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Negative Co-Receptors and Ligands

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TIM-3 and Its Regulatory Role in Immune Responses

Chen Zhu, Ana C. Anderson, and Vijay K. Kuchroo

Contents

Abstract T cell immunoglobulin mucin-(TIM)-3 was first identified as a molecule specifically expressed on IFN-γ-secreting $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic (Tc1) cells in both mice and humans. TIM-3 acts as a negative regulator of Th1/Tc1 cell function by triggering cell death upon interaction with its ligand, galectin-9. This negative regulatory function of TIM-3 has now been expanded to include its involvement in establishing and/or maintaining a state of T cell dysfunction or "exhaustion" observed in chronic viral diseases. In addition, it is now appreciated that TIM-3 has other ligands and is expressed on other cell types, where it may function differently. Given that an increasing body of data support an important role for TIM-3 in both autoimmune and chronic inflammatory diseases

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in humans, deciphering the function of TIM-3 on different cell types during different immune conditions and how these can be regulated will be critical for harnessing the therapeutic potential of TIM-3 for the treatment of disease.

1 Introduction

T-cell immunoglobulin and mucin-(TIM)-3 domain was first discovered in 2002 as a molecule specifically expressed on IFN-γ-producing $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic 1 (Tc1) cells in the mouse (Monney et al. 2002). Later, it was found that TIM-3 is also specifically expressed on IFN-γ-producing T cells in humans (Khademi et al. 2004). The specific expression of TIM-3 on Th1 cells catalyzed investigation into its potential role as a regulator of Th1 cells. Indeed, it is now known that ligation of TIM-3 triggers cell death in Th1 cells in mice (Zhu et al. 2005). Other studies support that TIM-3 also acts as a negative regulator of human Th1 T cells (Hastings et al. 2009; Koguchi et al. 2006; Yang et al. 2008).

The importance of TIM-3 in regulating T cell responses is underscored by the fact that both TIM-3 expression and its negative regulatory function is dysregulated in patients with multiple sclerosis (MS) and that both these defects are reversed following treatment (Koguchi et al. 2006; Yang et al. 2008). Moreover, the negative regulatory role for TIM-3 in T cells has recently been extended to dysfunctional or "exhausted" T cells in chronic viral infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) (Golden-Mason et al. 2009; Jones et al. 2008). Here, blockade of TIM-3 signaling has been shown to partially restore T cell function of otherwise "exhausted" T cells. Collectively, these data strongly support an important role for TIM-3 as a negative regulator of T cell responses and highlight the importance of this pathway as a therapeutic target in human diseases.

However, it is now appreciated that TIM-3 is not only expressed on T cells but also on other cell types such as dendritic cells (DCs) in both mice and humans and on monocytes in humans (Anderson et al. 2007). Further, TIM-3 is expressed on mast cells (Nakae et al. 2007), melanoma (Wiener et al. 2007), and on lymphoma-derived endothelium (Huang et al. 2010), where it may be involved in promoting tumor progression by inhibiting anti-tumor $CD4^+$ T cell responses (Huang et al. 2010). How these diverse functions of TIM-3 in different cell types are regulated and which one predominates in different disease states is not clear at this stage.

2 Protein Structure

Mouse TIM-3 is a 281 amino acid (aa) type I transmembrane glycoprotein that contains a membrane distal immunoglobulin variable (IgV) domain and a membrane proximal mucin domain. Human TIM-3 is 302 aa in length and shares 63% aa identity with mouse TIM-3 (Monney et al. 2002). Further, a putative soluble mouse TIM-3 splice variant has been identified in cDNA generated from concanavalin

A–activated splenocytes. The predicted protein sequence of this TIM-3 isoform contains only the signal peptide, immunoglobulin V (IgV), and cytoplasmic domain, lacking the mucin domain and transmembrane region (Sabatos et al. 2003).

TIM-3 belongs to the immunoglobulin super family (IgSF) (Bork et al. 1994) and recent studies have revealed the 3D structure of the IgV domain of TIM-3 as well as other TIM proteins (Cao et al. 2007; Santiago et al. 2007a, b). TIM-3 IgV domains consist of two anti-parallel β sheets that are tethered by a disulfide bond. Additional two disulfide bonds are formed by four noncanonical cysteines that are invariant within TIM proteins and unique among IgSF members. They stabilize the IgV domain of TIM-3 and reorient the CC′ loop so that it is in close proximity to the FG loop resulting in formation of a "cleft" or "pocket" structure in TIM-3 as well as other TIM proteins (Fig. 1) (Cao et al. 2007; Santiago et al. 2007b). This unique cleft structure is not found in other IgSF proteins and has been predicted to be involved in ligand binding (see below).

In the cytoplasmic region of both human and mouse TIM-3, there is a highly conserved region containing five tyrosine residues. Galectin-9 triggering of TIM-3 results in tyrosine phosphorylation of these residues, indicating that some, if not all, of these tyrosines are involved in TIM-3 signaling (van de Weyer et al. 2006). Otherwise, protein sequence analysis does not reveal any other homology to known inhibitory domains such as an immunoreceptor tyrosine-based inhibitory motif or immunoreceptor tyrosine-based switch motif. Thus, much remains to be elucidated regarding the signaling pathways recruited by TIM-3.

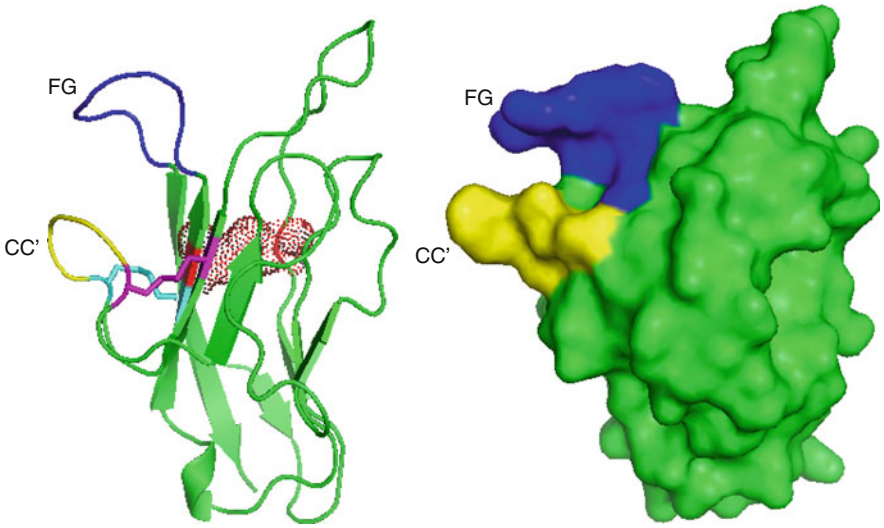

Fig. 1 Mouse TIM-3 IgV domain. (**a**) Ribbon diagram. *Sticks* represent two noncanonical disulfide bonds that reorient the CC′ loop thereby forming a "cleft" or "pocket" structure together with the FG loop in TIM-3 as well as other TIM proteins. Dots represent the disulfide bond that exist in all IgSF proteins. (**b**) Surface representation. Structure simulation was done by PyMOL

3 TIM-3 Ligands

3.1 Identification of Galectin-9 as a TIM-3 Ligand

In an attempt to identify the TIM-3 ligand, we screened a number of T cell lines and lymphomas for their ability to bind TIM-3-Ig fusion protein. TK-1 $CD8^+$ T cell lymphoma was found to have the strongest binding to TIM-3-Ig fusion protein, suggesting a high level of TIM-3 ligand expression on this cell type. Subsequent pull-down of proteins bound to TIM-3-Ig identified a 35-kDa cell surface molecule that only bound TIM-3-Ig but not control hIgG1. This molecule was later identified as galectin-9 by mass spectrometry (Zhu et al. 2005).

