{IL-10} and Arg-24^{IL-10}, respectively. The interface also contains a small hydrophobic surface comprised of Phe-143$^{IL-10R1}$ that packs into a cleft formed by Pro-20^{IL-10} and Ile-158^{IL-10}. Additional hydrophobic surface area is donated to the interface through packing of the aliphatic portions of Arg-191$^{IL-10R1}$ and Arg-27^{IL-10} (Josephson et al. 2001).

2.5 IL-10R2 Chain

Although IL-10R2 exhibits low-affinity for cIL-10, its overall architecture is similar to IL-10R1 (Figs. 2c, d) (Yoon et al. 2010). Like IL-10R1, IL-10R2 consists of two β-sandwich domains (D1 and D2) that are oriented at ~90° to one another. However, the IL-10R2 L2 and L5 loops adopt significantly different conformations than observed in IL-10R1. In particular, the L2$^{IL-10R2}$ adopts a β-hairpin structure and L5$^{IL-10R2}$ forms a "thumb-like" structure that extends away from the rest of the molecule. The conformations of the L2$^{IL-10R2}$ hairpin and L5$^{IL-10R2}$ thumb give rise to two clefts (L2/L3 and L3/L5 clefts) that are not observed in IL-10R1, or the ECDs of IL-22R1, IL-20R1, or IL-28R1 (Bleicher et al. 2008; Jones et al. 2008; Logsdon et al. 2012; Miknis et al. 2010; Yoon et al. 2010). In addition to unique structures, the L2, L3, and L5 loops are lined with aromatic residues (Tyr-56, Tyr-59, Tyr-82, Tyr-140, and Trp-143) that allow IL-10R2 to recognize IL-10, as well as IL-22, IL-26, IL-28a,b, and IL-29 (Fig. 4d) (Yoon et al. 2010). In support of these findings, structure-function studies, performed on the class 1 shared receptors gp130 and IL2γc, demonstrated aromatic residues Phe-169^{gp130} and Tyr-103$^{\gamma c}$ form critical contacts at the center of the IL-6/gp130, IL-2/γc, and IL-4/γc interfaces (Wang et al. 2009). Comparisons between IL-10R2, gp130, and IL2γc demonstrate Phe-169^{gp130}, Tyr-103$^{\gamma c}$, and Tyr-82^{IL10R2} are structurally conserved, which suggests the promiscuous binding cytokine receptors share a common origin (Yoon et al. 2010).

2.6 Model of the cIL-10/IL-10R1/IL-10R2 Signaling Complex

A structure-based model of the cIL-10/IL-10R1/IL-10R2 signaling complex has been generated using a mutagenesis /computational docking strategy (Fig. 1d) (Yoon et al. 2010). In the final model, the cIL-10 dimer assembles a symmetric

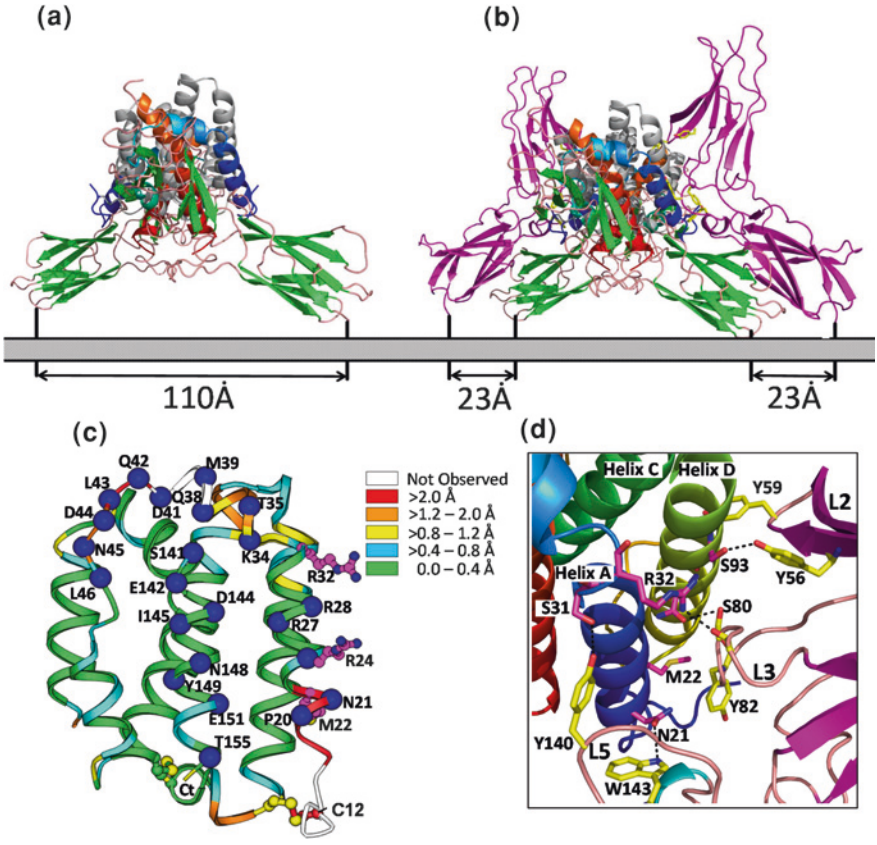

Fig. 4 IL-10R1 Binding induces conformational changes in the IL-10R2 binding site (Site 2). **a** Orthogonal view, relative to Fig. 1b, of the IL-10/IL-10R1 binary complex. **b** Orthogonal view, relative to Fig. 1c, of the IL-10/IL-10R1/IL-10R2 signaling complex derived from a data-driven computational docking strategy (Yoon et al. 2010). **c** Ribbon diagram of one subunit of IL-10 colored to show the extent of conformational changes observed upon IL-10R1 binding. Distances in the legend correspond to distances between α-carbon atoms in unbound cIL-10 and IL-10R1-bound cIL-10 (Yoon et al. 2006). "Not observed" regions (*white*) correspond to residues that were not observed in the final electron density maps of unbound cIL-10, but were present in the structure of the cIL-10/sIL-10R1 complex. Residues that form the IL-10R1 binding site 1 are denoted by blue spheres. The sidechains of residues important for IL-10R2 binding are shown in *magenta*. **d** Enlargement of the site 2 interface (see Fig. 1d), which shows interactions between aromatic IL-10R2 residues (*yellow*) and IL-10 residues determined to be important for IL-10R2 binding by mutagenesis (*magenta*). For all sidechains shown, oxygen atoms are colored *red* and nitrogen atoms are *blue*

complex containing two IL-10R1 chains and two IL-10R2 chains (Figs. 1c, 4b). In the ternary complex, IL-10R2 forms two contacts with the cIL-10/IL-10R1 binary complex. A site 2 interface between cIL-10 and IL-10R2 and a site 3 interface between the D2 domains of IL-10R1 and IL-10R2 (Fig. 1d). At this time, no

experimental data directly confirms the formation of the site 3 interface. This is because mutational data used for the docking experiment characterized the cIL-10/IL-10R2 site 2 interface but not residues in the putative site 3 contact (Yoon et al. 2010). Interestingly, modeling IL-10R2 onto the recently determined IL-20/IL-20R1/IL-20R2 ternary complex structure, another IL-10 family cytokine, suggests the site 3 interface may not form (Logsdon et al. 2012). Small angle x-ray scattering experiments confirmed IL-10R2 adopts a rigid structure with a fixed D1/D2 domain orientation (Logsdon et al. 2012). Thus, IL-10R2 flexibility cannot be used to explain problems with the docking model. This suggests IL-20R2 and IL-10R2 recognition strategies may be very different. Interestingly, the crystal structure of another class 2 cytokine ternary complex, IFNα2/IFNAR1/IFNAR2, revealed the membrane proximal domain of the IFNAR1 chain, equivalent to the IL-10R2 D2 domain, is disordered (Thomas et al. 2011). Together, the data suggests the IL-10R1/IL-10R2 site 3 interface either does not form or is extremely weak and/or transient in nature.

In contrast to site 3, the structural model of site 2 is consistent with mutational analysis of cIL-10 and IL-10R2, which provides insight into how IL-10R2 is recruited into the binary cIL-10/IL-10R1 complex (Yoon et al. 2010; Yoon et al. 2006). The main features of the site 2 interface are three IL-10R2 binding loops (L2, L3, and L5, Fig. 4d) that recognize cIL-10 helices A and D in a "vertical" manner, as described for the cIL-10/IL-10R1 interaction (Fig. 4b). Met-22^{cIL-10}, located between helices A and D, forms the center of the interface and packs against L3$^{IL-10R2}$ residue Tyr-82$^{IL-10R2}$ (Fig. 4d). On either side of L3$^{IL-10R2}$, L2$^{IL-10R2}$, and L5$^{IL-10R2}$ form contacts with helices D and A, respectively. As predicted from analysis of the IL-10R2 structure, IL-10R2 aromatic residues, that when mutated to alanine disrupt cIL-10 binding, form several key contacts in the interface (Yoon et al. 2010; Yoon et al. 2006). In particular, L3 residue Ser-80^{IL10R2} forms contacts with Arg-32^{cIL-10}, L2 residue Tyr-56^{IL10R2} forms contacts with Ser-93^{cIL-10} ,and L5 residue Tyr-140$^{IL-10R2}$ contacts Ser-31^{cIL-10} (Fig. 4d).

3 IL-10R1 and IL-10R2 Binding Properties and Impact on Signaling

3.1 Binding and Sensor Properties of IL-10R1 and IL-10R2

The affinity of cIL-10 for cell surface IL-10R1 ranges from 50 to 200 pM when measured on different cell lines (Ding et al. 2001; Liu et al. 1997; Tan et al. 1993). On a biacore chip surface, the K_D of the cIL-10/IL-10R1 interaction was determined to be 240 pM (Yoon et al. 2012). Thus, cell surface binding and in vitro surface plasmon resonance (SPR) analyses are in good agreement. Although IL-10R2 is essential for the biological activity of cIL-10 (Kotenko et al. 1997), cIL-10 cell binding affinity does not change whether the IL-10R2 chain is expressed on cells

or not (Ding et al. 2001). Subsequent SPR studies estimated the cIL-10/IL10R2 binding affinity to be approximately 3 mM (Logsdon et al. 2002). Additional in vitro binding studies measured a ~13-fold increase in the affinity of soluble IL-10R2 ($K_D = 234$ μM) for the cIL-10/IL-10R2 binary complex, relative to cIL-10 alone (Yoon et al. 2005). Even with the ~13-fold affinity increase, IL-10R2 interaction is approximately four orders of magnitude weaker than the cIL-10/IL-10R1 site 1 interaction.

As a result of the disparate IL-10R1 and IL-10R2 affinities, each receptor chain has a distinct function in activating IL-10 cellular responses. Specifically, IL-10R1 functions as the IL-10 binding chain which controls cell specificity and cellular targeting of IL-10 to immune cells that selectively express the IL-10R1 chain (Nagalakshmi et al. 2004; Wolk et al. 2002). The second function of the IL-10R1 chain is to regulate receptor occupancy time, which is controlled by the kinetics of the IL-10/IL-10R1 interaction. In contrast to IL-10R1, IL-10R2 functions as a sensor chain, which efficiently "senses" IL-10 bound to IL-10R1 (e.g., the IL-10/IL-10R1 complex). Thus, the role of the IL-10R2 chain is to activate signaling based on the kinetics of the IL-10/IL-10R1 interaction. Because of its singular function, IL-10R2 can be used in similar signaling strategies of the other IL-10R2 binding cytokines, IL-22, IL-26, IL-28a/b, and IL-29 (Donnelly et al. 2004; Jones et al. 2008; Yoon et al. 2010). This allows specific signaling responses from six cytokines using five receptors. Thus, the promiscuous binding IL-10R2, with its singular function, reduces the number of unique receptor chains required for IL-10 family cytokine signaling.

Not surprisingly, changes in site 1 (IL-10/IL-10R1) or site 2 (IL-10/IL-10R2) interfaces result in different biological outcomes (Ding et al. 2001; Raftery et al. 2004; Yoon et al. 2012; Yoon et al. 2006). Disruptions in IL-10 site 1, increases the effective concentration of the ligand required to induce one half of measured maximal biological responses (EC50). Despite increased EC50 values, site 1 mutants can still induce biological responses equivalent to cIL-10, at high-protein concentrations (Yoon et al. 2012). In contrast, mutations in IL-10 site 2 cannot induce the same response levels observed for cIL-10, despite the addition of extremely high-protein concentrations (Yoon et al. 2006). However, consistent with the low-affinity of the IL10R2 chain, most mutations in IL-10 site 2 have little impact on IL-10 signaling, whereas most mutations made in the high-affinity site 1 cause a measurable difference in biological activity. Overall, these findings are consistent with the roles of IL-10R1 and IL-10R2, as binding and sensor chains, respectively.

3.2 Reduced IL-10/IL-10R1 Affinity Prevents Signaling on Cells with Low-IL-10R1 Levels

The impact of reduced IL-10/IL-10R1 binding affinity on IL-10 cellular responses has been predominantly studied using ebvIL-10, which exhibits ~1000-fold lower affinity for IL-10R1 than cIL-10 (Ding et al. 2001; Liu et al. 1997; Yoon et al. 2012). Due to its weak affinity, ebvIL-10 was unable to signal on thymocytes,

which express very low-levels of IL-10R1, whereas cIL-10 signaled normally (Ding et al. 2001). Thus, ebvIL-10/IL-10R1 binding affinity is insufficient to assemble enough ebvIL-10/IL-10R1/IL-10R2 complexes to reach the threshold required for signaling. While EBV has presumably engineered ebvIL-10 to not be responsive on cells with low-IL-10R1 levels, regulation of cell surface IL-10R1 levels appears to be a normal mechanism to regulate IL-10 signaling. Specifically, human neutrophils do not respond to cIL-10 unless they are activated by danger signals, such as LPS, which upregulates IL-10R1 expression allowing cIL-10 signaling (Crepaldi et al. 2001; Tamassia et al. 2008). In contrast to neutrophils, activated DC express lower levels of IL-10R1 than immature DC (Corinti et al. 2001; Kalinski et al. 1998). In contrast to IL-10R1, IL-10R2 has been shown to be constitutively expressed on essentially all cells (Nagalakshmi et al. 2004; Wolk et al. 2002).

3.3 Reduced IL-10/IL-10R1 Affinity Stimulates Signaling on Cells with High-IL-10R1 Levels

Although ebvIL-10 cannot signal on cells expressing low-IL-10R1 levels, ebvIL-10 signaling is enhanced, relative to cIL-10, on cells (e.g., human B-cells) that express high-IL-10R1 levels (Liu et al. 1997; Rousset et al. 1992; Yoon et al. 2012). To address this counter intuitive observation, a series of ebvIL-10/cIL-10 chimeras were expressed, purified, and tested for receptor binding and biological activity (Yoon et al. 2012). A unique feature of this study was the ebvIL-10/cIL-10 chimeras were produced as monomers and dimers to further address the importance of the IL-10 dimer in activating IL-10 cellular responses. Yoon et al. found monomeric ebvIL-10/cIL-10 chimeras stimulated short-term proliferative responses that were directly proportional to IL-10R1 affinity (Yoon et al. 2012). Thus, IL-10 monomers exhibiting weak affinity for IL-10R1 exhibited weak cellular responses and monomers with high-affinity for IL-10R1 exhibited enhanced biological activity. In contrast to experiments performed with the monomers, the biological activity of ebvIL-10/cIL-10 dimers was inversely proportional to IL-10R1 affinity. For example, dimers with high-affinity for IL-10R1 (e.g., cIL-10) exhibited lower biological activity than dimers with reduced affinity for IL-10R1 (e.g., ebvIL-10). Thus, the ebvIL-10 dimer was essential for its enhanced signaling properties on cells expressing high-levels of IL-10R1.

Receptor binding kinetics revealed ebvIL-10 exhibits very transient interactions (~10 s) with IL-10R1, before the complex falls apart (Yoon et al. 2012). In contrast, cIL-10/IL-10R1 interactions are very stable (~50 min). As a result, ebvIL-10 is very inefficient in forming the 1:2 ebvIL-10/IL-10R1 complex (see Fig. 1b). This suggests ebvIL-10 may activate cellular responses primarily by formation of 1:1 ebvIL-10/IL-10R1 complexes that are recognized by IL-10R2 as shown by Josephson et al. (Josephson et al. 2000) (e.g., Fig. 1a). How transient receptor binding enhances biological activity remains to be determined. One hypothesis is transient ebvIL-10/IL-10R1 interactions prevent the engagement of

the IL-10R1 ubiquitination machinery, which prevents IL-10R1 internalization and signal termination (Jiang et al. 2011; Wei et al. 2006). A second hypothesis suggests that cells that express high-levels of IL-10R1 capture high-affinity cIL-10 in non-functional cIL-10/IL-10R1 complexes that reduce its biological activity. This idea originated from biochemical studies that demonstrated the soluble cIL-10/IL-10R1 complex contains two cIL-10 dimers and four sIL-10R1 molecules (Tan et al. 1993). The solution stoichiometry of the complex is also observed in the cIL-10/IL-10R1 crystals, where two 1:2 cIL-10/IL-10R1 complexes are positioned adjacent to one another such that they block IL-10R2 binding (Josephson et al. 2001).

4 Structural Mechanisms Regulating IL-10/IL-10R1/IL-10R2 Assembly

4.1 Conserved IL-10 Site 1 Residues

To identify essential molecular features of IL-10 required for IL-10 receptor binding, the diverse sequences and structures of the cellular and viral IL-10s were compared, since all share the ability to engage IL-10R1 and IL-10R2 and initiate IL-10 signaling responses (Slobedman et al. 2009). This analysis revealed the subunit structures of ebvIL-10 and cmvIL-10 exhibit root mean square deviations (r.m.s.d.) of 0.5 and 1.9 Å, respectively, with cIL-10 (Yoon et al. 2005). The structural comparison identified eight residues (Leu-23, Arg-27, Lys-34, Gln-38, Ser-141, Asp-142, Asp-144, Glu-151, see Fig. 3d) whose sequence and structure were conserved in the site 1 interface (Jones et al. 2002). Not surprisingly, these residues participate in the extensive hydrogen binding networks observed in site 1a (Fig. 3c) and site 1b (Fig. 3d). However, they also form interpeptide salt bridges between helices A and F in the domain-swapped dimer (Fig. 3e) (Jones et al. 2002; Zdanov et al. 1995). Thus, these conserved residues promote the folding and stability of IL-10, as well as forming critical interactions with IL-10R1.

4.2 IL-10/IL-10R1 Specificity: A Two-Point Recognition Model

While conserved residues in the IL-10 site 1 interface are important for IL-10R1 binding, they do not fully explain why IL-10 is specific for IL-10R1 and not other class 2 cytokine receptors, such as IL-22R1. To identify key molecular features that control IL-10/IL-10R1 specificity, structural comparisons of cellular and viral IL-10/IL-10R1 complexes (Jones et al. 2002; Josephson et al. 2001; Yoon et al. 2005), and IL-22/IL-22R1 complexes (Bleicher et al. 2008; Jones et al. 2008) were performed. This analysis identified the site 1a L2/AB loop recognition

motif, which consists of Tyr-43$^{IL-10R1}$ and Gly-44$^{IL-10R1}$, as a critical specificity determinant (Fig. 3c). This is because the precise geometry of the extensive site 1a hydrogen bonds, positions the IL-10 subunit for additional IL-10R1 interactions in site 1b (Fig. 3d), which cannot be accurately reproduced in noncognate complexes (e.g., IL-22/sIL-10R1 or IL-10/sIL-22R1) without steric clashes between other regions of the molecules. Thus, the requirement for specific contacts in two spatially distinct regions of the interface, site1a and site 1b (Fig. 3b), provides critical constraints that ensure specificity between IL-10/IL-10R1 and other IL-10 family complexes, such as IL-22/IL-22R1.

4.3 IL-10 Conformational Changes Regulate IL-10/IL-10R1 Affinity and IL-10R2 Recruitment

A static picture of IL-10/IL-10R1 binding does not explain ebvIL-10 binding affinity or recruitment of IL-10R2 into the IL-10/IL-10R1 complex. Both cIL-10 and ebvIL-10 share the 8 conserved residues within the IL-10R1 binding epitope. However, ebvIL-10 exhibits ~1000-fold lower affinity for IL-10R1 than cIL-10 (Ding et al. 2001; Liu et al. 1997). Comparison of the amount of surface area buried in the cIL-10R1/IL-10R1 and ebvIL-10/IL-10R1 site 1 interfaces are very similar (Yoon et al. 2005). Thus, buried surface area does not explain the differences in IL-10R1 affinity between cIL-10 and ebvIL-10. However, closer examination revealed the ebvIL-10 AB loop is partially disordered in the ebvIL10/IL-10R1 complex (Yoon et al. 2005). In contrast, the cIL-10 AB loop is completely ordered in the cIL-10/IL-10R1 complex (Josephson et al. 2001; Yoon et al. 2005). Furthermore, the cIL-10 and ebvIL-10 AB loops are completely disordered in structures determined without the IL-10R1 chain (Fig. 4c) (Walter and Nagabhushan 1995; Zdanov et al. 1997). Taken together, this data suggests IL-10R1 binding promotes the ordering of the cIL-10 and ebvIL-10 AB loops. However, in the case of ebvIL-10, the transition to the ordered state is only partially completed. As a result of the increased mobility of the ebvIL-10 AB loop, the hydrogen bonds in site 1a are less precise (e.g., increased hydrogen bond lengths and poor geometry) than observed in the cIL-10/IL-10R1 complex, which as described in the two-point recognition model, disrupts interactions in site 1b and overall ebv/IL-10R1 affinity (Yoon et al. 2005).

To substantiate this model, a series of ebvIL-10/cIL-10 chimeras were tested for IL-10R1 binding affinity (Yoon et al. 2012). This study revealed changing just two residues in the ebvIL-10 AB loop (V43$^{ebvIL-10}$ and A87$^{ebvIL-10}$) to cIL-10 residues (L43^{cIL-10} and I87^{cIL-10}) almost completely restored "cIL-10-like" IL-10R1 affinity (Yoon et al. 2012). Remarkably, residues L43^{cIL-10} and I87^{cIL-10} point into the IL-10 hydrophobic core and make no direct contacts with IL-10R1 (Yoon et al. 2005). Thus, ebvIL-10 contains packing defects in its hydrophobic core, which prevents the AB loop from effectively "locking" into an orientation that precisely positions ebvIL-10 site 1a and site 1b IL-10R1 contacts for high-affinity binding.

Conformational changes in cIL-10 that occur upon IL-10R1 binding were determined by comparing crystal structures of unbound cIL-10 and IL-10R1-bound cIL-10 (Fig. 4c) (Yoon et al. 2006). In addition to "ordering" the AB loop, IL-10R1 binding also induces conformational changes (exceeding 2Å) in the N-terminus of helix A and in the CD loop, which corresponds to the IL-10R2 site 2 binding site (Figs. 1d, 4d) (Yoon et al. 2006). Specific IL-10R2 binding residues (Fig. 4d) that undergo large conformational changes include Asn-21^{cIL-10}, Met-22^{cIL-10}, and Arg-32^{cIL-10}, which are adjacent to IL-10R1 site 1a and site 1b. These conformational changes provide a structural mechanism to explain the ~13-fold increase in IL-10R2 affinity for the cIL-10/IL-10R1 complex compared to cIL-10 alone (Logsdon et al. 2002). The conformational coupling between cIL-10 site 1 and site 2 revealed from crystal structure analysis is further validated by mutations in cIL-10 site 2, which increase the affinity of the site 1 cIL-10/IL-10R1 interaction (Yoon et al. 2006).

5 Conclusions

Using crystal structures, receptor binding, protein engineering and functional studies, a unifying mechanism of IL-10 receptor assembly is beginning to appear. These data provide a molecular framework for understanding the diverse biological functions of IL-10 under normal and pathologic conditions. To date, the structural data has been used to design nonfunctional versions of rhesus CMV IL-10 (RhcmvIL-10) for use in vaccination strategies against RhCMV, the non-human primate model of CMV (Crough and Khanna 2009; Yue and Barry 2008). Immunization of RhCMV-infected rhesus macaques with these nonfunction RhcmvIL-10 mutants stimulated antibodies that neutralize wildtype RhcmvIL-10 biological activity, but do not cross react with rhesus cellular IL-10 (Logsdon et al. 2011). Given the critical role of IL-10 in persistent viral infections, vaccine induced neutralization of cmvIL-10 biological activity could be an exciting new strategy to prevent CMV infection or reinfection.

Structural elucidation of the IL-10 signaling complex has also played an important role in understanding IL-10, IL-10R1, and IL-10R2 mutations, which are found in patients suffering from early onset IBD (Eberhardt et al. 2012; Grundtner et al. 2009). Further studies will refine the ability to predict the biological properties of newly discovered mutations and might ultimately lead to short-term therapies that restore IL-10 signaling to these patients while hematopoietic stem cell therapy is being considered.

While excellent progress has been made elucidating molecular mechanisms of IL-10 receptor binding and formation of the extracellular signaling complex, the accompanying intracellular steps required for activation remain largely unknown (Fig. 1). This problem is not specific to the IL-10 receptor complex, but is a general problem in the cytokine structural biology field. It should be noted that crystal structures of JAK1 and TYK2 kinase (JH1) domains, and a JAK pseudo kinase

(JH2) domain, have been determined (Bandaranayake et al. 2012; Chrencik et al. 2010; Williams et al. 2009). However, this represents only ~50 % of the full length JAK and TYK proteins (Haan et al. 2010). Furthermore, how these proteins assemble with the IL-10R1 and IL-10R2 ICDs, or how the JAKs alter their conformations upon IL-10 binding to the ECDs remains unknown. These are important fundamental questions that could lead to new strategies to harness IL-10 biological activities for therapies to treat inflammatory disease, viral infections, and even cancer.

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References

Bandaranayake RM, Ungureanu D, Shan Y, Shaw DE, Silvennoinen O, Hubbard SR (2012) Crystal structures of the JAK2 pseudokinase domain and the pathogenic mutant V617F. Nat Struct Mol Biol 19:754–759. doi:10.1038/nsmb.2348

Bazan JF (1990) Shared architecture of hormone binidng in type I and type II interferon receptors. Cell 61:753–754

Blackburn SD, Wherry EJ (2007) IL-10, T cell exhaustion and viral persistence. Trends Microbiol 15:143–146

Bleicher L, de Moura PR, Watanabe L, Colau D, Dumoutier L, Renauld JC, Polikarpov I (2008) Crystal structure of the IL-22/IL-22R1 complex and its implications for the IL-22 signaling mechanism. FEBS Lett 582:2985–2992. doi:10.1016/j.febslet.2008.07.046

Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB (2006) Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 12:1301–1309

Chang C, Magracheva E, Kozlov S, Fong S, Tobin G, Kotenko S, Wlodawer A, Zdanov A (2003) Crystal structure of interleukin-19 defines a new subfamily of helical cytokines. J Biol Chem 278:3308–3313

Chang WL, Baumgarth N, Yu D, Barry PA (2004) Human cytomegalovirus-encoded interleukin-10 homolog inhibits maturation of dendritic cells and alters their functionality. J Virol 78:8720–8731. doi:10.1128/JVI.78.16.8720-8731.2004

Chrencik JE, Patny A, Leung IK, Korniski B, Emmons TL, Hall T, Weinberg RA, Gormley JA, Williams JM, Day JE, Hirsch JL, Kiefer JR, Leone JW, Fischer HD, Sommers CD, Huang HC, Jacobsen EJ, Tenbrink RE, Tomasselli AG, Benson TE (2010) Structural and thermodynamic characterization of the TYK2 and JAK3 kinase domains in complex with CP-690550 and CMP-6. J Mol Biol 400:413–433. doi:10.1016/j.jmb.2010.05.020

Corinti S, Albanesi C, la Sala A, Pastore S, Girolomoni G (2001) Regulatory activity of autocrine IL-10 on dendritic cell functions. J Immunol 166:4312–4318

Crepaldi L, Gasperini S, Lapinet JA, Calzetti F, Pinardi C, Liu Y, Zurawski S, de Waal Malefyt R, Moore KW, Cassatella MA (2001) Up-regulation of IL-10R1 expression is required to render human neutrophils fully responsive to IL-10. J Immunol 167:2312–2322

Crough T, Khanna R (2009) Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev 22:76–98. Table of Contents. doi: 10.1128/CMR.00034-08

de Vos AM, Ultsch M, Kossiakoff AA (1992) Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. Science 255:306–312

Ding Y, Qin L, Kotenko SV, Pestka S, Bromberg JS (2000) A single amino acid determines the immunostimulatory activity of interleukin 10. J Exp Med 191:213–224

Ding Y, Qin L, Zamarin D, Kotenko SV, Pestka S, Moore KW, Bromberg JS (2001) Differential IL-10R1 expression plays a critical role in IL-10-mediated immune regulation. J Immunol 167:6884–6892

Donnelly RP, Dickensheets H, Finbloom DS (1999) The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. J Interferon Cytokine Res 19:563–573

Donnelly RP, Sheikh F, Kotenko SV, Dickensheets H (2004) The expanded family of class II cytokines that share the IL-10 receptor-2 (IL-10R2) chain. J Leukoc Biol 76(2):314–21

Duell BL, Tan CK, Carey AJ, Wu F, Cripps AW, Ulett GC (2012) Recent insights into microbial triggers of interleukin-10 production in the host and the impact on infectious disease pathogenesis. FEMS Immunol Med Microbiol 64:295–313. doi:10.1111/j.1574-695X.2012.00931.x

Eberhardt MK, Chang WL, Logsdon NJ, Yue Y, Walter MR, Barry PA (2012) Host immune responses to a viral immune modulating protein: immunogenicity of viral interleukin-10 in rhesus cytomegalovirus-infected rhesus macaques. PLoS ONE 7:e37931. doi:10.1371/journal.pone.0037931

Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, von Herrath MG (2006) Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 203:2461–2472

Engelhardt KR, Shah N, Faizura-Yeop I, Kocacik Uygun DF, Frede N, Muise AM, Shteyer E, Filiz S, Chee R, Elawad M, Hartmann B, Arkwright PD, Dvorak C, Klein C, Puck JM, Grimbacher B, Glocker EO (2013) Clinical outcome in IL-10- and IL-10 receptor-deficient patients with or without hematopoietic stem cell transplantation. J Allergy Clin Immunol 131:825–830. doi:10.1016/j.jaci.2012.09.025

Fickenscher H, Hor S, Kupers H, Knappe A, Wittmann S, Sticht H (2002) The interleukin-10 family of cytokines. Trends Immunol 23:89–96

Finbloom DS, Winestock KD (1995) IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. J Immunol 155:1079–1090

Gad HH, Dellgren C, Hamming OJ, Vends S, Paludan SR, Hartmann R (2009) Interferon-lambda is functionally an interferon but structurally related to the interleukin-10 family. J Biol Chem 284:20869–20875. doi:10.1074/jbc.M109.002923

Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, Perro M, Diestelhorst J, Allroth A, Murugan D, Hatscher N, Pfeifer D, Sykora KW, Sauer M, Kreipe H, Lacher M, Nustede R, Woellner C, Baumann U, Salzer U, Koletzko S, Shah N, Segal AW, Sauerbrey A, Buderus S, Snapper SB, Grimbacher B, Klein C (2009) Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med 361:2033–2045. doi:10.1056/NEJMoa0907206

Grundtner P, Gruber S, Murray SS, Vermeire S, Rutgeerts P, Decker T, Lakatos PL, Gasche C (2009) The IL-10R1 S138G loss-of-function allele and ulcerative colitis. Genes Immun 10:84–92. doi:10.1038/gene.2008.72

Haan C, Behrmann I, Haan S (2010) Perspectives for the use of structural information and chemical genetics to develop inhibitors of Janus kinases. J Cell Mol Med 14:504–527. doi:10.1111/j.1582-4934.2010.01018.x

Ho AS, Liu Y, Khan TA, Hsu DH, Bazan JF, Moore KW (1993) A receptor for interleukin 10 is related to interferon receptors. Proc Natl Acad Sci USA 90:11267–11271

Ho AS, Wei SH, Mui AL, Miyajima A, Moore KW (1995) Functional regions of the mouse interleukin-10 receptor cytoplasmic domain. Mol Cell Biol 15:5043–5053

Hor S, Pirzer H, Dumoutier L, Bauer F, Wittmann S, Sticht H, Renauld JC, de Waal Malefyt R, Fickenscher H (2004) The T-cell lymphokine interleukin-26 targets epithelial cells through the interleukin-20 receptor 1 and interleukin-10 receptor 2 chains. J Biol Chem 279:33343–33351. doi:10.1074/jbc.M405000200

Hsu DH, de Waal Malefyt R, Fiorentino DF, Dang MN, Vieira P, de Vries J, Spits H, Mosmann TR, Moore KW (1990) Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. Science 250:830–832

Jiang H, Lu Y, Yuan L, Liu J (2011) Regulation of interleukin-10 receptor ubiquitination and stability by beta-TrCP-containing ubiquitin E3 ligase. PLoS ONE 6:e27464. doi:10.1371/journal.pone.0027464

Jones BC, Logsdon NJ, Josephson K, Cook J, Barry PA, Walter MR (2002) Crystal structure of human cytomegalovirus IL-10 bound to soluble human IL-10R1. Proc Natl Acad Sci USA 99:9404–9409

Jones BC, Logsdon NJ, Walter MR (2008) Structure of IL-22 bound to its high-affinity IL-22R1 chain. Structure 16:1333–1344. doi:10.1016/j.str.2008.06.005

Josephson K, DiGiacomo R, Indelicato SR, Iyo AH, Nagabhushan TL, Parker MH, Walter MR (2000) Design and analysis of an engineered human interleukin-10 monomer. J Biol Chem 275:25054

Josephson K, Logsdon NJ, Walter MR (2001) Crystal structure of the IL-10/IL-10R1 complex reveals a shared receptor binding site. Immunity 15:35–46

Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML (1998) Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a + CD83 + dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. J Immunol 161:2804–2809

Kotenko SV (2002) The family of IL-10-related cytokines and their receptors: related, but to what extent? Cytokine Growth Factor Rev 13:223–240

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP (2003) IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4:69–77

Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S (2001) Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. J Biol Chem 276:2725–2732

Kotenko SV, Krause CD, Izotova LS, Pollack BP, Wu W, Pestka S (1997) Identification and functional characterization of a second chain of the interleukin-10 receptor complex. EMBO J 16:5894–5903

Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, Pestka S (2000) Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci USA 97:1695–1700 97/4/1695 [pii]

Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274

Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJ, van der Poll T (2000) Proinflammatory effects of IL-10 during human endotoxemia. J Immunol 165:2783–2789

Liu Y, de Waal Malefyt R, Briere F, Parham C, Bridon JM, Banchereau J, Moore KW, Xu J (1997) The EBV IL-10 homologue is a selective agonist with impaired binding to the IL-10 receptor. J Immunol 158:604–613

Liu Y, Wei SH, Ho AS, de Waal Malefyt R, Moore KW (1994) Expression cloning and characterization of a human IL-10 receptor. J Immunol 152:1821–1829

Livnah O, Johnson DL, Stura EA, Farrell FX, Barbone FP, You Y, Liu KD, Goldsmith MA, He W, Krause CD, Pestka S, Jolliffe LK, Wilson IA (1998) An antagonist peptide-EPO receptor complex suggests that receptor dimerization is not sufficient for activation. Nat Struct Biol 5:993–1004

Lockridge KM, Zhou SS, Kravitz RH, Johnson JL, Sawai ET, Blewett EL, Barry PA (2000) Primate cytomegaloviruses encode and express an IL-10-like protein. Virology 268:272–280

Logsdon NJ, Deshpande A, Harris BD, Rajashankar KR, Walter MR (2012) Structural basis for receptor sharing and activation by interleukin-20 receptor-2 (IL-20R2) binding cytokines. Proc Natl Acad Sci USA 109:12704–12709. doi:10.1073/pnas.1117551109

Logsdon NJ, Eberhardt MK, Allen CE, Barry PA, Walter MR (2011) Design and analysis of rhesus cytomegalovirus IL-10 mutants as a model for novel vaccines against human cytomegalovirus. PLoS ONE 6:e28127. doi:10.1371/journal.pone.0028127

Logsdon NJ, Jones BC, Josephson K, Cook J, Walter MR (2002) Comparison of interleukin-22 and interleukin-10 soluble receptor complexes. J Interferon Cytokine Res 22:1099–1112

Lutfalla G, Gardiner K, Uze G (1993) A new member of the cytokine receptor gene family maps on chromosome 21 at less than 35 kb from IFNAR. Genomics 16:366–373. doi:10.1006/geno.1993.1199

Miknis ZJ, Magracheva E, Li W, Zdanov A, Kotenko SV, Wlodawer A (2010) Crystal structure of human interferon-lambda1 in complex with its high-affinity receptor interferon-lambdaR1. J Mol Biol 404:650–664. doi:10.1016/j.jmb.2010.09.068

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science 248:1230–1234

Mumm JB, Emmerich J, Zhang X, Chan I, Wu L, Mauze S, Blaisdell S, Basham B, Dai J, Grein J, Sheppard C, Hong K, Cutler C, Turner S, LaFace D, Kleinschek M, Judo M, Ayanoglu G, Langowski J, Gu D, Paporello B, Murphy E, Sriram V, Naravula S, Desai B, Medicherla S, Seghezzi W, McClanahan T, Cannon-Carlson S, Beebe AM, Oft M (2011) IL-10 elicits IFNgamma-dependent tumor immune surveillance. Cancer Cell 20:781–796. doi:10.1016/j.ccr.2011.11.003

Nagalakshmi ML, Murphy E, McClanahan T, de Waal Malefyt R (2004) Expression patterns of IL-10 ligand and receptor gene families provide leads for biological characterization. Int Immunopharmacol 4:577–592

Nagem RA, Colau D, Dumoutier L, Renauld JC, Ogata C, Polikarpov I (2002) Crystal structure of recombinant human interleukin-22. Structure (Camb) 10:1051–1062

Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB (2004) Interleukin-10 and Related Cytokines and Receptors. Annu Rev Immunol 22:929–979

Raftery MJ, Wieland D, Gronewald S, Kraus AA, Giese T, Schonrich G (2004) Shaping phenotype, function, and survival of dendritic cells by cytomegalovirus-encoded IL-10. J Immunol 173:3383–3391

Rennick DM, Fort MM (2000) Lessons from genetically engineered animal models. XII. IL-10-deficient (IL-10(-/-) mice and intestinal inflammation. Am J Physiol Gastrointest Liver Physiol 278:G829–G833

Riley JK, Takeda K, Akira S, Schreiber RD (1999) Interleukin-10 receptor signaling through the JAK-STAT pathway. Requirement for two distinct receptor-derived signals for anti-inflammatory action. J Biol Chem 274:16513–16521

Rousset F, Garcia E, Defrance T, Peronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Banchereau J (1992) Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 89:1890–1893

Santin AD, Hermonat PL, Ravaggi A, Bellone S, Pecorelli S, Roman JJ, Parham GP, Cannon MJ (2000) Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8(+) cytotoxic T lymphocytes. J Virol 74:4729–4737

Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB (1998) Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 66:5224–5231

Sheikh F, Baurin VV, Lewis-Antes A, Shah NK, Smirnov SV, Anantha S, Dickensheets H, Dumoutier L, Renauld JC, Zdanov A, Donnelly RP, Kotenko SV (2004) Cutting edge: IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. J Immunol 172:2006–2010

Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J, Ostrander C, Dong D, Shin J, Presnell S, Fox B, Haldeman B, Cooper E, Taft D, Gilbert T, Grant FJ, Tackett M, Krivan W, McKnight G, Clegg C, Foster D, Klucher KM (2003) IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 4:63–68

Slobedman B, Barry PA, Spencer JV, Avdic S, Abendroth A (2009) Virus-encoded homologs of cellular interleukin-10 and their control of host immune function. J Virol 83:9618–9629. doi:10.1128/JVI.01098-09

Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I, Akira S (1999) Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 10:39–49

Tamassia N, Calzetti F, Menestrina N, Rossato M, Bazzoni F, Gottin L, Cassatella MA (2008) Circulating neutrophils of septic patients constitutively express IL-10R1 and are promptly responsive to IL-10. Int Immunol 20:535–541. doi:10.1093/intimm/dxn015

Tan JC, Indelicato SR, Narula SK, Zavodny PJ, Chou CC (1993) Characterization of interleukin-10 receptors on human and mouse cells. J Biol Chem 268:21053–21059

Thomas C, Moraga I, Levin D, Krutzik PO, Podoplelova Y, Trejo A, Lee C, Yarden G, Vleck SE, Glenn JS, Nolan GP, Piehler J, Schreiber G, Garcia KC (2011) Structural linkage between ligand discrimination and receptor activation by type I interferons. Cell 146:621–632. doi:10.1016/j.cell.2011.06.048

Thompson-Snipes L, Dhar V, Bond MW, Mosmann TR, Moore KW, Rennick DM (1991) Interleukin 10: a novel stimulatory factor for mast cells and their progenitors. J Exp Med 173:507–510

Trivella DB, Ferreira-Junior JR, Dumoutier L, Renauld JC, Polikarpov I (2010) Structure and function of interleukin-22 and other members of the interleukin-10 family. Cell Mol Life Sci 67:2909–2935. doi:10.1007/s00018-010-0380-0

Walter MR (2004) Structural analysis of IL-10 and Type I interferon family members and their complexes with receptor. Adv Protein Chem 68:171–223

Walter MR, Nagabhushan TL (1995) Crystal structure of interleukin 10 reveals an interferon gamma-like fold. Biochemistry 34:12118–12125

Wang X, Lupardus P, Laporte SL, Garcia KC (2009) Structural biology of shared cytokine receptors. Annu Rev Immunol 27:29–60. doi:10.1146/annurev.immunol.24.021605.090616

Weber-Nordt RM, Egen C, Wehinger J, Ludwig W, Gouilleux-Gruart V, Mertelsmann R, Finke J (1996) Constitutive activation of STAT proteins in primary lymphoid and myeloid leukemia cells and in Epstein-Barr virus (EBV)-related lymphoma cell lines. Blood 88:809–816

Wehinger J, Gouilleux F, Groner B, Finke J, Mertelsmann R, Weber-Nordt RM (1996) IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. FEBS Lett 394:365–370

Wei SH, Ming-Lum A, Liu Y, Wallach D, Ong CJ, Chung SW, Moore KW, Mui AL (2006) Proteasome-mediated proteolysis of the interleukin-10 receptor is important for signal down-regulation. J Interferon Cytokine Res 26:281–290. doi:10.1089/jir.2006.26.281

Williams NK, Bamert RS, Patel O, Wang C, Walden PM, Wilks AF, Fantino E, Rossjohn J, Lucet IS (2009) Dissecting specificity in the Janus kinases: the structures of JAK-specific inhibitors complexed to the JAK1 and JAK2 protein tyrosine kinase domains. J Mol Biol 387:219–232. doi:10.1016/j.jmb.2009.01.041

Wilson EB, Brooks DG (2011) The role of IL-10 in regulating immunity to persistent viral infections. Curr Top Microbiol Immunol 350:39–65. doi:10.1007/82_2010_96

Wolk K, Kunz S, Asadullah K, Sabat R (2002) Cutting edge: immune cells as sources and targets of the IL-10 family members? J Immunol 168:5397–5402

Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, Gurney AL (2000) Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. J Biol Chem 275:31335–31339

Xu T, Logsdon NJ, Walter MR (2005) Structure of insect-cell-derived IL-22. Acta Crystallogr D Biol Crystallogr 61:942–950

Yoon SI, Jones BC, Logsdon NJ, Harris BD, Deshpande A, Radaeva S, Halloran BA, Gao B, Walter MR (2010) Structure and mechanism of receptor sharing by the IL-10R2 common chain. Structure 18:638–648. doi:10.1016/j.str.2010.02.009

Yoon SI, Jones BC, Logsdon NJ, Harris BD, Kuruganti S, Walter MR (2012) Epstein-Barr virus IL-10 engages IL-10R1 by a two-step mechanism leading to altered signaling properties. J Biol Chem 287:26586–26595. doi:10.1074/jbc.M112.376707

Yoon SI, Jones BC, Logsdon NJ, Walter MR (2005) Same structure, different function crystal structure of the Epstein-Barr virus IL-10 bound to the soluble IL-10R1 chain. Structure 13:551–564

Yoon SI, Logsdon NJ, Sheikh F, Donnelly RP, Walter MR (2006) Conformational changes mediate interleukin-10 receptor 2 (IL-10R2) binding to IL-10 and assembly of the signaling complex. J Biol Chem 281:35088–35096