Galectins, a group of S-type lectins, are a family of carbohydrate-binding proteins that exhibit important functions in regulating immune cell homeostasis and inflammation (Rabinovich and Toscano 2009). Galectin-9 binding to TIM-3 is dependent on its carbohydrate recognition domain that recognizes the oligosaccharide chains on the TIM-3 IgV domain. Galectin-9 is expressed on a variety of cell types and it is up-regulated by IFN-γ (Imaizumi et al. 2002). In vitro analyses revealed that galectin-9 predominantly induces intracellular calcium flux, cell aggregation, and death of Th1 but not Th2 cells, and this process is dependent on the presence of TIM-3 on Th1 cells, as TIM-3 deficient Th1 cells are relatively resistant to galectin-9-induced cell death. Furthermore, administration of galectin-9 in vivo during an ongoing immune response dampens inflammation by specifically eliminating antigen specific IFN-γ-producing T cells, thereby attenuating disease progress in experimental autoimmune encephalomyelitis (EAE) (Zhu et al. 2005). Thus, the galectin-9–TIM-3 pathway provides a negative feedback loop by which Th1 cells are regulated to prevent uncontrolled Th1 responses which otherwise could be detrimental to the host.

Subsequent studies in other disease models have demonstrated that galectin-9 triggering of TIM-3 attenuates Th1 and Th17 responses, thereby exhibiting therapeutic potential in skin inflammation (Niwa et al. 2009), experimental autoimmune arthritis (Seki et al. 2008), and herpes simplex virus (HSV)-induced ocular inflammation (Sehrawat et al. 2009). Thus, although TIM-3 is expressed at low levels on Th17 cells (Chen et al. 2006), it may have a role in attenuating Th17 responses. Further, galectin-9 has been shown to induce cell death in $TIM\text{-}3^+CD8^+$ alloreactive T cells, thereby reducing cytotoxicity and prolonging survival of skin grafts (Wang et al. 2007).

Interestingly, the galectin-9–TIM-3 interaction does not always lead to suppression of immune responses. Triggering of TIM-3 on innate immune cells in both mice and humans exhibits an opposite role (Anderson et al. 2007) (see below). The galectin-9–TIM-3 pathway in DCs actually synergizes with Toll-like receptor (TLR) signaling and promotes Th1 immunity. In addition, administration of galectin-9 prolongs the survival of Meth-A tumor-bearing mice by increasing the number of IFN-γ-producing $TIM\text{-}3^+CD8^+$ T cells with enhanced cytolytic function as a result of an increase in the number of $TIM\text{-}3^+CD86^+$ mature DCs (Nagahara et al. 2008).

These observations support another interesting biological role for the galectin-9–TIM-3 pathway, enhancement of adaptive immunity via galectin-9-induced maturation of TIM-3^+ antigen presenting cells.

Although the function of the galectin-9–TIM-3 pathway in immune responses has been extensively studied, multiple lines of evidence suggested the existence of other TIM-3 ligands. (1) At least one additional membrane protein specifically associates with TIM-3-Ig (our unpublished data); (2) Bacterially expressed TIM-3 tetramer that lacks carbohydrate modification binds to a broad panel of cell types. That these interactions do not require TIM-3 carbohydrate moieties excludes the possibility of them being galectin-9-dependent. (3) The crystal structure of the TIM-3 IgV domain revealed a potential ligand binding site at the CC′-FG cleft; however, the potential N and O-linked glycosylation sites in the TIM-3 IgV domain are not proximal to this region. Overall, the topological features of TIM-3 indicate the existence of other independent TIM-3 ligands that bind to discrete regions on the TIM-3 IgV domain.

3.2 Phosphatidylserine as a TIM-3 Ligand

As mentioned above, it has been predicted that the unique cleft present in TIM family proteins participates in ligand binding. Indeed, it is now known that phosphatidylserine (PtdSer) binds the cleft region of both TIM-1 and TIM-4 and is functionally involved in recognition and uptake of apoptotic cells (Miyanishi et al. 2007; Santiago et al. 2007a). TIM-3, on the other hand, exhibits a different FG loop structure and thus the cleft present in TIM-3 is a bit different from that of the other TIMs (Santiago et al. 2007a). Nevertheless, Nakayama and colleagues recently reported that phosphatidylserine (PtdSer) may be another ligand for TIM-3 (Nakayama et al. 2009). Expression of TIM-3 in NKR cells, a rat kidney cell line that does not express TIM-3, resulted in gain of PtdSer binding and internalization of apoptotic cells. They further found that expression of TIM-3 on peritoneal exudate $Mac1^+$ cells (PEMs), monocytes, and splenic $CD8^+$ DCs was found to be involved in apoptotic cell uptake. Accordingly, blockade of the TIM-3 pathway by an anti-TIM-3 antibody resulted in increased anti-dsDNA autoantibody in the serum and reduced cross-presentation of apoptotic cell-associated antigens (Nakayama et al. 2009). Although PtdSer can bind to the TIM-3 cleft region, its binding affinity is much weaker than that of TIM-1 or TIM-4. Furthermore, allelic variants of TIM-3 display a differential capacity to bind to PtdSer and to phagocytose apoptotic cells, with the BALB/c allele demonstrating stronger affinity and phagocytic properties than the C.D2Es-Hba (HBA) allele (DeKruyff et al. 2010). Lastly, TIM-3 mediated phagocytic function is cell type dependent, as TIM-3 transfected T cell or B cell lines were able to form conjugates with but failed to engulf apoptotic cells (DeKruyff et al. 2010). Given that the bulk of the data showing TIM-3 binding to PtdSer come from experiments with transfected cell lines and that there is no obvious defect in apoptotic cell uptake in TIM-3 deficient mice (unpublished observations), raises the

question as to how physiologically relevant is the binding of TIM-3 to PtdSer. Further investigation will help clarify this issue.

3.3 Carbohydrate Ligands for TIM-3

Other lines of evidence suggest that TIM-3 may also bind carbohydrate moieties. Wilker and colleagues performed a glycan array screen and identified a set of glycan moieties exhibiting high affinity for the TIM-3 IgV domain (Wilker et al. 2007). Direct evidence was found using IdlD CHO cells, a UDP-galactose/UDP-*N*-acetylglucosamine 4-epimerase defective cell line. While IdlD CHO cells lack the ability to synthesize complete N-linked, O-linked, and lipid-linked glycoconjugates de novo, the cells can uptake galactose (Gal) and *N*-acetylgalactosamine (GalNAc) from culture medium and generate these glycoconjugates through salvage pathways. When these cells were stained with TIM-3 tetramer, it was found that TIM-3 retained binding to the cells grown in media with either 10% or 3% serum. However, the binding of TIM-3 tetramer to the cells grown in 1% serum was significantly reduced. Importantly, TIM-3 tetramer binding to IdlD CHO cells was restored when 1% serum was supplemented with Gal and GalNAc (Wilker et al. 2007). These results support that certain glycan moieties can act as TIM-3 ligands. The functional role of such interactions remains unknown.

4 Expression of TIM-3

Since its discovery on T cells, it is now appreciated that TIM-3 is expressed constitutively on other cell types and can be induced on some cells in pathological conditions. In the naïve or unimmunized state in mice, TIM-3 is expressed primarily on DCs at high levels (Anderson et al. 2007) and on a small percentage of effector/memory ($CD44^{hi}CD62L^{low}$) CD4 and CD8 T cells (Zhu et al. 2005).

During in vitro Th1 polarization, TIM-3 expression gradually increases until it reaches a stable, high expression level on terminally differentiated Th1 cells (Monney et al. 2002; Sanchez-Fueyo et al. 2003). When EAE is induced in mice, TIM-3 expression is found on $CD4^+$ and $CD8^+$ T cells that infiltrate the central nervous system (CNS) during the disease onset. These TIM-3^+ T cells decrease in the CNS as disease progresses, indicating an active role for TIM-3 in the initiation of EAE (Monney et al. 2002). Interestingly, recent studies of viral antigen specific $CD4^+$ and $CD8^+$ T cells from patients with chronic viral infection, showed that TIM-3 is expressed by a distinct population of "exhausted" T cells that fail to respond to viral antigens (Jones et al. 2008; Golden-Mason et al. 2009) (discussed below). The expression of TIM-3 on both functional and non-functional or "exhausted" T cells in two different disease states may indicate that TIM-3 integrates different extracellular signals present in these different immune milieus thereby delivering distinct signaling events to regulate T cell function.

In the naïve state, TIM-3 is not expressed on peripheral $CD11b^+$ cells but is expressed in $CD11b^+$ microglia that are resident in the CNS (Anderson et al. 2007) and can be induced in $CD11b^+$ peritoneal macrophages after treatment with thioglycollate (Nakayama et al. 2009). In the peritoneum, TIM-3 is additionally expressed on peritoneal mast cells (Nakae et al. 2007).

In humans, TIM-3 is also expressed on IFN-γ-secreting cells (Khademi et al. 2004) and is expressed constitutively at high levels on DCs and at lower levels on monocytes (Anderson et al. 2007). In cancer, TIM-3 expression has been noted on melanoma cells (Wiener et al. 2007) and lymphoma associated endothelium (Huang et al. 2010). While the differential roles and contributions of TIM-3 expression on T cells versus other innate and non-immune cells types remains to be ironed out, the wealth of data supporting an important role for TIM-3 in regulating the immune responses in both animal models and in human diseases, prompted us to begin examining the transcriptional regulation of TIM-3 expression in the two major cell types that express TIM-3, T cells and DCs.

4.1 Transcriptional Control of TIM-3 Expression

We have examined the role of Th1-associated transcription factors in regulating TIM-3 expression and found that TIM-3 expression is in part regulated by the Th1-specific transcription factor T-bet in both T cells and DCs (Anderson et al. 2010). We have found that T-bet directly binds to the TIM-3 promoter. In addition, we have found that the role of T-bet is not secondary to its induction of IFN-γ as T-bet can drive TIM-3 expression in the absence of IFN-γ and IFN-$\gamma R^{-/-}$ cells do not exhibit defects in TIM-3 expression. We have also examined a role for STAT-4 in regulating TIM-3 expression but found that STAT-$4^{-/-}$ cells exhibit only a modest, if any, defect in TIM-3 expression. Given that TIM-3 is stably expressed in Th1 cells only after several rounds of in vitro polarization but T-bet is upregulated early during Th1 differentiation (Szabo et al. 2000) suggests that other transcription factors may be involved in TIM-3 expression. Indeed, that T-$bet^{-/-}$ cells are not completely deficient in TIM-3 expression points to the involvement of other transcription factors in transactivating TIM-3 expression; however, these remain to be identified. In addition, it remains to be seen how TIM-3 expression is regulated in non-immune cell types.

5 TIM-3 in Disease

5.1 Genetic Basis for Role of TIM-3 in Disease

Genetic data suggest a role for TIM-3 expression and/or function in immune-mediated diseases in animal models and humans. The locus that encodes the TIM

gene family has shown linkage to disease susceptibility in several different autoimmune disease models such as EAE (locus EAE 6a), diabetes (Idd4), and SLE (lbw8) (Butterfield et al. 1998; Grattan et al. 2002; Kono et al. 1994). Similarly, a major locus for airway hyper-reactivity in mice and a syntenic locus on 5q33 in humans associated with asthma overlaps with the TIM gene locus (McIntire et al. 2001). Furthermore, comparisons of the TIM family genes in different strains of mice have revealed polymorphisms in TIM-1 and TIM-3, with Th1 prone strains (i.e., C57BL/6) and Th2 prone strains (i.e., Balb/c) expressing different TIM-1 and TIM-3 alleles, further supporting that genetic differences in the genes encoding TIM family proteins impact on disease.

In humans, several single nucleotide polymorphisms (SNPs) have been identified in the TIM-3 gene; one is found in the coding region (exon 3) and results in an amino acid change. An analysis of TIM-3 genotype and allelic frequencies among several hundred patients with rheumatoid arthritis and control subjects suggested that a SNP in the coding region of TIM-3 may be associated with susceptibility to rheumatoid arthritis (Chae et al. 2004b). This group has similarly identified SNPs in the promoter and coding regions of TIM-3 that may be associated with atopic disease (Chae et al. 2004a). Another group has also observed that a SNP in the coding region of TIM-3 is highly associated with atopic disease (Graves et al. 2005). While the functional and biological consequences of TIM-3 SNPs are presently unknown, current data in both humans and mice point to the TIM family genes, specifically TIM-1 and TIM-3, as important regulators of Th1/Th2 immunity, and possibly important determinants of susceptibility to both autoimmune and allergic diseases.

6 TIM-3 in Autoimmune Diseases

The importance of TIM-3 in regulating the immune response was first suggested by experiments that involved manipulation of the TIM-3 pathway in experimental disease models (Monney et al. 2002; Sabatos et al. 2003; Sanchez-Fueyo et al. 2003). Since then, several observations regarding TIM-3 expression and function in different disease states in humans further support the importance of TIM-3 in immune regulation. First, it has been noted that TIM-3 expression is dysregulated in patients with MS in that T cell clones isolated from the cerebrospinal fluid (CSF) clones from MS patients secrete significantly higher levels of IFN-γ than clones from the CSF of control subjects, yet the CSF clones from MS patients express lower levels of TIM-3 (Koguchi et al. 2006). Moreover, further Th1 polarization in vitro significantly augmented IFN-γ secretion but not TIM-3 expression among CSF clones from MS patients relative to those from control subjects. Tolerance induced by costimulatory blockade in vitro was less effective among CSF clones from MS patients that expressed lower amounts of TIM-3, consistent with previous reports that TIM-3 influences tolerance induction in a variety of murine models (Sabatos et al. 2003; Sanchez-Fueyo et al. 2003). Interestingly, T cells from MS

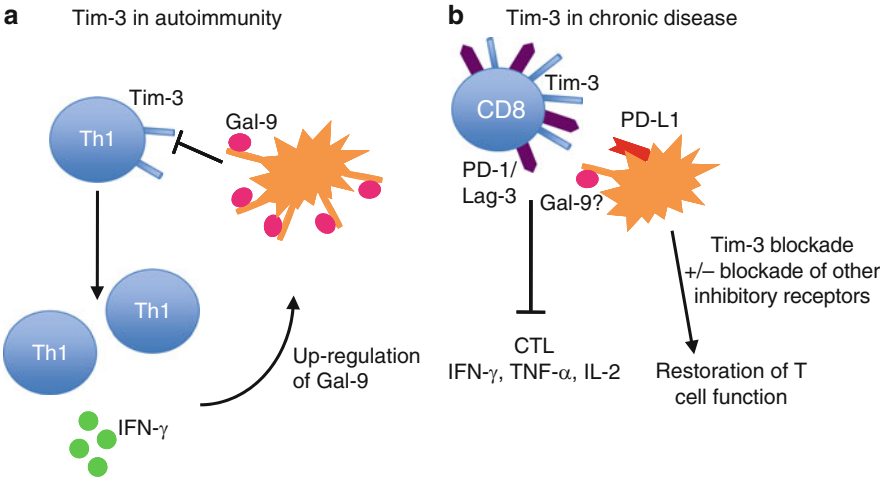

Fig. 2 Dysregulation of TIM-3 in different disease states. (**a**) In autoimmunity, IFN-γ secretion by $CD4^+$ Th1 cells up-regulates expression of the TIM-3 ligand, galectin-9. However, low T cell expression of TIM-3 allows cells to escape galectin-9-induced cell death. Consequently, autoreactive proinflammatory cells expand. Treatments that increase TIM-3 expression restore galectin-9-mediated negative regulation of IFN-γ-secreting $CD4^+$ Th1 T cells. The factor(s) responsible for the dysregulation of TIM-3 expression in $CD4^+$ Th1 cells in patients with autoimmune disease are not known. (**b**) In chronic conditions, TIM-3 expression on $CD8^+$ T cells either with or without co-expression of other inhibitory ligands, such as PD-1 or Lag-3, is associated with T cell exhaustion. Blockade of TIM-3/TIM-3–ligand interactions either alone or in combination with blockade of other inhibitory receptors restores effector function to T cells. Whether galectin-9 is involved in this function of TIM-3 and, if so, how these cells escape galectin-9-mediated cell death is not known

patients who have undergone treatment with glatiramer acetate or IFN-β for MS exhibit a restoration of TIM-3 expression (Yang et al. 2008). Furthermore, the ability of TIM-3 blockade to augment T cell proliferation and IFN-γ production is also restored in T cells from MS patients after treatment. Collectively, these data support that TIM-3 is an important negative regulator of T cell function and suggest that low-level expression of TIM-3 in T cells from MS patients allows pathogenic, autoreactive T cells to escape negative regulation by TIM-3 (Fig. 2).