Yue Y, Barry PA (2008) Rhesus cytomegalovirus a nonhuman primate model for the study of human cytomegalovirus. Adv Virus Res 72:207–226. doi:10.1016/S0065-3527(08)00405-3

Zdanov A (2010) Structural analysis of cytokines comprising the IL-10 family. Cytokine Growth Factor Rev 21:325–330. doi:10.1016/j.cytogfr.2010.08.003

Zdanov A, Schalk-Hihi C, Gustchina A, Tsang M, Weatherbee J, Wlodawer A (1995) Crystal structure of interleukin-10 reveals the functional dimer with an unexpected topological similarity to interferon gamma. Structure 3:591–601

Zdanov A, Schalk-Hihi C, Menon S, Moore KW, Wlodawer A (1997) Crystal structure of Epstein-Barr virus protein BCRF1, a homolog of cellular interleukin-10. J Mol Biol 268:460–467

Zdanov A, Schalk-Hihi C, Wlodawer A (1996) Crystal structure of human interleukin-10 at 1.6 A resolution and a model of a complex with its soluble receptor. Protein Sci 5:1955–1962

Role of IL-22 in Microbial Host Defense

Celine Eidenschenk, Sascha Rutz, Oliver Liesenfeld and Wenjun Ouyang

Abstract Interleukin (IL)-22 is a member of the IL-10 family of cytokines, which, besides IL-10, contains seven additional cytokines. Although the founding member IL-10 is an important immunoregulatory cytokine that represses both innate and adaptive immunity, the other family members preferentially target epithelial cells and enhance innate host defense mechanisms against various pathogens such as bacteria, yeast, and viruses. Based on their functions, the IL-10 family can be further divided into three subgroups, IL-10 itself, the IL-20 subfamily, and the IFNλ subfamily. IL-22 is the best-studied member of the IL-20 subfamily, and exemplifies the diverse biological effects of this subfamily. IL-22 elicits various innate immune responses from epithelial cells and is essential for host defense against several invading pathogens, including *Citrobacter rodentium* and *Klebsiella pneumonia*. IL-22 also protects tissue integrity and maintains the mucosal homeostasis. On the other hand, IL-22 is a proinflammatory cytokine with the capacity to amplify inflammatory responses, which might result in tissue damage, e.g., the IL-22-dependent necrosis of the small intestine during *Toxoplasma gondii* infection.

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Contents

1 Introduction

Since the identification of IL-10 more than 20 years ago, the IL-10 cytokine family has grown to include nine cytokines with diverse immune regulatory functions (Ouyang et al. 2011). IL-10 itself is important in repressing inflammatory responses and restoring normal tissue homeostasis after various immune responses. The other IL-10 family members have diverse functions, ranging from host defense and maintenance of mucosal integrity to tissue regeneration and wound healing. Among them, IL-22 is the best-studied cytokine. Research on the biology of IL-22 revealed several new frontiers in the field of immunology. For example, the identification of IL-22 as a Th17 cytokine (Chung et al. 2006; Liang et al. 2006; Zheng et al. 2007) provided the molecular basis for how T helper cells regulate host defense against extracellular pathogens and tissue inflammation. The pursuit of innate sources of IL-22 led to the discovery of new innate lymphoid subsets (Satoh-Takayama et al. 2008; Cella et al. 2009). In addition, the elucidation of the biological effects of IL-22 on epidermal keratinocytes and the regulation of IL-22 by IL-23 revealed how skin-infiltrating leukocytes trigger epidermal hyperplasia in psoriasis (Boniface 2005; Zheng et al. 2007). The role of IL-22 in chronic inflammation and autoimmunity in intestine and the skin has been extensively reviewed (Wolk et al. 2004; Zenewicz and Flavell 2008; Sonnenberg et al. 2011). Here, we focus on the roles of IL-22 in host defense against bacterial, fungal, viral, and parasitic pathogens.

2 IL-10 Family Cytokines

Members of the IL-10 family belong to the class II family of cytokines (Ouyang et al. 2011). The founding member IL-10 was identified in 1989 (Fiorentino et al. 1989) as a cytokine produced by Th2 cells and able to inhibit IFN-γ production from Th1 cells. IL-10 binds and signals through a heterodimeric receptor complex composed of IL-10R1 and IL-10R2 chains, which is expressed on leukocytes, such

as T cells, B cells, and myeloid cells. Leukocytes are also the source of IL-10. Production of IL-10 by T cells, B cells, or myeloid cells is crucial for inhibiting both innate and adaptive immune responses, especially during the resolution stage. Overall, IL-10 is considered as an anti-inflammatory cytokine. For instance, IL-10 represses Th1 differentiation and inhibits IL-12 production from dendritic cells (DCs) and macrophages (Fiorentino et al. 1991a, b). In consequence, deficiency in IL-10 signaling results in the development of spontaneous colitis in both mouse and human (Kühn et al. 1993; Mitsuyama et al. 2006; Franke et al. 2008). Conversely, increased IL-10 expression is associated with chronic bacterial and viral infections. In fact, some viruses take advantage of the anti-inflammatory properties of the IL-10 pathway and encode a viral variant of IL-10 (vIL-10) that can repress host antiviral responses (Moore et al. 1990).

In contrast to IL-10 itself, the other cytokines of the IL-10 family contribute to host defense against various pathogens, but do not have direct anti-inflammatory functions (Ouyang et al. 2011). These cytokines include IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29, all of which were identified about a decade after the discovery of IL-10. IL-26 exists in human and other vertebrates but not in mouse (Wang et al. 2010). IL-28A, IL-28B, and IL-29 are also named as IFNλ2, IFNλ3, and IFNλ1, respectively, because they can induce similar antiviral activities as type I IFN (Fox et al. 2009).

All of these cytokines are structurally related to IL-10 and moreover, many of them share IL-10R2 as one chain of their heterodimeric receptor complex (Fig. 1). In fact, IL-10R2 is the common β chain for IL-22, IL-26, IL-28A, IL-28B, and IL-29, while IL-19, IL-20, and IL-24 use IL-20R2 as the β chain. The IL-22R1 chain is shared by IL-22, IL-20, and IL-24 as the α chain. IL-20R1 is the functional α chain for IL-19, IL-20, IL-24, and IL-26. IL-28R pairs with IL-10R2 to form the receptor complex for IL-28A, IL-28B, and IL-29 (Xie et al. 2000; Blumberg et al. 2001; Dumoutier et al. 2001; Kotenko et al. 2001; Parrish-Novak et al. 2002; Wang et al. 2002; Kotenko et al. 2003; Sheppard et al. 2003; Sheikh et al. 2004). Finally, IL-22R2, also referred to as IL-22BP, is a soluble receptor and shares structural similarity with the extracellular domain of IL-22R1 (Kotenko et al. 2001a, b; Xu et al. 2001; Dumoutier et al. 2003). Functionally, IL-22R2 serves as an antagonist by binding to IL-22 and blocking its biological activities. While IL-10R1 is mainly expressed on leukocytes, IL-22R1, IL-20R1, and IL-28R1 are primarily detected on epithelial cells (Ho et al. 1993; Liu et al. 1994; Blumberg et al. 2001; Wolk et al. 2004; Sommereyns et al. 2008; Witte et al. 2009). As a consequence all of these newly identified IL-10 family cytokines preferentially target tissue epithelial cells rather than leukocytes. In addition, they do not have direct immunosuppressive functions, in contrast to IL-10. Instead, these cytokines can elicit innate immune responses from tissue epithelia against invading pathogens, such as bacteria, fungi, or viruses.

IL-10 family cytokines can be subdivided into three groups based on their distinct functions. The first subfamily is composed of IL-10 itself, an important immune regulator that silences inflammatory responses. The IFN-λ subfamily of cytokines (or type III IFN), which includes IL-28A, IL-28B, and IL-29, drives a typical antiviral response by inducing the formation of the ISGF3 complex comprising STAT1,

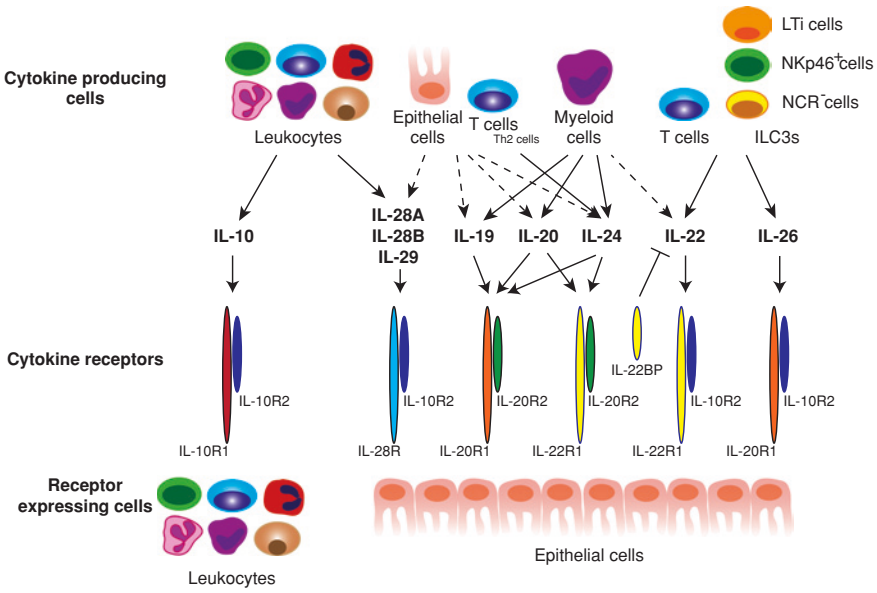

Fig. 1 IL-10 family of cytokines and their receptors. IL-10 can be produced by almost all leukocytes such as T, B, NK cells, cells from myeloid origin as well as neutrophils and eosinophils. IL-10 signals through an heterodimaric receptor composed of IL-10R1 and R2 which is expressed on leukocytes. The type III IFN subfamily, composed of IL-28A, IL-28B and IL-29, is produced by leukocytes and, under certain circumstances by epithelial cells, and acts mainly on epithelial cells. The main producer of IL-19, 20 and 24 are myeloid cells. Epithelial cells as well as T cells can contribute, but to a lesser extent. IL-24 has been shown to be also produced by Th2 cells. T cells and ILC3s are the main source of IL-22 and IL-26. Similarly to type III IFN receptors, the receptors for IL-19, IL-20, IL-22, IL-24 and IL-26 are expressed on epithelial cells

STAT2, and IRF9, as do type I IFNs, despite signaling through different receptors. Accordingly, type III IFNs can induce antiviral responses similar to, although weaker than type I IFNs (Kotenko et al. 2003; Onoguchi et al. 2007; Gad et al. 2009). Moreover, IFN-λ cytokines can synergize with type I IFNs during viral infection. The importance of the λ IFNs is supported by human genetics studies. As an example, IL-28B has been linked with the clinical response to IFN-α and ribavirin treatments in patients with chronic hepatitis C infection (Miller et al. 2009). The IL-20 subfamily is comprised of IL-19, IL-20, IL-22, IL-24, and IL-26. These cytokines have similar downstream biological effects, especially in targeting epidermal keratinocytes. IL-20 subfamily cytokines are all upregulated in lesional skin of psoriatic patients (Rømer et al. 2003; Wolk et al. 2004; Otkjaer et al. 2005; Boniface et al. 2007). Interestingly, IL-20, IL-22, and IL-24 transgenic mice display skin phenotypes similar to those observed psoriatic skin (Blumberg et al. 2001; Wolk et al. 2009; He and Liang 2010). In vitro, IL-19, IL-20, IL-22, and IL-24 promote hyperplasia of epidermal keratinocyte, and induce the expression of many genes associated with psoriasis (Boniface et al. 2005; Liang et al. 2006; Wolk et al. 2006; Sa et al. 2007). All the cytokines in this subfamily promote the expression of genes

involved in epithelial host defense. To date, however, IL-22 is the only member of this subgroup that has been well characterized in terms of its physiological functions in host defense, tissue homeostasis, and repair.

3 Cellular Sources of IL-22

Mouse IL-22 cDNA was first isolated by Ronald and colleagues from T cell clones stimulated with IL-9 and named IL-10-related T cell-derived inducible factor (IL-TIF) (Dumoutier et al. 2000). The human gene was subsequently cloned based on sequence homology (Dumoutier et al. 2000). The same human gene was independently identified by Gurney and colleagues and named IL-22. Gurney's group also reported the receptor complex for IL-22 (Gurney 2004). From these early reports, leukocytes, especially lymphocytes were found to produce IL-22. Among them, T cells and NK cells were potential cellular sources of IL-22. To date, T cells and group 3 innate lymphocytes (ILC3) are considered to be the major producers of IL-22 (Ouyang et al. 2011; Rutz and Ouyang 2011). Among T cells, either $\alpha\beta TCR^+$ $CD4^+$ and $CD8^+$ T cells, or $\gamma\delta TCR^+$ T cells can secrete IL-22 upon TCR activation and stimulation with proper cytokines such as IL-23 and IL-6. Within the $CD4^+$ T helper subsets, Th17 cells were first reported to preferentially produce IL-22, and IL-22 thus became one of the signature cytokine for this subset, along with IL-17 (Chung et al. 2006; Liang et al. 2006; Zheng et al. 2007). However, many other subsets such as Th1 and Th2 cells can also secrete IL-22 at lower levels (Rutz et al. 2013). In fact, Th17 polarizing conditions are not optimal for IL-22 production. TGF-β, which in combination with IL-6 is necessary for de novo Th17 differentiation and IL-17 production from naïve $CD4^+$ T cells, inhibits IL-22 production through a c-MAF-dependent mechanism (Zheng et al. 2007; Rutz et al. 2011). IL-6 alone or in combination with IL-23 promotes maximal IL-22 production from activated naïve T cells. Under this condition, however, there is minimal IL-17 expression. These "IL-22 only" secreting T cells have been identified in human, and named Th22 cells (Duhen et al. 2009; Eyerich et al. 2009; Trifari et al. 2009). Similar cells were also reported in murine models and can exert important host defensive roles during bacterial infection (Basu et al. 2012). In addition to IL-6, IL-21 can also induce IL-22 production through the activation of STAT3 in $CD4^+$ T cells (Sutton et al. 2009). Other pathways, such as Notch and aryl hydrocarbon receptor (AhR) pathways, can also positively regulate the expression of IL-22 from T cells (Rutz and Ouyang 2011).