6.1 TIM-3 in Chronic Viral Infection

A second disease state where TIM-3 appears to play a critical negative regulatory role in T cells is chronic viral infection. Here, it has been observed that virus-specific T cells develop an impaired or dysfunctional phenotype characterized by failure to proliferate and exert effector functions such as cytotoxicity and cytokine secretion in response to antigen stimulation. This phenomenon has been termed

T cell "exhaustion" and was first described in T cells in mice chronically infected with lymphocytic choriomeningitis virus (LCMV) (Zajac et al. 1998). Further studies identified that "exhausted" T cells exhibit sustained expression of the inhibitory molecule programmed cell death 1 (PD-1) and that blockade of PD-1 and PD-1 ligand (PD-L1) interactions can partially reverse T cell "exhaustion" and restore antigen specific T cell responses in LCMV infected mice (Barber et al. 2006). Importantly, T cell "exhaustion" also occurs during chronic viral infections in humans (Klenerman and Hill 2005) and $CD8^+$ T cells in humans chronically infected with HIV (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006), hepatitis B virus (HBV) (Boettler et al. 2006), and HCV (Urbani et al. 2006) express high levels of PD-1 and blockade of PD-1/PD-L interactions can partially restore T cell function in vitro.

Interestingly, a recent study in patients with HIV has shown that TIM-3 is also upregulated on "exhausted" $CD8^+$ T cells (Fig. 2) and that TIM-3 and PD-1 mark distinct populations of "exhausted" cells (Jones et al. 2008). T cells positive for both PD-1 and TIM-3 were rare. Similarly, another group has shown that TIM-3 is upregulated on "exhausted" T cells in patients with HCV (Golden-Mason et al. 2009). In this case, cells that co-express TIM-3 and PD-1 are the most abundant fraction among HCV-specific $CD8^+$ T cells. In both studies, blocking TIM-3 partially restored T cell proliferation and enhanced cytokine production. Given that blockade of the TIM-3 and PD-1 pathways has each been shown individually to partially restore function to "exhausted" T cells and the fact that these molecules are expressed on distinct and overlapping T cell populations in chronically infected patients raises the possibility that blockade of both pathways may prove most effective in restoring function to "exhausted" T cells. Indeed, combined blockade of PD-1 and TIM-3 during the priming/differentiation phase of Friend virus (FV) infection has been shown to restore $CD8^+$ T cell functionality and virus control to otherwise nonresponsive or "exhausted" T cells (Takamura et al. 2010).

While it is clear that TIM-3 plays an important role in T cell exhaustion, many questions remain. Whether galectin-9 is involved in this function of TIM-3 has not been addressed experimentally. If galectin-9 is involved, then why do these $TIM\text{-}3^+$ cells persist and escape galectin-9-induced cell death? Some answers may lie in the elucidation of the TIM-3 signaling cascade in exhausted T cells versus bona fide $TIM\text{-}3^+$ IFN-γ-secreting Tc1 cells. Another possibility is that integration of signals through other inhibitory receptors changes the response to TIM-3 ligation in exhausted T cells. Further breakdown of the distribution of inhibitory receptors (PD-1, TIM-3, Lag-3, and CTLA-4) on exhausted T cells and how these define different subpopulations of exhausted cells will advance our understanding of how exhaustion is induced, maintained, and most effectively reversed.

Several lines of evidence also suggest that during chronic viral infection and virus-associated malignancy, an elevated expression of galectin-9, may be related to suppression of adaptive immune responses. In chronic HCV infection, it was reported that an increased expression of galectin-9 in serum and in Kupffer cells during chronic infection is associated with expansion of $CD4^+CD25^+FoxP3^+C\text{-}D127^{lo}$ regulatory T cells, contraction of $CD4^+$ effector T cells, and apoptosis

of HCV-specific CTLs (Mengshol et al. 2010). In Epstein–Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC), one of the most common virus-associated human malignancies, it has been reported that NPC cells release galectin-9-containing exosomes that induce massive apoptosis in EBV-specific $CD4^+$ T cells, which can be inhibited by both anti-TIM-3 and anti-galectin-9 blocking antibodies (Klibi et al. 2009). These observations indicate that the galectin-9–TIM-3 pathway can be adopted to escape immune surveillance during both chronic viral infection and tumor progression. Thus, blockade of the galectin-9–TIM-3 pathway might help to reinvigorate anti-viral and anti-tumor immunity and thereby improving the clinical efficacy of current immunotherapies.

6.2 TIM-3 in Other Diseases

It has been shown in a mouse model of acute graft-versus-host disease (aGVHD) that TIM-3 expression is dramatically upregulated in both donor and host-derived hepatic $CD8^+$ T cells. Blockade of the TIM-3 signaling pathway with anti-TIM-3 antibodies results in significantly increased IFN-γ expression by splenic and hepatic $CD4^+$ and $CD8^+$ T cells and exacerbates aGVHD (Oikawa et al. 2006). This result demonstrates that TIM-3 is crucial in the regulation of hepatic $CD8^+$ T cell homeostasis and tolerance.

In a mouse model of coxsackievirus B3 (CVB3)-induced autoimmune heart disease, the TIM-3 signaling pathway has been shown to affect the adaptive immune system through effects on the innate immune system. Specifically, blockade of TIM-3 with anti-TIM-3 antibody in vivo exacerbates acute myocarditis due to reduced TIM-3 and CD80 expression on mast cells and macrophages and the amount of intracellular CTLA-4 in $CD4^+$ T cells (Frisancho-Kiss et al. 2006), resulting in increased macrophages/neutrophils and reduced Treg populations in the heart (Frisancho-Kiss et al. 2006).

7 Conclusions

Since the initial discovery of TIM-3, much progress has been made in characterizing TIM-3 ligands and TIM-3 function in immune responses in different disease states. It is now well appreciated that besides Th1 and Tc1 cells, TIM-3 is also expressed on DCs and macrophages, and even on non-immune cells during tumor development, suggesting a complex biological role for TIM-3. While the role of TIM-3 on non-lymphoid cells is still being investigated, accumulating evidence suggests that TIM-3 negatively regulates the functions of Th1 and Tc1 cells. Reduced TIM-3 expression correlates with increased IFN-γ production of CSF T cell clones in MS patients and escape from TIM-3-mediated regulation. In contrast, sustained expression of TIM-3 contributes to the exhausted phenotype of viral

antigen specific $CD8^+$ T cells in chronic HIV and HCV infection. Elucidating the mechanism(s) by which TIM-3 impacts on T cell function in different human autoimmune diseases and chronic viral infections will provide new therapeutic targets for treating these diseases.