During the early phase of infection with *Citrobacter rodentium*, a Gram-negative bacterium that specifically targets mouse colon epithelial cells, the majority of IL-22 is not produced by T cells since the level of IL-22 remained unchanged in $Rag2^{-/-}$ mice (Zheng et al. 2008). Consequent studies have revealed that group 3 innate lymphoid cells (ILC3) are the dominant source of IL-22 in the gastrointestinal tract (Satoh-Takayama et al. 2008; Cella et al. 2009; Cupedo et al. 2009; Takatori et al. 2009). ILCs are a heterogeneous group of cells that share

lymphoid morphology but lack Rag-dependent rearranged antigen receptors as well as major lymphoid lineage markers (Spits and Di Santo 2011). The development of ILCs depends on the common cytokine receptor γ chain (γc) and inhibitor of DNA binding 2 (ID2) (Yokota et al. 1999; Sun et al. 2000). Group 3 ILCs include the previously identified lymphoid tissue-inducer (LTi) cells (Veldhoen et al. 2008; Takatori et al. 2009), NCR^+ ILC3s characterized by their expression of the NK cell marker NKp46 (Satoh-Takayama et al. 2008; Cella et al. 2009), and NCR^- ILC3s (Sonnenberg et al. 2011; Spits et al. 2013). Although these ILCs share different cell surface markers, they are developmentally related since they all require IL-7 signaling and the expression of RORγt. Both LTi cells and NCR^+ ILC3s seem to be essential for IL-22 production during *C. rodentium* infection (Satoh-Takayama et al. 2008; Luci et al. 2009; Sanos et al. 2009; Lee et al. 2012). It is still unclear whether these cells represent distinct stable lineages or different states of the same cell type.

Unlike the optimal IL-22 conditions in $CD4^+$ T helper cells, IL-23, but not IL-6, is indispensable for the induction of IL-22 from ILC3s (Spits and Cupedo 2012) as illustrated by the complete lack of IL-22 induction in IL-23p40 or p19 deficient mice during *C. rodentium* infection (Zheng et al. 2008). Interestingly, the lymphotoxin (LT) pathway regulates IL-22 production in ILC3s. Blocking the interaction between LTαβ and LTβR by LTβR-Fc fusion protein in vivo abolishes IL-22 induction after *C. rodentium* infection (Spahn et al. 2004; Ota et al. 2011). LTαβ is expressed on ILCs, whereas LTβR is expressed on intestinal epithelial cells and DCs. It is, thus, unlikely that the LT signal directly induces IL-22 expression from ILCs. Instead, it seems that the expression of LTβR on both epithelial cells and DCs is necessary for optimal IL-22 induction and host defense against *C. rodentium*. LT signaling is important for the induction of various chemokines that can promote the formation of lymphoid follicles in the intestine (Fu and Chaplin 1999; Eberl et al. 2004; Randall et al. 2008). Lymphoid tissue-inducer (LTi) cells are the cornerstones for these intestinal lymphoid follicles. It is possible that the LT signal is essential to bring DC and group 3 ILCs into proximity in order to facilitate the induction of IL-22 from ILCs by IL-23 produced by DCs upon infection.

4 Biological Functions of IL-22

As discussed above, members of the IL-20 subfamily of cytokines preferentially act on epithelial cells. However, the expression pattern of IL-22R1 does not completely overlap with that of IL-20R1. In addition to epidermal keratinocytes, IL-22R1 is highly expressed on intersectional and lung epithelial cells, hepatic cells, and acinar cells in pancreas (Wolk et al. 2004). Being produced by innate lymphocytes and T cells, IL-22 emerges as a key cytokine bridging the immune system to epithelial surfaces (Witte et al. 2010; Zenewicz and Flavell 2011).

IL-22 functions in several ways to maintain the homeostasis of tissue epithelia. First, it promotes innate host defense mechanisms from epithelial cells, which

sequester, control, and eliminate invading pathogens. For example, IL-22 promotes the survival of goblet cells and mucin production by these cells (Sugimoto et al. 2008). At mucosal surfaces, mucin plays an essential role in preventing luminal pathogens from directly contacting epithelial cells. Moreover, IL-22 was initially described to induce the production of proteins involved in antimicrobial host defense from skin keratinocytes (Wolk et al. 2006; Boniface et al. 2007). Subsequently, it has been shown that it acts similarly on epithelial cells of intestinal and bronchial origins. An exhaustive list of antimicrobial peptides, including S100A7, S100A8, S100A9, β-defensin (−2 and −3) (Wolk et al. 2004; Liang et al. 2006; Wolk et al. 2006; Boniface et al. 2007), RegIIIβ, and RegIIIγ (Zheng et al. 2008), is induced by IL-22 from epithelial cells. These antimicrobial peptides can either directly destroy invading pathogens, or deprive them of essential nutrients thus impeding their growth. Second, IL-22 prevents apoptosis of epithelial cells during inflammation and infection. It also induces the proliferation of various epithelial cells, contributing to the maintenance of epithelial integrity (Ouyang et al. 2011). All these mechanisms keep pathogens from penetrating the epithelial layer.

Third, IL-22, by itself or in synergy with other cytokines such as IL-17 or tumor necrosis factor-α (TNF-α), induces the release of proinflammatory cytokines and chemokines, for example, IL-6, G-CSF, CXCL1, CXCL5, and CXCL9 (Wolk et al. 2006; Boniface et al. 2007; Aujla et al. 2008; Liang et al. 2010; Ouyang et al. 2011), from epithelial cells. These cytokines and chemokines recruit and activate leukocytes that further eliminate those pathogens that successfully breached the epithelial barrier. However, uncontrolled induction of these cytokines by IL-22 can result in inflammatory symptoms. A pathogenic role of IL-22 has been described in psoriasis, a chronic inflammatory disease of the skin characterized by keratinocyte hyperproliferation and leukocyte infiltration (Lowes et al. 2007). IL-22 is overexpressed in psoriatic lesions from patients and has been shown to be produced by T cells (Wolk et al. 2004; Boniface et al. 2007). IL-22 acts on skin keratinocytes to amplify the inflammation (Liang et al. 2006). Overexpression of IL-22 in mouse is sufficient to induce a skin phenotype similar to psoriasis (Wolk et al. 2009). IL-22 plays also a pathogenic role during rheumatoid arthritis (RA) (Geboes et al. 2009). IL-22 is upregulated in synovial fluid mononuclear cells of RA patients (Ikeuchi et al. 2005). Moreover, increase numbers of Th17 have been found in peripheral blood mononuclear cells (PBMCs) and joints of patients with RA, which may contribute to the increased production of IL-22 (Pène et al. 2008; Shen et al. 2009). IL-22 levels are also elevated in a mouse model of collagen-induced arthritis (Geboes et al. 2009) and IL-22 deficient mice show decreased levels of proinflammatory cytokines in this model, leading to reduced disease severity. Overall, if well controlled, IL-22 is essential for the protection of epithelial barriers against infection but excessive production of IL-22 can cause inflammation.

IL-22 can exert important roles in wound healing and tissue repair if infection or inflammation results in epithelial damage. IL-22 stimulates the expression of various proteinases (Boniface et al. 2005; Wolk et al. 2006) that may participate in tissue remodeling process. Furthermore, the proliferation of epithelial cells

triggered by IL-22 helps the re-epithelialization process. Consistently, IL-22 knockout mice have defects in wound healing (Brand et al. 2006; Sugimoto et al. 2008; Pickert et al. 2009; Neufert et al. 2010). Finally, IL-22 plays an important role in organ regeneration by directly promoting epithelial stem cells proliferation. The IL-22 pathway is activated after a partial hepatectomy in mice and required for optimal liver regeneration (Ren et al. 2010; Yang et al. 2010). Furthermore, IL-22 is involved in thymus regeneration after radiation (Dudakov et al. 2012). In a murine graft-versus-host disease (GVHD) model, IL-22 is able to protect intestinal stem cells, which help to maintain the intestinal epithelial integrity (Kapessidou et al. 2008). Mechanistically, the host defense functions and tissue repair effects of IL-22 may not be separated. Together, IL-22 is a critical leukocyte-derived mediator that helps to maintain epithelial homeostasis.

5 IL-22 in Controlling Extracellular Bacterial Infections at Epithelial Surfaces

The critical role of IL-22 during bacterial infections targeting epithelial cells has been first established using two models: *C. rodentium* infection of the colon and *Klebsiella pneumoniae* infection of the lung. As mentioned earlier, *C. rodentium* is a Gram-negative bacterium that specifically infects epithelial cells in the murine colon. It belongs to the attaching and effacing bacterial pathogens, which also include enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli*. Both EHEC and EHPC pose a threat to human health because they can cause diarrhea, morbidity, and mortality especially among infants in the developing world (Mead and Griffin 1998). In mouse, *C. rodentium* infection usually results in acute colitis. The infection is normally self-resolving within a month in wild-type mice involving the development of adaptive immune responses, such as anti-*C. rodentium* antibodies. In contrast, IL-22 deficient mice are susceptible and mostly succumb to the infection within the second week postinfection (Zheng et al. 2008). These mice show increased colonic epithelial damage and systemic dissemination of bacteria into various organs, including liver and spleen. IL-22 expression is induced by *C. rodentium* in the colon of wild-type mice in an IL-23-dependent manner. As we discussed before, ILCs but not T cells are the source of IL-22 during the early phase of infection (Satoh-Takayama et al. 2008; Zheng et al. 2008; Cella et al. 2009). However, T cells can still produce some IL-22 and contribute to host defense. This T cell-derived IL-22 is dependent on IL-6, but not IL-23 (Basu et al. 2012). IL-22 induces many innate effector functions from epithelial cells contributing to host defense against *C. rodentium* (Fig. 2). One of the best-characterized downstream effects of IL-22 in this model is the induction of Reg peptides, a family including seven secreted c-type lectins. Six of them, including RegI, RegII, RegIIIα, RegIIIβ, RegIIIγ, and RegIIIδ, are upregulated in the colon during *C. rodentium* infection in an IL-22-dependent manner. Exactly how these proteins help to control the dissemination of *C. rodentium* is still unclear but it has been shown that RegIIIγ is able to

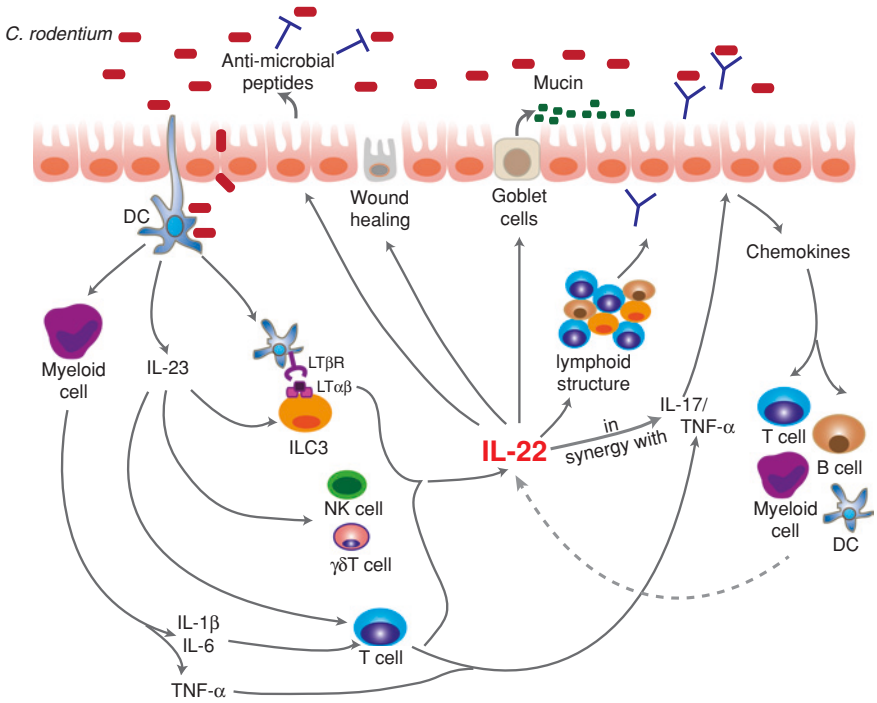

Fig. 2 Central role of IL-22 in the immune response to *C. rodentium* infection. *C. rodentium* can be sensed by DCs, which subsequently are activated and produce IL-23. IL-23 induces the expression of IL-22 from different cells such as NK, γδT cells, ILC3s, and in combination with IL-1β and IL-6, from T cells. Presumably, signal through the LT pathway is important for induction of IL-22 from ILC3s by DC derived IL-23. IL-22 acts on epithelial cells to induce the production of antimicrobial peptides, and the production of mucin by Goblets cells. It also functions to preserve the integrity of the epithelium by promoting tissue regeneration and wound healing. IL-22 is required for the activation and maintenance of lymphoid structures in the gut. Finally, IL-22 synergizes with IL-17 (produced by Th17 cells) and TNF-α (produced by myeloid cells) to stimulate the production of cytokines and chemokines from epithelial cells, which in turn act on leukocytes to amplify the immune response

directly kill Gram-positive bacteria, and RegIIIγ can cause aggregation of Gram-bacteria. IL-22 is also rapidly induced in peritoneal cavity and serum after intraperitoneal infection with *Salmonella typhimurium* (Siegemund et al. 2009). Infection with this Gram-negative bacterium leads to gastroenteritis and/or systemic infection. In the absence of IL-12, IL-22 plays a protective role against systemic dissemination of *S. enterica* (Schulz et al. 2008). Again, IL-22 production has been shown to be IL-23-dependent in this model.

IL-22 has been shown to be essential for host defense in several models of lung infection such as *Klebsiella pneumonia* infection. The source of IL-22 in this model of Gram-negative lung infection still needs to be clearly identified. $Rag^{-/-}\gamma c^{-/-}$ mice, which are deficient in IL-22 production, were found to be more susceptible than wild-type mice but the contribution of T cells versus innate lymphocytes has not

been resolved. γδT cells have been shown to be required for resistance to *K. pneumonia* infection. However, the production of IL-22 by these cells has not been assessed (Moore et al. 2000). IL-22 is induced in an IL-23-dependent manner in this model. Animals treated with IL-22 neutralizing antibodies succumb within 24 h post-*K. pneumonia* infection showing massive bacterial dissemination. IL-17 is also involved in resistance to infection in this model. IL-22 and IL-17 are both required to induce the full panel of cytokines, chemokines, and the antimicrobial protein Lipocalin-2 involved in protection against *K. pneumonia* infection (Aujla et al. 2008).

IL-22 and IL-17 have also been suggested to be beneficial in *Staphylococcus aureus* infection in human. Interestingly, patients with hyper IgE syndrome, also called Job's syndrome, harbor a mutation in STAT3 (Minegishi et al. 2007), leading to impaired Th17 development (Milner et al. 2008) and reduced IL-17 and IL-22 production. These patients are susceptible to *S. aureus* infection of the skin and lung (Minegishi et al. 2009). The protective role of IL-22 and IL-17 has been confirmed in a *S. aureus* pneumoniae model in mice (Kudva et al. 2011). Both cytokines are required for bacterial clearance from the lung and for limiting bacterial dissemination to the spleen. Similarly IL-22 is protective in a *Bacillus subtilis*-induced lung inflammation model. Repetitive exposure of mice to *B. subtilis* results in hypersensitivity pneumonitis, which progresses to lung fibrosis. IL-22 is produced by γδT cells in an AhR-dependent manner (Simonian et al. 2010). Treatment of TCR-$\delta^{-/-}$ or $Ahr^{d/d}$ mice with recombinant IL-22 partially ameliorates the inflammatory phenotype in these mice.