References

Anderson AC, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, Chandwaskar R, Karman J, Su EW, Hirashima M et al (2007) Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science 318:1141–1143

Anderson AC, Lord GM, Dardalhon V, Lee DH, Sabatos-Peyton CA, Glimcher LH, Kuchroo VK (2010) T-bet, a Th1 transcription factor regulates the expression of Tim-3. Eur J Immunol 40:859–866

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

Boettler T, Panther E, Bengsch B, Nazarova N, Spangenberg HC, Blum HE, Thimme R (2006) Expression of the interleukin-7 receptor alpha chain (CD127) on virus-specific $CD8^+$ T cells identifies functionally and phenotypically defined memory T cells during acute resolving hepatitis B virus infection. J Virol 80:3532–3540

Bork P, Holm L, Sander C (1994) The immunoglobulin fold. Structural classification, sequence patterns and common core. J Mol Biol 242:309–320

Butterfield RJ, Sudweeks JD, Blankenhorn EP, Korngold R, Marini JC, Todd JA, Roper RJ, Teuscher C (1998) New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J Immunol 161:1860–1867

Cao E, Zang X, Ramagopal UA, Mukhopadhaya A, Fedorov A, Fedorov E, Zencheck WD, Lary JW, Cole JL, Deng H et al (2007) T cell immunoglobulin mucin-3 crystal structure reveals a galectin-9-independent ligand-binding surface. Immunity 26:311–321

Chae SC, Park YR, Lee YC, Lee JH, Chung HT (2004a) The association of TIM-3 gene polymorphism with atopic disease in Korean population. Hum Immunol 65:1427–1431

Chae SC, Park YR, Shim SC, Yoon KS, Chung HT (2004b) The polymorphisms of Th1 cell surface gene Tim-3 are associated in a Korean population with rheumatoid arthritis. Immunol Lett 95:91–95

Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, Blumenschein W, Churakovsa T, Low J, Presta L et al (2006) Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J Clin Invest 116:1317–1326

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

DeKruyff RH, Bu X, Ballesteros A, Santiago C, Chim YL, Lee HH, Karisola P, Pichavant M, Kaplan GG, Umetsu DT et al (2010) T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells. J Immunol 184:1918–1930

Frisancho-Kiss S, Nyland JF, Davis SE, Barrett MA, Gatewood SJ, Njoku DB, Cihakova D, Silbergeld EK, Rose NR, Fairweather D (2006) Cutting edge: T cell Ig mucin-3 reduces inflammatory heart disease by increasing CTLA-4 during innate immunity. J Immunol 176:6411–6415

Golden-Mason L, Palmer BE, Kassam N, Townshend-Bulson L, Livingston S, McMahon BJ, Castelblanco N, Kuchroo V, Gretch DR, Rosen HR (2009) Negative immune regulator Tim-3

is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional $CD4^+$ and $CD8^+$ T cells. J Virol 83:9122–9130

Grattan M, Mi QS, Meagher C, Delovitch TL (2002) Congenic mapping of the diabetogenic locus Idd4 to a 5.2-cM region of chromosome 11 in NOD mice: identification of two potential candidate subloci. Diabetes 51:215–223

Graves PE, Siroux V, Guerra S, Klimecki WT, Martinez FD (2005) Association of atopy and eczema with polymorphisms in T-cell immunoglobulin domain and mucin domain-IL-2-inducible T-cell kinase gene cluster in chromosome 5 q 33. J Allergy Clin Immunol 116:650–656

Hastings WD, Anderson DE, Kassam N, Koguchi K, Greenfield EA, Kent SC, Zheng XX, Strom TB, Hafler DA, Kuchroo VK (2009) TIM-3 is expressed on activated human $CD4^+$ T cells and regulates Th1 and Th17 cytokines. Eur J Immunol 39:2492–2501

Huang X, Bai X, Cao Y, Wu J, Huang M, Tang D, Tao S, Zhu T, Liu Y, Yang Y et al (2010) Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. J Exp Med 207:505–520

Imaizumi T, Kumagai M, Sasaki N, Kurotaki H, Mori F, Seki M, Nishi N, Fujimoto K, Tanji K, Shibata T et al (2002) Interferon-gamma stimulates the expression of galectin-9 in cultured human endothelial cells. J Leukoc Biol 72:486–491

Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, Wong JC, Satkunarajah M, Schweneker M, Chapman JM et al (2008) Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. J Exp Med 205:2763–2779

Khademi M, Illes Z, Gielen AW, Marta M, Takazawa N, Baecher-Allan C, Brundin L, Hannerz J, Martin C, Harris RA et al (2004) T Cell Ig- and mucin-domain-containing molecule-3 (TIM-3) and TIM-1 molecules are differentially expressed on human Th1 and Th2 cells and in cerebrospinal fluid-derived mononuclear cells in multiple sclerosis. J Immunol 172:7169–7176

Klenerman P, Hill A (2005) T cells and viral persistence: lessons from diverse infections. Nat Immunol 6:873–879

Klibi J, Niki T, Riedel A, Pioche-Durieu C, Souquere S, Rubinstein E, Le Moulec S, Guigay J, Hirashima M, Guemira F et al (2009) Blood diffusion and Th1-suppressive effects of galectin-9-containing exosomes released by Epstein-Barr virus-infected nasopharyngeal carcinoma cells. Blood 113:1957–1966

Koguchi K, Anderson DE, Yang L, O'Connor KC, Kuchroo VK, Hafler DA (2006) Dysregulated T cell expression of TIM3 in multiple sclerosis. J Exp Med 203:1413–1418

Kono DH, Burlingame RW, Owens DG, Kuramochi A, Balderas RS, Balomenos D, Theofilopoulos AN (1994) Lupus susceptibility loci in New Zealand mice. Proc Natl Acad Sci USA 91:10168–10172

McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT, DeKruyff RH (2001) Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nat Immunol 2:1109–1116

Mengshol JA, Golden-Mason L, Arikawa T, Smith M, Niki T, McWilliams R, Randall JA, McMahan R, Zimmerman MA, Rangachari M, Dobrinskikh E, Busson P, Polyak SJ, Hirashima M, Rosen HR (2010) A crucial role for Kupffer cell-derived galectin-9 in regulation of T cell immunity in hepatitis C infection. PLoS One 5(3):e9504

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura, Nagata S (2007) Identification of Tim4 as a phosphatidylserine receptor. Nature 450:435–439

Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA et al (2002) Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 415:536–541

Nagahara K, Arikawa T, Oomizu S, Kontani K, Nobumoto A, Tateno H, Watanabe K, Niki T, Katoh S, Miyake M et al (2008) Galectin-9 increases Tim-3+ dendritic cells and $CD8^+$ T cells and enhances antitumor immunity via galectin-9-Tim-3 interactions. J Immunol 181:7660–7669

Nakae S, Iikura M, Suto H, Akiba H, Umetsu DT, Dekruyff RH, Saito H, Galli SJ (2007) TIM-1 and TIM-3 enhancement of Th2 cytokine production by mast cells. Blood 110:2565–2568

Nakayama M, Akiba H, Takeda K, Kojima Y, Hashiguchi M, Azuma M, Yagita H, Okumura K (2009) Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. Blood 113:3821–3830

Niwa H, Satoh T, Matsushima Y, Hosoya K, Saeki K, Niki T, Hirashima M, Yokozeki H (2009) Stable form of galectin-9, a Tim-3 ligand, inhibits contact hypersensitivity and psoriatic reactions: a potent therapeutic tool for Th1- and/or Th17-mediated skin inflammation. Clin Immunol 132:184–194

Oikawa T, Kamimura Y, Akiba H, Yagita H, Okumura K, Takahashi H, Zeniya M, Tajiri H, Azuma M (2006) Preferential involvement of Tim-3 in the regulation of hepatic $CD8^+$ T cells in murine acute graft-versus-host disease. J Immunol 177:4281–4287

Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, Precopio ML, Schacker T, Roederer M, Douek DC et al (2006) PD-1 is a regulator of virus-specific $CD8^+$ T cell survival in HIV infection. J Exp Med 203:2281–2292

Rabinovich GA, Toscano MA (2009) Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol 9:338–352

Sabatos CA, Chakravarti S, Cha E, Schubart A, Sanchez-Fueyo A, Zheng XX, Coyle AJ, Strom TB, Freeman GJ, Kuchroo VK (2003) Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. Nat Immunol 4:1102–1110

Sanchez-Fueyo A, Tian J, Picarella D, Domenig C, Zheng XX, Sabatos CA, Manlongat N, Bender O, Kamradt T, Kuchroo VK et al (2003) Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. Nat Immunol 4:1093–1101

Santiago C, Ballesteros A, Martinez-Munoz L, Mellado M, Kaplan GG, Freeman GJ, Casasnovas JM (2007a) Structures of T cell immunoglobulin mucin protein 4 show a metal-Ion-dependent ligand binding site where phosphatidylserine binds. Immunity 27:941–951

Santiago C, Ballesteros A, Tami C, Martinez-Munoz L, Kaplan GG, Casasnovas JM (2007b) Structures of T cell immunoglobulin mucin receptors 1 and 2 reveal mechanisms for regulation of immune responses by the TIM receptor family. Immunity 26:299–310

Sehrawat S, Suryawanshi A, Hirashima M, Rouse BT (2009) Role of Tim-3/galectin-9 inhibitory interaction in viral-induced immunopathology: shifting the balance toward regulators. J Immunol 182:3191–3201

Seki M, Oomizu S, Sakata KM, Sakata A, Arikawa T, Watanabe K, Ito K, Takeshita K, Niki T, Saita N et al (2008) Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin Immunol 127:78–88

Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100:655–669

Takamura S, Tsuji-Kawahara S, Yagita H, Akiba H, Sakamoto M, Chikaishi T, Kato M, Miyazawa M (2010) Premature terminal exhaustion of Friend virus-specific effector $CD8^+$ T cells by rapid induction of multiple inhibitory receptors. J Immunol 184:4696–4707

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

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

van de Weyer PS, Muehlfeit M, Klose C, Bonventre JV, Walz G, Kuehn EW (2006) A highly conserved tyrosine of Tim-3 is phosphorylated upon stimulation by its ligand galectin-9. Biochem Biophys Res Commun 351:571–576

Wang F, He W, Zhou H, Yuan J, Wu K, Xu L, Chen ZK (2007) The Tim-3 ligand galectin-9 negatively regulates CD8+ alloreactive T cell and prolongs survival of skin graft. Cell Immunol 250:68–74

Wiener Z, Kohalmi B, Pocza P, Jeager J, Tolgyesi G, Toth S, Gorbe E, Papp Z, Falus A (2007) TIM-3 is expressed in melanoma cells and is upregulated in TGF-beta stimulated mast cells. J Invest Dermatol 127:906–914

Wilker PR, Sedy JR, Grigura V, Murphy TL, Murphy KM (2007) Evidence for carbohydrate recognition and homotypic and heterotypic binding by the TIM family. Int Immunol 19:763–773

Yang L, Anderson DE, Kuchroo J, Hafler DA (2008) Lack of TIM-3 immunoregulation in multiple sclerosis. J Immunol 180:4409–4414

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

Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK (2005) The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol 6:1245–1252

Role of PD-1 in Regulating T-Cell Immunity

Hyun-Tak Jin, Rafi Ahmed, and Taku Okazaki

Contents

Abstract Programmed cell death-1 (PD-1) is a member of the CD28 superfamily that delivers negative signals upon interaction with its two ligands, PD-L1 or PD-L2. PD-1 and its ligands are broadly expressed and exert a wider range of immunoregulatory roles in T cells activation and tolerance compared with other CD28 family members. Subsequent studies show that PD-1–PD-L interaction regulates the induction and maintenance of peripheral tolerance and protect tissues from autoimmune attack. PD-1 and its ligands are also involved in attenuating infectious immunity and tumor immunity, and facilitating chronic infection and tumor progression. The biological significance of PD-1 and its

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ligand suggests the therapeutic potential of manipulation of PD-1 pathway against various human diseases. In this review, we summarize our current understanding of PD-1 and its ligands ranging from discovery to clinical significance.

1 Identification of PD-1 and Its Ligands

In 1992, programmed cell death-1 (PD-1) was identified as a molecule whose expression was strongly induced upon apoptotic stimuli (Ishida et al. 1992). Based on the observation that LyD9 (hematopoietic progenitor cell line) and 2B4.11 cell lines (T-cell hybridoma) died of apoptosis upon interleukin-3 deprivation and phorbol 12-myristate 13-acetate (PMA) and ionomycin treatment, respectively, and that apoptosis of both cell lines required *de novo* RNA and protein synthesis, Honjo and colleagues performed subtractive-hybridization to identify the gene(s) that plays critical roles in apoptosis. A complementary DNA (cDNA) library of dying LyD9 cells subtracted with messenger RNA (mRNA) of resting LyD9 cells was screened by a labeled cDNA library of dying 2B4.11 cells subtracted with mRNA of resting LyD9 cells. Four clones were isolated and all of them encoded PD-1 cDNA. Deduced amino acid sequence predicted that PD-1 is a type I transmembrane protein with a single IgV domain in the extracellular region. However, overexpression of PD-1 cDNA in these cell lines failed to induce apoptosis (Agata et al. 1996) and the function of PD-1 was intangible for many years. In 1999, Honjo and colleagues found that PD-1 negatively regulates immune responses based on the observation that PD-1-deficient mice spontaneously developed lupus-like arthritis and glomerulonephritis (Nishimura et al. 1999).

The identification of PD-1 ligands rests on several chance events. Honjo and colleagues collaborated with Genetic Institute to identify the ligand of PD-1 using the Biacore system. At that time, Freeman in Harvard University identified a B7-like molecule (clone 129) by database search and collaborated with Genetic Institute independently. The group in Genetic Institute accidentally found that these molecules interacted with each other. T cells from PD-1 sufficient but not PD-1-deficient mice showed lower proliferative response against anti-CD3 antibody stimulation in the presence of 129-Ig chimeric protein. Based on these physical and functional experiments, clone 129 was confirmed to be the ligand of PD-1 and was named PD-L1 (*Pdcd1lg1*) for programmed cell death 1 ligand 1 (Freeman et al. 2000). Later, PD-L1 was endowed CD number 274. The collaboration further identified another ligand, PD-L2 (*Pdcd1lg2,* CD273) (Latchman et al. 2001). The identification of PD-L1 added PD-1 to the list of CD28 family (Fig. 1). At the same time, the other groups reported B7-H1 and B7-DC, which were identical to PD-L1 and PD-L2, respectively, costimulated T cells, the mechanisms of which still remain unknown (Dong et al. 1999; Tseng et al. 2001).

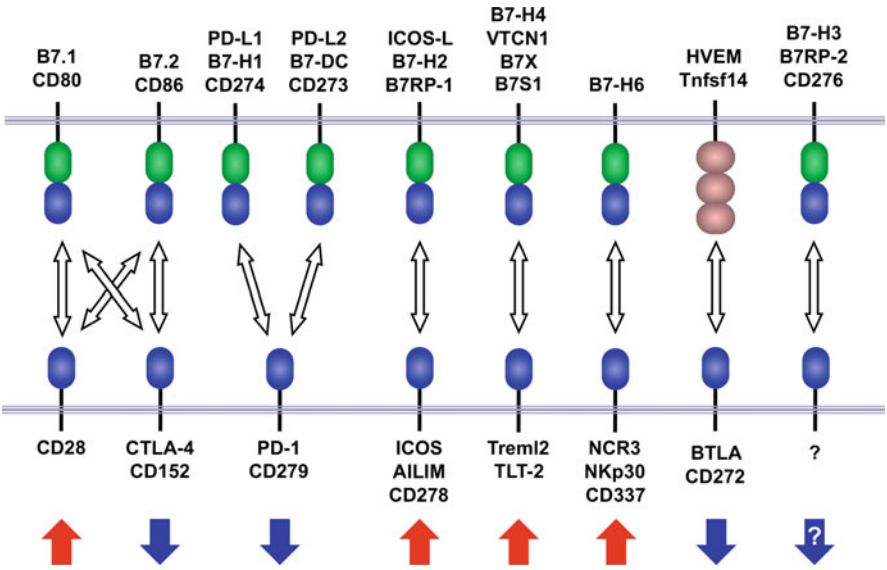

Fig. 1 Summary of CD28 family members and their ligands. *Upward* and *downward arrows* indicate stimulatory and inhibitory signals, respectively

2 Structure of PD-1 and Its Ligands

PD-1 is a 50–55 kDa type I transmembrane glycoprotein composed of an IgV domain sharing 21–33% sequence identity with CTLA-4, CD28, and inducible costimulatory molecule. Unlike CTLA-4 that forms homodimer, PD-1 lacks the membrane proximal cysteine residue required for homodimerization of other members of the CD28 family and is supposed to exist as monomer on the cell surface (Zhang et al. 2004). The PD-1 cytoplasmic region has two tyrosine residues, the membrane–proximal one constitutes an immunoreceptor tyrosine-based inhibitory motif (ITIM) and the other an immunoreceptor tyrosine-based switch motif (ITSM), which is essential for the inhibitory function of PD-1 (Long 1999; Sidorenko and Clark 2003). The ligands of PD-1 (PD-L1 and PD-L2, PD-Ls) are type I transmembrane glycoproteins composed of IgC and IgV domains. The amino acid identity between PD-L1 and PD-L2 is about 40%, while the amino acid identity between PD-Ls and B7s is about 20%. The amino acid identities between human and murine orthologues of PD-Ls are about 70%. B7-1 is also reported to associate with PD-L1 and transduce inhibitory signal (Butte et al. 2007). However, the precise mechanism how PD-L1 transduces inhibitory signals is currently unknown.