In contrast, IL-22 was found to be dispensable for host defense against the intracellular pathogen *Listeria monocytogenes,* despite the fact that IL-22 has well-documented potent regenerative effects on liver cells, and despite a strong CD4 T cell response triggered by the infection. However, *L. monocytogenes* infects preferentially macrophages, which do not express IL-22R. Similarly, IL-22 is not required in response to *Mycobacterium avium* infection in the liver. Furthermore, lung infection with *Mycobacterium tuberculosis* induces an IL-22-independent immune response (Wilson et al. 2010). These pathogens are not in direct contact with epithelial surfaces, possibly explaining the absence of IL-22-driven immune protection in these models.

In conclusion, IL-22 exerts important functions in protecting epithelial cells from various invading bacteria. IL-22 produced by ILCs and T cells likely provide the first regulatory mechanisms to enhance innate epithelial defense. In doing so, IL-22 not only enhances the ability to eliminate the invading pathogens by the epithelial barrier itself, but also helps to gain time for the adaptive immune responses to mature and eventually join the combat.

6 IL-22 in Containing Commensal Bacteria and Maintaining Mucosal Homeostasis

As discussed above IL-22 plays a key role in the protective immune response of the gastrointestinal tract in a variety of bacterial infections. Accumulating evidence suggests that IL-22 is also critical in maintaining the integrity of the mucosal barrier in

the gut in homeostasis, protecting against the dissemination of commensal bacteria (Kranich et al. 2011; Bird 2012). The intestinal microbiota and the immune system are in constant close interaction and it is imperative to maintain a well-regulated balance. On one hand, commensals provide certain essential nutrients to the host. They help to develop and shape the mucosal immune system to prevent the colonization of the intestine with certain pathogens. On the other hand, the immune system needs to sufficiently constrain the commensals so that they don't cause damage to the host as a result of bacterial dissemination and subsequent systemic inflammation, pathologies often observed during progressive HIV and hepatitis virus infection. In addition, the immune system has to be tolerant to the presence of commensals in the intestine, since uncontrolled local inflammatory responses may lead to sustained tissue damage, as observed in inflammatory bowel disease.

IL-22 has important functions in containing and shaping the commensal microbiota in the intestine. It is constitutively produced in parts of the intestine and helps to maintain various mucosal lymphoid structures. An essential role of IL-22 in shaping the commensal microbiota is evident from IL-22-deficient mice, which have altered colonic microbiota, for example, a decreased abundance of Lactobacillus (Zenewicz et al. 2007). Interestingly, this alteration in microbiota can be transmitted to wild-type mice that are co-housed with IL-22 deficient mice. As a result, not only the IL-22 knockout mice, but also the co-housed wild-type mice are more susceptible to DSS-induced colitis. Interestingly, similar findings were also observed in Toll-like receptor 5 (TLR5)-deficient mice. Triggering of the TLR5 pathway, which is upstream of IL-22, by flagellin can induce IL-22 production from ILC3s in an IL-23-dependent manner (Kinnebrew et al. 2012). Similar to IL-22-deficient mice, TLR5-knockout mice have altered microflora in the intestine, and are more susceptible to colitis and metabolic syndromes (Vijay-Kumar et al. 2007, 2010).

Deficiency in IL-22 also leads to increased colonization by the commensal segmented filamentous bacteria (SFB) in the small intestine. SFB is a Gram-positive bacterium, which can promote Th17 differentiation and the development of other T helper subsets in the lamina propria of infected mice (Ivanov et al. 2009; Upadhyay et al. 2012). Increased SFB colonization contributes to disease severity in many autoimmune preclinical models, such as experimental autoimmune encephalomyelitis (EAE) and arthritis models. In addition to the changes in intestinal commensals, lack of IL-22, is also associated with systemic dissemination of commensal bacteria. ILCs have been identified as the main source of IL-22 in the gastrointestinal tract of wild-type mice. Depletion of ILCs induces a peripheral dissemination of commensal bacteria, leading to systemic inflammation. Administration of IL-22 is sufficient to prevent this process. The disseminating bacterium has been identified as *Alcaligenes*. Inoculation of ILC-deficient mice with this bacterium promotes inflammation (Sonnenberg et al. 2012). *Alcaligenes* has also been associated with Crohn's disease and Hepatitic C virus infection.

As we discussed before, IL-22 provides many beneficial mechanisms for maintaining mucosal homeostasis. It enhances epithelial barrier integrity through the activation of STAT3, and induces innate antimicrobial responses (Ouyang et al. 2011). Consequently, IL-22-deficient mice are prone to developing colitis, whereas exogenous

IL-22 can ameliorate intestinal inflammation (Zenewicz et al. 2008). The role of IL-22 in shaping the commensals may also contribute to colitis, given that changes in the intestinal flora are associated with human inflammatory bowel diseases (IBD). Indeed, a recent study revealed a decrease of IL-22-producing T cells in patients with active ulcerative colitis (Broadhurst et al. 2010; Leung et al. 2014). The reduced levels of IL-22 are associated with an altered intestinal microbiota. The development of many other autoimmune diseases and metabolic diseases is also influenced by the intestinal microflora. It will be interesting to study the impact of IL-22 in these diseases through its regulation of mucosal homeostasis.

7 IL-22 in Yeast Infection

Similar to its protective functions in pathogenic bacterial infections and in containing commensals at mucosal surfaces, IL-22 can contribute to protective immunity against yeast infection. In a model of lung infection by *Aspergillus fumigatus*, IL-22 is required to control the fungal burden. In the absence of IL-22, production of IL-1β, TNF-α, MIP1α, and MIP1β is reduced, whereas IL-17A levels remain unchanged. In this model, IL-22 production is Dectin-1/IL-23 dependent (Gessner et al. 2012). In the case of *Candida albicans* infection, IL-22 is dispensable for immunity if the pathogen is inoculated by the oropharyngeal route (Conti et al. 2009), the same is true in a model of skin infection (Kagami et al. 2010). In contrast, in a murine model of intragastric infection with *C. albicans*, IL-22 is rapidly induced in the stomach of infected mice, and is required for the production of antimicrobial peptides, such as S100A8, S100A9, RegIIIβ, and RegIIIγ in response to the immune challenge. IL-22 is necessary to prevent the dissemination of *C. albicans* in the stomach and kidney (De Luca et al. 2010).

IL-22 has also been implicated in *C. albicans* infection in humans. Patients with autoimmune polyendocrine syndrome type I (APS-1), who have high titers of neutralizing auto-antibodies against IL-22, IL-17A, and IL-17F, are prone to develop chronic mucocutaneous candidiasis (CMC) (Kisand et al. 2010; Puel et al. 2010). PBMCs from patients suffering from CMC were found to be impaired in their production of IL-22 and IL-17 (Eyerich et al. 2008). Patients with mutations in the IL-17 receptor or IL-17F do also develop CMC (Puel et al. 2011). The potential role of IL-22 in these patients has not been assessed. Several other mutation that impair either the development of Th17 cells, such as mutations in STAT3 in patients with hyper IgE syndrome (Puel et al. 2010), or those that interfere with the IL-23 pathway, such as mutations in IL-12p40 or IL-12Rβ1, predispose for the development of CMC (de Beaucoudrey et al. 2010; Prando et al. 2013). From these reports, it appears that both IL-22 as well as IL-17 are required for full protection against *C. albicans* infection. The specific role and requirement of each individual cytokine still need further clarification (Zelante et al. 2011).

8 IL-22 in Viral Infection

Type III IFNs not only share one chain of their receptor with IL-22, but they also act on the same cells as IL-22. In fact, IL-28Rα expression is restricted mainly to epithelial cells, making these cells responsive to type III IFN stimulation. Given the close relation between IL-22 and these potent antiviral proteins, it was reasonable to speculate that IL-22 can play a protective role during viral infection (Ouyang et al. 2011).

Indeed IL-22 was found to be induced in viral infections, as seen in a murine model of T cell-mediated liver injury induced by mouse cytomegalovirus (MCMV) infection (Brand et al. 2007) or in patients with chronic Hepatitis B Virus (HBV) infection (Feng et al. 2012). In HBV infection, IL-22 levels correlate with severity of inflammation. In a corresponding mouse model, IL-22 has been shown to promote liver stem/progenitor cell proliferation and survival in vitro as well as in vivo. IL-22 is produced mainly by $CD3^+$ T cells, and to a lesser extent by NKT and NK cells. In Hepatitis C Virus (HCV) infection, IL-22 levels are increased in the liver of patients with viral hepatitis (Dambacher et al. 2008; Foster et al. 2012). CD4 T cells-producing IL-22 as well as IFN-γ are enriched in the liver compared to blood whereas the percentage of IL-22/IL-17-producing CD4 cells does not differ between liver and blood (Kang et al. 2012), suggesting a specific role of these IL-22/IFN-γ double-positive cells.

HBV, HCV, and lymphocytic choriomeningitis virus (LCMV) infections preferentially target the liver. Hepatocytes express high levels of IL-22R, and the role of IL-22 to protect hepatocytes by promoting their survival and proliferation is well documented. It is this tissue-protective and regenerative functions of IL-22 that are the basis for any protective role in viral infections. A direct antiviral activity of IL-22 has not been demonstrated (Dambacher et al. 2008; Pellegrini et al. 2011). In fact, IL-22 did not significantly change the expression levels of IFN-α/β and of the antiviral proteins MxA and 2′,5′-OAS in in vitro models of HCV replication and infection (Dambacher et al. 2008).

However, IL-22 might function to prevent opportunistic infections by protecting epithelial layers. During progression of Simian immunodeficiency virus (SIV) or HIV infection, the epithelial barrier of the gastrointestinal tract is damaged allowing microbial dissemination and chronic immune activation. Two simultaneous publications describe the loss of IL-22 producing cells in a model of SIV infection (Klatt et al. 2012) as well as in HIV patients (Kim et al. 2012). In the SIV model, the loss of integrity of the colonic epithelial barrier correlates with depletion of IL-22/IL-17-producing lymphocytes as well as with loss of $CD103^+$ DCs, required for the development of these IL-22/IL-17-producing cells. Similarly, IL-22 production from T cell is lost during HIV infection since these cells are progressively depleted during the early stages of the infection. Non-T cell populations producing IL-22 compensate for this loss and maintain epithelial integrity. During chronic infection, all $IL\text{-}22^+$ cell populations disappear, which correlates with the loss of epithelial barrier integrity. Moreover, in vitro, Kim et al.

showed that IL-22 protects the epithelium against damages induced by HIV infection (Kim et al. 2012).

IL-22 exerts a similar function during influenza virus infection. In this model, IL-22 is produced in the bronchoalveolar lavage (BAL), trachea as well as in the lung and spleen by conventional NK cells (Kumar et al. 2013). In the absence of IL-22, the regeneration and proliferation of tracheal epithelial cells is impaired resulting in increased susceptibility to influenza infection in IL-22-deficient mice and increased body weight loss. Moreover, IL-22 KO mice show decreased lung function together with increased fibrotic phenotype in the lung upon influenza infection (Pociask et al. 2013). Consistent with the idea that IL-22 does not possess direct antiviral activity, no differences in the viral load postinfection were observed between IL-22 deficient mice and wild-type mice. Interestingly, in a co-infection model, Influenza infection resulted in substantially decreased IL-17, IL-22, and IL-23 production associated with increased inflammation and impaired clearance after *S. aureus* infection (Kudva et al. 2011). The loss of IL-17/IL-22-driven immunity in patients can be lethal in the case of subsequent *S. aureus* infection.

These findings suggest that IL-22 production can be elicited during viral infections, and although IL-22 lacks direct antiviral functions, it helps to protect the barrier functions of epithelial surfaces, which not only reduces viral dissemination, but is also critical in preventing opportunistic infections, as often seen in HIV and influenza infections.

9 IL-22 and IL-10 in Infection with *Toxoplasma gondii*

As discussed above, IL-22 and IL-10 function very differently in that IL-22 mediates the cross-talk between the immune system and the tissue, whereas IL-10 is a negative regulator of the adaptive immune response itself. The murine model of acute infection with the parasite *T. gondii* is a good example to compare the function of these cytokines as both IL-10 and IL-22 are induced during this infection. The immunopathology observed in the small intestine during oral infection with a high inoculum of *T. gondii* resembles key features of the inflammatory responses in IBD in humans and in models of experimental colitis in rodents (Liesenfeld 2002). In fact, susceptible C57BL/6 mice develop massive necrosis in the ileum characterized by a $CD4^+$ T cell-dependent overproduction of proinflammatory mediators including IFN-γ, TNF-α, and nitric oxide (NO), leading to death within eight days postinfection (Liesenfeld et al. 1996; Khan et al. 1997; Mennechet et al. 2002). Activation of $CD4^+$ T cells by IL-12 and IL-18 is critical for the development of small intestinal pathology (Vossenkämper et al. 2004).

Interestingly, we found that, in contrast to its protective role in *C. rodentium* infection in the colon or in chronic inflammation in IBD, IL-22 is actually pathogenic in this model, as it contributes to the induction of massive necrosis observed in the small intestine of infected mice. In fact, IL-23-induced upregulation of IL-22 is essential for the development of small intestinal immunopathology since

IL-22-deficient mice do not develop small intestinal necrosis although they harbor the same number of parasites as both wild-type and IL-17 KO mice. Sources of IL-22 in the ileum are $CD4^+$ T cells but also a $CD4^-$ $CD11c^-$, $CD8^-$, $NK1.1^-$, $\gamma\delta^-$ cell population in the lamina propria of the small intestine; in contrast, in the mesenteric lymph nodes, IL-22 is exclusively produced by $CD4^+$ but not $CD4^-$ cells. Consistent with the phenotype of IL-22 deficient mice, anti-IL-22 treatment results in significantly reduced intestinal pathology associated with reduced IL-17A, IL-17F, TNF-α, and IFN-γ expression. In this model, IL-22 and IL-17 are inversely regulated. Whereas IL-22 is upregulated in an IL-23-dependent manner, IL-17 production is turned off in the ileum 7 days after infection. The high concentrations of IFN-γ in the small intestine may contribute to the down-regulation of IL-17 production as previously shown in models of adjuvant-induced arthritis (Kim et al. 2008) and mycobacterial infection (Cruz et al. 2006).