Recently, the crystal structures of PD-1 and PD-Ls were clarified (Fig. 2) (Zhang et al. 2004; Lazar-Molnar et al. 2008; Lin et al. 2008). In contrast to CTLA-4 that

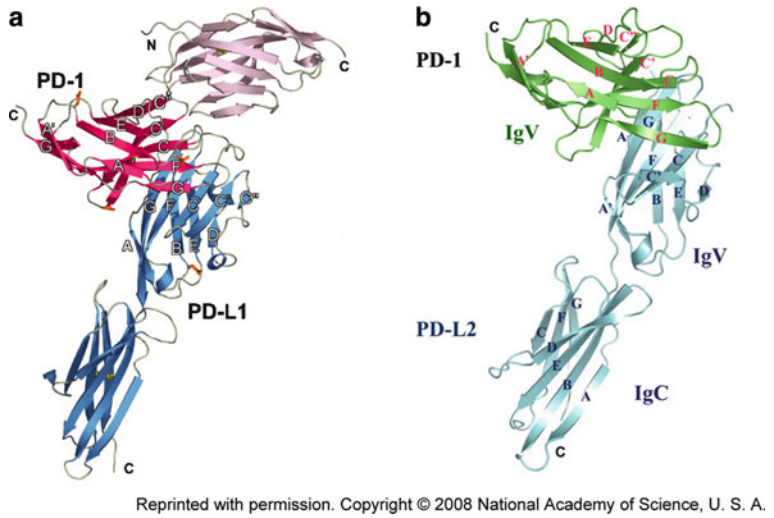

Fig. 2 Crystal structure of PD-1/PD-L1 and PD-1/PD-L2 complexes. (**a**) The structure of PD-1/PD-L1 complex; the loop at the ends of the PD-1 domain and the first domain of PD-L1 are located on the same side of the complex. The strands of the two β-sheets of PD-1 are labeled ABED and A′GFCC′C″. The strands of the two β-sheets of PD-L1 V domain are labeled AGFCC′C″ and BED. (**b**) The structure of PD-1/PD-L2 complex; the face-to-face interaction of PD-1 and PD-L2 makes the large contact area and the PD-1/PD-L2 complex more compact. The strands of PD-1 and PD-L2 are labeled in *red* and *blue*, respectively. Figures are taken from the original paper (Lazar-Molnar et al. 2008; Lin et al. 2008) with permission

uses a hydrophobic sequence, MYPPY, in the loop connecting F and G β-strands (FG loop, corresponding to CDR3 region of antigen receptors) of its IgV domain to bind B7-1 and B7-2, PD-1 uses its front β-face (AGFCC′ β-strands) to bind to the β-face of PD-L1 (AGFCC′) or PD-L2 (AGFC). This face-to-face interaction of PD-1 and PD-Ls makes the contact area (1,870 $Å^2$) larger than that of CTLA-4 and B7s (~1,200 $Å^2$) and makes the PD-1/PD-Ls complex more compact (76 Å and 100 Å for PD-1/PD-Ls and B7-1/CTLA-4, respectively). The total length of receptors and ligands should be compatible with the dimension of pMHC/TCR complex (~140 Å) in the immunological synapse. The longer linker segment connecting PD-1 IgV domain and transmembrane region (20 a.a. and 6 a.a. for PD-1 and CTLA-4, respectively) may help adjust the total size of the PD-1/PD-Ls complex allowing its appropriate co-localization with pMHC/TCR complex. The structure of PD-1 and PD-Ls showed high similarity to those of T-cell receptor (TCR), antibody, and CD8 dimer. Especially, CDR-like loops (BC, C′C″, and FG loops for CDR1, 2, and 3, respectively) of PD-1/PD-Ls are in positions similar to those of the antigen-binding loops of TCR and antibody. Because TCRs and antibodies bind antigens and CD8 dimers bind major histocompatibility complex (MHC) class I using these loops, it is possible that these loops of PD-1/PD-Ls complex may bind another molecule.

3 Regulation of PD-1 Expression

PD-1 can be expressed on CD4 and CD8 T cells, B cells, monocytes, natural killer (NK) cells, and dendritic cells (DCs) (Agata et al. 1996; Yamazaki et al. 2002; Keir et al. 2008). PD-1 is not expressed on resting T cells but is inducibly expressed within 24 h after stimulation (Chemnitz et al. 2004). PD-1 expression can also be induced on antigen-presenting cells (APCs) on myeloid $CD11c^+$ DCs and monocytes (Petrovas et al. 2006). Recently, it was found that PD-1 expression was elevated on NKT cells in response to CD1d-restricted lipid antigen (Moll et al. 2009). The two known ligands for PD-1, PD-L1 (B7-H1; CD274), and PD-L2 (B7-DC; CD273) have differential expression. PD-L1 is constitutively expressed on T and B cells, DCs, macrophages, mesenchymal stem cells, and bone marrow-derived mast cells (Yamazaki et al. 2002). In addition, PD-L1 is expressed on a wide variety of nonhematopoietic cells including lung, vascular endothelium, liver nonparenchymal cells, mesenchymal stem cells, pancreatic islets, and keratinocytes (Keir et al. 2008). In contrast, the expression of PD-L2 is restricted to activated DCs, macrophages, bone marrow-derived mast cells, and more than 50% of peritoneal B1 cells (Zhong et al. 2007). The comparative studies with PD-L1- or PD-L2-deficient mice and with blocking antibody against PD-L1 and PD-L2 demonstrate overlapping inhibitory functions on APCs and different features on tissues for these two ligands (Kanai et al. 2003; Matsumoto et al. 2004; Keir et al. 2006; Habicht et al. 2007).

The broader expressions of PD-1 and its ligands suggest that PD-1 regulates a wider spectrum of immune response compared with other CD28 family members. The spontaneous development of autoimmune diseases by PD-1-deficient mice implies that PD-1 is involved in the establishment and maintenance of immunological tolerance (Nishimura et al. 1999, 2001). In the thymus, PD-L1 is expressed broadly in the cortex, whereas PD-L2 expression is restricted to medullary stromal cells (Brown et al. 2003; Liang et al. 2003). PD-1 is expressed on $CD4^-CD8^-$ double-negative thymocytes and is required for the normal selection of thymocytes (Nishimura et al. 2000). PD-1 expression is upregulated after TCR ligation on $CD4^+CD8^+$ double-positive thymocytes, and PD-1 can participate in selection of the αβ TCR repertoire by controlling TCR signaling thresholds. PD-1–PD-L1 interactions modulate positive selection (Keir et al. 2005), and PD-1 also regulates negative selection (Blank et al. 2003). Collectively, these findings implicate that PD-1 and its ligand play a crucial role in central as well as peripheral tolerance. PD-L1 and PD-L2 is also expressed on placental syncytiotrophoblasts and vascular endothelial cells, respectively (Guleria et al. 2005). PD-L1 is differentially expressed across gestation and functions in the placenta to induce fetal–maternal tolerance (Guleria et al. 2005; Holets et al. 2006). PD-L1 is expressed constitutively in the cornea, and PD-1–PD-L1 interaction protects the eye from activated T cells (Hori et al. 2006; Meng et al. 2006; Watson et al. 2006; Sugita et al. 2009). So, PD-1-PD-L pathway may protect immune-privileged sites, such as the placenta and the eye, from immune responses. Given that PD-L is found on various tumor cells and PD-1 expression is upregulated and sustained on virus-specific T cells during

chronic viral infection, PD-1-PD-L pathway may play important roles in tumor immunity and infectious immunity, which are addressed in depth in the following section of this review.