IL-10, on the other hand, plays a well-established anti-inflammatory role in *T. gondii* infection. IL-10 was first found to antagonize the ability of IFN-γ-primed macrophages to kill intracellular *T. gondii* (Gazzinelli et al. 1992). IL-10 is induced by *T. gondii* infection (Gazzinelli et al. 1992; Hunter et al. 1993; Burke et al. 1994; Gazzinelli et al. 1994) and contributes to suppression of T cell functions (Candolfi et al. 1995; Khan et al. 1995), thereby favoring parasite replication. However, $IL\text{-}10^{-/-}$ mice infected with *T. gondii* succumb to systemic inflammatory disease (Gazzinelli et al. 1996a, b; Neyer et al. 1997) mediated by $CD4^+$ T cells (Gazzinelli et al. 1996; Suzuki et al. 2000; Wilson et al. 2005) establishing IL-10 as a key factor in the control of immunopathology during toxoplasmosis. Initially, production of IL-10 by macrophages (Khan et al. 1995; Grunvald et al. 1996; Bliss et al. 2000) and B cells (Mun et al. 2003) following infection with *T. gondii* was described and was considered as a critical mechanism responsible for controlling the Th1 cell response to *T. gondii*. To address the role of T cell-derived IL-10 mice with an inactivation of the IL-10 gene restricted to T cells were generated; these mice succumbed to severe immunopathology upon infection with *T. gondii* highlighting the importance of T cell-derived IL-10 in the regulation of T cell responses (Roers et al. 2004). A $CD4^+Tbet^-Foxp3^-$ Th1 cell population was described producing IFN-γ plus IL-10 (Jankovic et al. 2007). Using $CD4^+$ T cell lines and clones derived from *T. gondii*-vaccinated mice Jankovic et al. (2007) showed that *T. gondii*-reactive Th1 cells differentiate during the adaptive response to infection. These cells do not appear to be regulatory T cells, but rather effectors that also possess the capacity to produce IL-10 and limit immune pathology. T cell production of IL-10 thus has a key role in maintaining balanced, yet efficacious, immune responses to *T. gondii,* and other pathogens (O'Garra and Vieira 2007). Similarly, NK cells were also shown to rapidly express IL-10 in an IL-12-dependent manner following systemic infection with *T. gondii* via inhibition of DC release of IL-12 (Perona-Wright et al. 2009). Thus, IL-12 appears to limit its own production by eliciting IL-10 from NK cells to limit inflammation.

Development of small intestinal pathology following high-dose oral infection with 100 cysts occurs in genetically susceptible C57BL/6 but not in resistant BALB/c mice (Liesenfeld et al. 1996). Of interest, we observed that C57BL/6-background

IL-10$^{-/-}$ mice succumb to an inoculum as low as 20 cysts; moreover, resistant BALB/c-background IL-10$^{-/-}$ mice also succumbed to the low-dose oral infection developing small intestinal pathology (Suzuki et al. 2000). Treatment with anti-IFN-γ mAb prevents the pathology and prolonged time to death in infected IL-10$^{-/-}$ mice. Thus, IL-10 plays a critical role in down-regulating IFN-γ production in the small intestine following low-dose oral infection with *T. gondii* in both genetically resistant BALB/c and susceptible C57BL/6 mice.

In this model, IL-10 and IL-22 play opposite roles: IL-10 prevents ileitis whereas IL-22 promotes it. The underlying mechanism of the pathogenic role of IL-22 still needs further clarification but this model highlights that IL-22 can be pathogenic in some circumstances.

10 Conclusion

Cytokines from the IL-10 family have diverse functions that all contribute to an essential protection against infections and inflammation. While IL-10 inhibits the immune response and thus, protects against excessive inflammation, type III IFN subfamily induces antiviral responses from epithelia of different origins. IL-22, which belongs to the IL-20 subfamily and is our main focus for this review, acts on epithelial cells to induce an antimicrobial response upon bacterial or yeast infection. Moreover, IL-22 maintains the integrity of the epithelium by promoting its regeneration as well as wound healing. In doing so, IL-22 plays a protective role against viral infection at the epithelial barrier, even if IL-22 does not have any direct antiviral activity. Recently, IL-22 has also been shown to be essential for the maintenance of the mucosal homeostasis. Despite these protective functions, IL-22 clearly has a pathogenic role during, for example, *T. gondii* infection and in chronic inflammation of the skin. A better comprehension of the underlying mechanisms is required to precisely understand what makes IL-22 protective versus pathogenic, a prerequisite to exploit this pathway therapeutically.

References

Aujla SJ, Chan YR et al (2008) IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med 14(3):275–281

Basu R, O'Quinn DB et al (2012) Th22 Cells Are an Important Source of IL-22 for Host Protection against Enteropathogenic Bacteria. Immunity 37(6):1061–1075

Bird L (2012) Mucosal immunology: IL-22 keeps commensals in their place. Nat Rev Immunol

Bliss SK, Butcher BA, Denkers EY (2000) Rapid recruitment of neutrophils containing prestored IL-12 during microbial infection. J Immunol 165(8):4515–4521

Blumberg H, Conklin D et al (2001) Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell 104(1):9–19

Boniface K, Bernard F-X et al (2005) IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. J Immunol 174(6):3695–3702

Boniface K, Guignouard E et al (2007) A role for T cell-derived interleukin 22 in psoriatic skin inflammation. Clin Exp Immunol 150(3):407–415

Brand S, Beigel F et al (2006) IL-22 is increased in active Crohn's disease and promotes pro-inflammatory gene expression and intestinal epithelial cell migration. Am J Physiol Gastrointest Liver Physiol 290(4):G827–G838

Brand S, Dambacher J, Beigel F, Zitzmann K, Heeg MH, Weiss TS, Prüfer T, Olszak T, Steib CJ, Storr M, Göke B, Diepolder H, Bilzer M, Thasler WE, Auernhammer CJ (2007) IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. Am J Physiol Gastrointest Liver Physiol 292(4):G1019–G1028

Broadhurst MJ, Leung JM et al (2010) IL-22 + CD4 + T cells are associated with therapeutic trichuris trichiura infection in an ulcerative colitis patient. Sci Transl Med 2(60):60–88

Burke JM, Roberts CW, Hunter CA, Murray M, Alexander J (1994) Temporal differences in the expression of mRNA for IL-10 and IFN-gamma in the brains and spleens of C57BL/10 mice infected with Toxoplasma gondii. Parasite Immunol 16(6):305–314

Candolfi E, Hunter CA, Remington JS (1995) Roles of gamma interferon and other cytokines in suppression of the spleen cell proliferative response to concanavalin A and toxoplasma antigen during acute toxoplasmosis. Infect Immun 63(3):751–756

Cella M, Fuchs A et al (2009) A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 457(7230):722–725

Chung Y, Yang X et al (2006) Expression and regulation of IL-22 in the IL-17-producing CD4 + T lymphocytes. Cell Res 16(11):902–907

Conti HR, Shen F et al (2009) Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med 206(2):299–311

Cruz A, Khader SA, Torrado E, Fraga A, Pearl JE, Pedrosa J, Cooper AM, Castro AG (2006) Cutting edge: IFN-gamma regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. J Immunol 177(3):1416–1420

Cupedo T, Crellin NK et al (2009) Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC + CD127 + natural killer-like cells. Nat Immunol 10(1):66–74

Dambacher J, Beigel F, Zitzmann K, Heeg MH, Göke B, Diepolder HM, Auernhammer CJ, Brand S (2008) The role of interleukin-22 in hepatitis C virus infection. Cytokine 41(3):209–216

de Beaucoudrey L, Samarina A et al (2010) Revisiting human IL-12Rbeta1 deficiency: a survey of 141 patients from 30 countries. Medicine (Baltimore) 89(6):381–402

De Luca A, Zelante T et al (2010) IL-22 defines a novel immune pathway of antifungal resistance. Mucosal Immunol 3(4):361–373

Dudakov JA, Hanash AM et al (2012) Interleukin-22 drives endogenous thymic regeneration in mice. Science 336(6077):91–95

Duhen T, Geiger R et al (2009) Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat Immunol 10(8):857–863

Dumoutier L, Leemans C, Lejeune D, Kotenko SV, Renauld JC (2001) Cutting edge: STAT activation by IL-19, IL-20 and mda-7 through IL-20 receptor complexes of two types. J Immunol 167(7):3545–3549

Dumoutier L, Lejeune D et al (2003) Cloning of a new type II cytokine receptor activating signal transducer and activator of transcription (STAT)1, STAT2 and STAT3. Biochem J 370(Pt 2):391–396

Dumoutier L, Louahed J et al (2000a) Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. J Immunol 164(4):1814–1819

Dumoutier L, Van Roost E et al (2000b) IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes. Genes Immun 1(8):488–494

Eberl G, Marmon S et al (2004) An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol 5(1):64–73

Eyerich K, Foerster S et al (2008) Patients with chronic mucocutaneous candidiasis exhibit reduced production of Th17-associated cytokines IL-17 and IL-22. J Invest Dermatol 128(11):2640–2645

Eyerich S, Eyerich K et al (2009) Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Investig 119(12):3573–3585
Feng D, Park O et al (2012) Interleukin-22 ameliorates cerulein-induced pancreatitis in mice by inhibiting the autophagic pathway. Int J Biol Sci 8(2):249–257
Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170(6):2081–2095
Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A (1991a) IL-10 inhibits cytokine production by activated macrophages. J Immunol 147:11
Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A (1991b) IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 146(10):3444–3451
Foster RG, Golden-Mason L, Rutebemberwa A, Rosen HR (2012) Interleukin (IL)-17/IL-22-producing T cells enriched within the liver of patients with chronic hepatitis C viral (HCV) infection. Dig Dis Sci 57(2):381–389
Fox BA, Sheppard PO, O'Hara PJ (2009) The role of genomic data in the discovery, annotation and evolutionary interpretation of the interferon-lambda family. PLoS ONE 4(3):e4933
Franke A, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, Domingues FS, Albrecht M, Nothnagel M, Ellinghaus D, Sina C, Onnie CM, Weersma RK, Stokkers PC, Wijmenga C, Gazouli M, Strachan D, McArdle WL, Vermeire S, Rutgeerts P, Rosenstiel P, Krawczak M, Vatn MH, IBSEN study group, Mathew CG, Schreiber S (2008) Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. Nat Genent 40(11):1319–1323
Fu Y, Chaplin D (1999) Development and maturation of secondary lymphoid tissues. Annu Rev Immunol 17:399–433
Gad HH, Dellgren C, Hamming OJ, Vends S, Paludan SR, Hartmann R (2009) Interferon-lambda is functionally an interferon but structurally related to the interleukin-10 family. J Biol Chem 284(31):20869–20875
Gazzinelli RT, Amichay D, Sharton-Kersten T, Grunwald E, Farber JM, Sher A (1996) Role of macrophage-derived cytokines in the induction and regulation of cell-mediated immunity to Toxoplasma gondii. Curr Top Microbiol Immunol 219:127–139
Gazzinelli RT, Oswald IP, James SL, Sher A (1992) IL-10 inhibits parasite killing and nitrogen oxide production by IFN-gamma-activated macrophages. J Immunol 148(6):1792–1796
Gazzinelli RT, Wysocka M, Hayashi S, Denkers EY, Hieny S, Caspar P, Trinchieri G, Sher A (1994) Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with Toxoplasma gondii. J Immunol 153(6):2533–2543
Geboes L, Dumoutier L et al (2009) Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. Arthritis Rheum 60(2):390–395
Gessner MA, Werner JL et al (2012) Dectin-1-dependent interleukin-22 contributes to early innate lung defense against Aspergillus fumigatus. Infect Immun 80(1):410–417
Grunvald E, Chiaramonte M, Hieny S, Wysocka M, Trinchieri G, Vogel SN, Gazzinelli RT, Sher A (1996) Biochemical characterization and protein kinase C dependency of monokine-inducing activities of Toxoplasma gondii. Infect Immun 64(6):2010–2018
Gurney AL (2004) IL-22, a Th1 cytokine that targets the pancreas and select other peripheral tissues. Int Immunopharmacol 4(5):669–677
He M, Liang P (2010) IL-24 transgenic mice: in vivo evidence of overlapping functions for IL-20, IL-22, and IL-24 in the epidermis. J Immunol 184(4):1793–1798
Ho AS, Liu Y, Khan TA, Hsu DH, Bazan JF, Moore KW (1993) A receptor for interleukin 10 is related to interferon receptors. Proc Natl Acad Sci USA 90(23):11267–11271
Hunter CA, Abrams JS, Beaman MH, Remington JS (1993) Cytokine mRNA in the central nervous system of SCID mice infected with Toxoplasma gondii: importance of T-cell-independent regulation of resistance to T. gondii. Infect Immun 61(10):4038–4044
Ikeuchi H, Kuroiwa T et al (2005) Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum 52(4):1037–1046

Ivanov II, Atarashi K et al (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139(3):485–498

Jankovic D, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, Wilson M, Wynn TA, Kamanaka M, Flavell RA, Sher A (2007) Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J Exp Med 204(2):273–283

Kagami S, Rizzo HL et al (2010) IL-23 and IL-17A, but not IL-12 and IL-22, are required for optimal skin host defense against Candida albicans. J Immunol 185(9):5453–5462

Kang YH, Seigel B, Bengsch B, Fleming VM, Billerbeck E, Simmons R, Walker L, Willberg CB, Barnes EJ, Bhagwanani A, Oo YH, Blum HE, Adams DH, Thimme R, Klenerman P (2012) CD161(+)CD4(+) T cells are enriched in the liver during chronic hepatitis and associated with co-secretion of IL-22 and IFN-γ. Front Immunol 3

Kapessidou P, Poulin L et al (2008) Interleukin-22 deficiency accelerates the rejection of full major histocompatibility complex-disparate heart allografts. Transpl Proc 40(5):1593–1597

Khan IA, Matsuura T, Kasper LH (1995) IL-10 mediates immunosuppression following primary infection with Toxoplasma gondii in mice. Parasite Immunol 17(4):185–195

Khan IA, Schwartzman JD, Matsuura T, Kasper LH (1997) A dichotomous role for nitric oxide during acute Toxoplasma gondii infection in mice. Proc Natl Acad Sci USA 94(25):13955–13960

Kim CJ, Nazli A et al (2012) A role for mucosal IL-22 production and Th22 cells in HIV-associated mucosal immunopathogenesis. Mucosal Immunol 5(6):670–680

Kim EY, Chi HH, Bouziane M, Gaur A, Moudgil KD (2008) Regulation of autoimmune arthritis by the pro-inflammatory cytokine interferon-gamma. Clin Immunol 127(1):98–106

Kinnebrew MA, Buffie CG et al (2012) Interleukin 23 production by intestinal CD103(+) CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. Immunity 36:276–287

Kisand K, Boe Wolff AS et al (2010) Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 207(2):299–308

Klatt NR, Estes JD et al (2012) Loss of mucosal CD103 + DCs and IL-17 + and IL-22 + lymphocytes is associated with mucosal damage in SIV infection. Mucosal Immunol 5(6):646–657

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP (2003) IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4(1):69–77

Kotenko SV, Izotova LS et al (2001a) Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. J Biol Chem 276(4):2725–2732

Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S (2001b) Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. J Immunol 166(12):7096–7103

Kranich J, Maslowski KM et al (2011) Commensal flora and the regulation of inflammatory and autoimmune responses. Semin Immunol 23(2):139–145

Kudva A, Scheller EV, Robinson KM, Crowe CR, Choi SM, Slight SR, Khader SA, Dubin PJ, Enelow RI, Kolls JK, Alcorn JF (2011) Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. J Immunol 186(3):1666–1674

Kühn R, Löhler J et al (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75(2):263–274

Kumar P, Thakar MS et al (2013) IL-22 from conventional NK cells is epithelial regenerative and inflammation protective during influenza infection. Mucosal Immunol 6(1):69–82

Lee JS, Cella M et al (2012) AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. Nat Immunol 13(2):144–151

Leung JM, Davenport M et al (2014) IL-22-producing CD4 + cells are depleted in actively inflamed colitis tissue. Mucosal Immunol 7(1):124–133

Liang SC, Nickerson-Nutter C et al (2010) IL-22 induces an acute-phase response. J Immunol 185(9):5531–5538

Liang SC, Tan X-Y et al (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 203(10):2271–2279

Liesenfeld O (2002) Oral infection of C57BL/6 mice with Toxoplasma gondii: a new model of inflammatory bowel disease? J Infect Dis 185(Suppl 1):S96–S101

Liesenfeld O, Kosek J, Remington JS, Suzuki Y (1996) Association of CD4 + T cell-dependent, interferon-gamma-mediated necrosis of the small intestine with genetic susceptibility of mice to peroral infection with Toxoplasma gondii. J Exp Med 184(2):597–607

Liu Y, Wei SH, Ho AS, de Waal Malefyt R, Moore KW (1994) Expression cloning and characterization of a human IL-10 receptor. J Immunol 152(4):1821–1829

Lowes MA, Bowcock AM, Krueger JG (2007) Pathogenesis and therapy of psoriasis. Nature 445(7130):866–873

Luci C, Reynders A et al (2009) Influence of the transcription factor RORgammat on the development of NKp46 + cell populations in gut and skin. Nat Immunol 10(1):75–82

Mead PS, Griffin PM (1998) Escherichia coli O157:H7. Lancet 352(9135):1207–1212

Mennechet FJ, Kasper LH, Rachinel N, Li W, Vandewalle A, Buzoni-Gatel D (2002) Lamina propria CD4 + T lymphocytes synergize with murine intestinal epithelial cells to enhance proinflammatory response against an intracellular pathogen. J Immunol 168(6):2988–2996

Miller DM, Klucher KM, Freeman JA, Hausman DF, Fontana D, Williams DE (2009) Interferon lambda as a potential new therapeutic for hepatitis C. Ann N Y Acad Sci. 80(7)

Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, Kanno Y, Spalding C, Elloumi HZ, Paulson ML, Davis J, Hsu A, Asher AI, O'Shea J, Holland SM, Paul WE, Douek DC (2008) Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 452(7188):773–776

Minegishi Y, Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, Agematsu K, Yamada M, Kawamura N, Ariga T, Tsuge I, Karasuyama H (2009) Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. J Exp Med 206(6):1291–1301

Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, Kawamura N, Ariga T, Pasic S, Stojkovic O, Metin A, Karasuyama H (2007) Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 448(7157):1058–1062

Mitsuyama K, Tomiyasu N, Takaki K, Masuda J, Yamasaki H, Kuwaki K, Takeda T, Kitazaki S, Tsuruta O, Sata M (2006) Interleukin-10 in the pathophysiology of inflammatory bowel disease: increased serum concentrations during the recovery phase. Mediators Inflamm 6:26875

Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science 248(4960):1230–1234

Moore TA, Moore BB, Newstead MW, Standiford TJ (2000) Gamma delta-T cells are critical for survival and early proinflammatory cytokine gene expression during murine Klebsiella pneumonia. J Immunol 165(5):2643–2650

Mun HS, Aosai F, Chen M, Piao LX, Norose K, Iwakura Y, Yano A (2003) Pathogenicity of Toxoplasma gondii through B-2 cell-mediated downregulation of host defense responses. Microbiol Immunol 47(7):533–542

Neufert C, Pickert G et al (2010) Activation of epithelial STAT3 regulates intestinal homeostasis. Cell Cycle 9(4):652–655

Neyer LE, Grunig G, Fort M, Remington JS, Rennick D, Hunter CA (1997) Role of interleukin-10 in regulation of T-cell-dependent and T-cell-independent mechanisms of resistance to Toxoplasma gondii. Infect Immun 65(5):1675–1682

O'Garra A, Vieira P (2007) T(H)1 cells control themselves by producing interleukin-10. Nat Rev Immunol 7(6):425–428

Onoguchi K, Yoneyama M, Takemura A, Akira S, Taniguchi T, Namiki H, Fujita T (2007) Viral infections activate types I and III interferon genes through a common mechanism. J Biol Chem 282(10):7576–7581

Ota N, Wong K et al (2011) IL-22 bridges the lymphotoxin pathway with the maintenance of colonic lymphoid structures during infection with Citrobacter rodentium. Nat Immunol 12(10):941–948

Otkjaer K, Kragballe K, Funding AT, Clausen JT, Noerby PL, Steiniche T, Iversen L (2005) The dynamics of gene expression of interleukin-19 and interleukin-20 and their receptors in psoriasis. Br J Dermatol 153(5):911–918

Ouyang W, Rutz S et al (2011) Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 29:71–109

Parrish-Novak J, Xu W, Brender T, Yao L, Jones C, West J, Brandt C, Jelinek L, Madden K, McKernan PA, Foster DC, Jaspers S, Chandrasekher YA (2002) Interleukins 19, 20, and 24 signal through two distinct receptor complexes. Differences in receptor-ligand interactions mediate unique biological functions. J Biol Chem 277(49):47517–47523

Pellegrini M, Calzascia T et al (2011) IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. Cell 144(4):601–613

Pène J, Chevalier S et al (2008) Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. J Immunol 180(11):7423–7430

Perona-Wright G, Mohrs K, Szaba FM, Kummer LW, Madan R, Karp CL, Johnson LL, Smiley ST, Mohrs M (2009) Systemic but not local infections elicit immunosuppressive IL-10 production by natural killer cells. Cell Host Microbe 6(6):503–512

Pickert G, Neufert C et al (2009) STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. J Exp Med 206(7):1465–1472

Pociask DA, Scheller EV, Mandalapu S, McHugh KJ, Enelow RI, Fattman CL, Kolls JK, Alcorn JF (2013) IL-22 is essential for lung epithelial repair following influenza infection. Am J Pathol 182(4):1286–1296

Prando C, Samarina A et al (2013) Inherited IL-12p40 deficiency: genetic, immunologic, and clinical features of 49 patients from 30 kindreds. Medicine (Baltimore) 92(2):109–122

Puel A, Cypowyj S et al (2011) Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 332(6025):65–68

Puel A, Doffinger R et al (2010a) Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 207(2):291–297

Puel A, Picard C et al (2010b) Inborn errors of mucocutaneous immunity to Candida albicans in humans: a role for IL-17 cytokines? Curr Opin Immunol 22(4):467–474

Randall TD, Carragher DM et al (2008) Development of secondary lymphoid organs. Annu Rev Immunol 26:627–650

Ren X, Hu B et al (2010) IL-22 is involved in liver regeneration after hepatectomy. Am J Physiol Gastrointest Liver Physiol 298(1):G74–G80

Roers A, Siewe L, Strittmatter E, Deckert M, Schlüter D, Stenzel W, Gruber AD, Krieg T, Rajewsky K, Müller W (2004) T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. J Exp Med 200(10):1289–1297

Rømer J, Hasselager E, Nørby PL, Steiniche T, Thorn Clausen J, Kragballe K (2003) Epidermal overexpression of interleukin-19 and -20 mRNA in psoriatic skin disappears after short-term treatment with cyclosporine a or calcipotriol. J Invest Dermatol 121(6):1306–1311

Rutz S, Eidenschenk C, Ouyang W (2013) IL-22, not simply a Th17 cytokine. Immunol Rev 252(1):116–132

Rutz S, Noubade R et al (2011) Transcription factor c-Maf mediates the TGF-beta-dependent suppression of IL-22 production in T(H)17 cells. Nat Immunol 12(12):1238–1245

Rutz S, Ouyang W (2011) Regulation of interleukin-10 and interleukin-22 expression in T helper cells. Curr Opin Immunol 23(5):605–612

Sa SM, Valdez PA et al (2007) The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. J Immunol 178(4):2229–2240

Sanos SL, Bui VL et al (2009) RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46 + cells. Nat Immunol 10(1):83–91

Satoh-Takayama N, Vosshenrich CA et al (2008) Microbial flora drives interleukin 22 production in intestinal NKp46 + cells that provide innate mucosal immune defense. Immunity 29(6):958–970

Schulz SM, Köhler G, Schütze N, Knauer J, Straubinger RK, Chackerian AA, Witte E, Wolk K, Sabat R, Iwakura Y, Holscher C, Müller U, Kastelein RA, Alber G (2008) Protective immunity to systemic infection with attenuated Salmonella enterica serovar enteritidis in the absence of IL-12 is associated with IL-23-dependent IL-22, but not IL-17. J Immunol 181(11):7891–7901

Sheikh F, Baurin VV et al (2004) Cutting edge: IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. J Immunol 172(4):2006–2010

Shen H, Goodall JC et al (2009) Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. Arthritis Rheum 60(6):1647–1656

Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J, Ostrander C, Dong D, Shin J, Presnell S, Fox B, Haldeman B, Cooper E, Taft D, Gilbert T, Grant FJ, Tackett M, Krivan W, McKnight G, Clegg C, Foster D, Klucher KM (2003) IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 4(1):63–68

Siegemund S, Schutze N et al (2009) Differential IL-23 requirement for IL-22 and IL-17A production during innate immunity against Salmonella enterica serovar Enteritidis. Int Immunol 21(5):555–565

Simonian PL, Wehrmann F et al (2010) gammadelta T cells protect against lung fibrosis via IL-22. J Exp Med 207(10):2239–2253

Sommereyns C, Paul S, Staeheli P, Michiels T (2008) IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. PLoS Pathog 4(3):1000017

Sonnenberg GF, Fouser LA et al (2011a) Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol 12(5):383–390

Sonnenberg GF, Monticelli LA et al (2012) Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. Science 336(6086):1321–1325

Sonnenberg GF, Monticelli LA et al (2011b) CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. Immunity 34(1):122–134

Spahn TW, Maaser C et al (2004) The lymphotoxin-beta receptor is critical for control of murine Citrobacter rodentium-induced colitis. Gastroenterology 127(5):1463–1473

Spits H, Artis D et al (2013) Innate lymphoid cells - a proposal for uniform nomenclature. Nat Rev Immunol 13(2):145–149

Spits H, Cupedo T (2012) Innate Lymphoid Cells: Emerging Insights in Development, Lineage Relationships, and Function. Annu Rev Immunol 30:647–675

Spits H, Di Santo JP (2011) The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. Nat Immunol 12(1):21–27

Sugimoto K, Ogawa A et al (2008) IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest 118(2):534–544

Sun Z, Unutmaz D et al (2000) Requirement for RORgamma in thymocyte survival and lymphoid organ development. Science 288(5475):2369–2373

Sutton CE, Lalor SJ et al (2009) Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity 31(2):331–341

Suzuki Y, Sher A, Yap G, Park D, Neyer LE, Liesenfeld O, Fort M, Kang H, Gufwoli E (2000) IL-10 is required for prevention of necrosis in the small intestine and mortality in both genetically resistant BALB/c and susceptible C57BL/6 mice following peroral infection with Toxoplasma gondii. J Immunol 164(10):5375–5382

Takatori H, Kanno Y et al (2009) Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. J Exp Med 206(1):35–41

Trifari S, Kaplan CD et al (2009) Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. Nat Immunol 10(8):864–871

Upadhyay V, Poroyko V et al (2012) Lymphotoxin regulates commensal responses to enable diet-induced obesity. Nat Immunol 13(10):947–953

Veldhoen M, Hirota K et al (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453(7191):106–109

Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 328(5975):228–231

Vijay-Kumar M, Sanders CJ, Taylor RT, Kumar A, Aitken JD, Sitaraman SV, Neish AS, Uematsu S, Akira S, Williams IR, Gewirtz AT (2007) Deletion of TLR5 results in spontaneous colitis in mice. J Clin Invest 117(12):3909–3921

Vossenkämper A, Struck D, Alvarado-Esquivel C, Went T, Takeda K, Akira S, Pfeffer K, Alber G, Lochner M, Förster I, Liesenfeld O (2004) Both IL-12 and IL-18 contribute to small intestinal Th1-type immunopathology following oral infection with Toxoplasma gondii, but IL-12 is dominant over IL-18 in parasite control. Eur J Immunol 34(11):3197–3207

Wang M, Tan Z, Zhang R, Kotenko SV, Liang P (2002) Interleukin 24 (MDA-7/MOB-5) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. J Biol Chem 277(9):7341–7347

Wang T, Díaz-Rosales P, Martin SA, Secombes CJ (2010) Cloning of a novel interleukin (IL)-20-like gene in rainbow trout Oncorhynchus mykiss gives an insight into the evolution of the IL-10 family. Dev Comp Immunol 34(2):158–167

Wilson EH, Wille-Reece U, Dzierszinski F, Hunter CA (2005) A critical role for IL-10 in limiting inflammation during toxoplasmic encephalitis. J Neuroimmunol 165(1–2):63–74

Wilson MS, Feng CG, Barber DL, Yarovinsky F, Cheever AW, Sher A, Grigg M, Collins M, Fouser L, Wynn TA (2010) Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. J Immunol 184(8):4378–4390

Witte E, Witte K et al (2010) Interleukin-22: a cytokine produced by T, NK and NKT cell subsets, with importance in the innate immune defense and tissue protection. Cytokine Growth Factor Rev 21(5):365–379

Witte K, Gruetz G, Volk HD, Looman AC, Asadullah K, Sterry W, Sabat R, Wolk K (2009) Despite IFN-lambda receptor expression, blood immune cells, but not keratinocytes or melanocytes, have an impaired response to type III interferons: implications for therapeutic applications of these cytokines. Genes Immun 10(8):702–714

Wolk K, Haugen HS et al (2009) IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. J Mol Med 87(5):523–536

Wolk K, Kunz S et al (2004) IL-22 increases the innate immunity of tissues. Immunity 21(2):241–254

Wolk K, Witte E et al (2006) IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol 36(5):1309–1323

Xie MH, Aggarwal S et al (2000) Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. J Biol Chem 275(40):31335–31339

Xu W, Presnell SR et al (2001) A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. Proc Natl Acad Sci USA 98(17):9511–9516

Yang L, Zhang Y et al (2010) Amelioration of high fat diet induced liver lipogenesis and hepatic steatosis by interleukin-22. J Hepatol 53(2):339–347

Yokota Y, Mansouri A et al (1999) Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. Nature 397(6721):702–706

Zelante T, Iannitti R et al (2011) IL-22 in antifungal immunity. Eur J Immunol 41(2):270–275

Zenewicz LA, Flavell RA (2008) IL-22 and inflammation: leukin' through a glass onion. Eur J Immunol 38(12):3265–3268
Zenewicz LA, Flavell RA (2011) Recent advances in IL-22 biology. Int Immunol 23(3):159–163
Zenewicz LA, Yancopoulos GD et al (2007) Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity 27(4):647–659
Zenewicz LA, Yancopoulos GD et al (2008) Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity 29(6):947–957
Zheng Y, Danilenko DM et al (2007) Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 445(7128):648–651
Zheng Y, Valdez PA et al (2008) Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med 14(3):282–289

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