PD-1 expression is induced by TCR- or B-cell receptor (BCR)-mediated signaling and is augmented by stimulation with tumor necrosis factor (TNF) (Nakae et al. 2006). Particularly, the expression of PD-1 on virus-specific CD8 T cells is dependent upon continued epitope recognition during chronic infections (Blattman et al. 2009). However, molecular mechanism for the regulation of PD-1 expression relatively remains unclear. Recent study demonstrates that NFATc1 is a critical transcription factor in promoting the induction of PD-1 expression following T cells activation (Oestreich et al. 2008), and interferon (IFN)-sensitive responsive element and STAT1/2 are primarily responsible for the regulation of constitutive and IFN-α-mediated PD-1 expression on macrophages (Cho et al. 2008). PD-L1 is upregulated by IFN-α, IFN-β, and IFN-γ (Eppihimer et al. 2002; Schreiner et al. 2004). IL-4 and granulocyte macrophage colony-stimulating factor stimulate the expression of PD-L2 on DCs, and IL-10 can induce the expression of PD-L1 on monocytes (Selenko-Gebauer et al. 2003). Analyses of PD-L1 promoter show that PD-L1 expression is dependent on two IFN regulatory factor-1 binding sites (Lee et al. 2006b). In addition, MyD88, TRAF6, MEK, and JAK2 have been implicated in signaling pathway for PD-L1 expression (Lee et al. 2006b; Liu et al. 2007; Parsa et al. 2007).

4 PD-1 Signaling: Molecular Mechanisms of Inhibition

ITSM (TxYxxL) is essential for the inhibitory function of PD-1. The inhibitory mechanism of PD-1 was initially analyzed in B cell line, IIA1.6 by using a chimeric molecule of PD-1 cytoplasmic region and $Fc_{\gamma}RIIB$ extracellular region (FcPD) (Okazaki et al. 2001). In IIA1.6 cells, crosslinking of BCRs induced strong Ca^{2+} mobilization, which was almost completely suppressed by co-crosslinking of FcPD and BCR. Tyrosine residues in both ITIM and ITSM of PD-1 were phosphorylated by Lyn upon BCR crosslinking and the phosphorylated tyrosine residue in ITSM but not ITIM recruited SHP-2 (SH2-domain containing tyrosine phosphatase 2) through its SH2 domain (Fig. 3a). Then SHP-2 dephosphorylated BCR-proximal signaling molecules including Igα/β and Syk, which attenuated the activation of downstream molecules including $PLC_{\gamma}2$, PI3K, vav, and ERK1/2. Later, two groups reported that PD-1 inhibited TCR signaling by similar mechanisms (Fig. 3b) (Chemnitz et al. 2004; Sheppard et al. 2004; Parry et al. 2005). Interestingly, one of them reported that phosphorylated ITSM recruited SHP-1 in addition to SHP-2 in T cells, while the other group reported that SHP-1 associated with phosphorylated ITSM only in the artificial system using synthetic peptide. SHP-1 may also bind to phosphorylated ITSM of PD-1 but its contribution to the inhibitory function of PD-1 can be much less compared to that of SHP-2. ITSM has been first defined in CD150 (SLAM) for its dual function (Sidorenko and Clark 2003). CD150 preferentially recruits either SHIP or SHP-2 in the presence or absence of

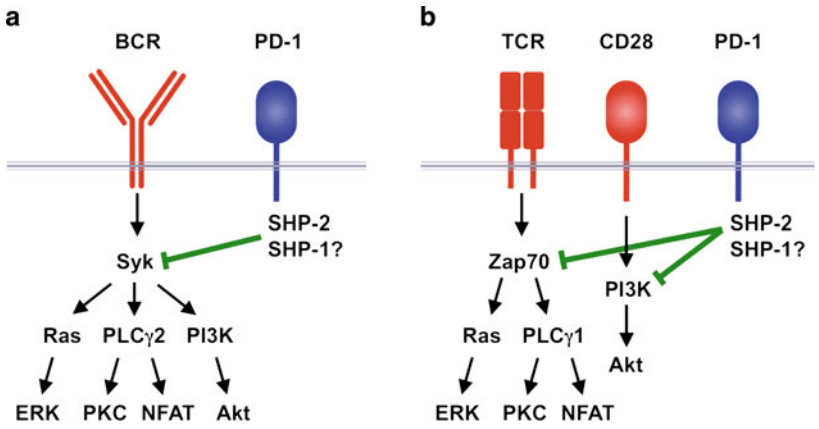

Fig. 3 Signaling of programmed cell death-1 (PD-1) pathway. (**a**) Upon B-cell receptor (BCR) crosslinking, the phosphorylated tyrosine residue in immunoreceptor tyrosine-based switch motif (ITSM) recruits SHP-2. And then, SHP-2 dephosphorylates BCR-proximal signaling molecules including Syk, which attenuates the activation of downstream molecules, such as PLCγ2, PI3K, and ERK. (**b**) PD-1 engagement on T cell surface also leads to phosphorylation of PD-1 cytoplasmic tyrosines and increases SHP-2 association with the ITSM of PD-1. Recruitment of SHPP-2 dephosphorylates signaling through the PI3K or Zap70 pathways

SAP (SH2D1A), a small SH2-domain-containing adaptor protein, respectively. Because ITSM of PD-1 has been reported to be unable to bind SAP, it is currently unknown whether ITSM of PD-1 can really "switch" to transduce positive signal or not.

PD-1 signal has been shown to play critical roles in the induction of anergy and the development of induced regulatory T cells (iTregs). Recently, Francisco et al. reported that engagement of PD-1 preferentially induced the development of iTregs (Francisco et al. 2009). In addition, PD-1 signal maintained FoxP3 expression and enhanced the efficiency of their suppressive function. During the induction of iTregs by PD-1, they found the downregulation of phopho-Akt, mTOR, S6, and ERK2 and concomitant upregulation of PTEN, suggesting that PD-1 signal promoted the development of iTregs by antagonizing the Akt-mTOR signaling pathway. The mechanism of anergy induction is also becoming clearer. Chikuma et al. reported that PD-1-sufficient but not PD-1-deficient 2C CD8 T cells could be anergyzed by single injection of an antigenic peptide *in vivo* (Chikuma et al. 2009). Interestingly, PD-1-deficient 2C T cells preferentially produced IL-2 upon anergy induction. The blockade of IL-2 during the anergy induction phase prevented the anergy resistance of PD-1-deficient 2C T cells and the IL-2 complementation resulted in anergy resistance in PD-1-sufficient 2C T cells. Therefore, PD-1 may induce anergy by negatively regulating autonomous production of IL-2 by CD8 T cells. Bishop et al. also found that PD-1 induced anergy on CD4 T cells by regulating IL-2 production using an *in vitro* model of A.E7 T cells (Bishop et al.

2009). Further studies are required to understand the precise molecular mechanisms how PD-1 regulate the production of IL-2.

5 Biological Significance of PD-1

As mentioned above, PD-1 and its ligands are broadly expressed and exert a vital and diverse range of immunoregulatory roles in T cells activation and tolerance. In this section, to address the biological significance of PD-1 pathway, we summarize our current understanding of the roles of PD-1 and its ligands in disease model including autoimmunity, chronic viral infection, and tumor.

5.1 PD-1 in Autoimmunity

Involvement of PD-1 in autoimmunity was first demonstrated by the autoimmune phenotype of PD-1-deficient mice (Nishimura et al. 1999, 2001; Okazaki et al. 2003). PD-1 deficiency results in the development of a spontaneous, late-onset lupus-like disease with deposition of IgG_3 in the glomeruli and a dilated cardiomyopathy owing to the production of an autoantibody against cardiac troponin-I. Interestingly, different target organs were affected by the autoimmune response when the genetic background of the PD-1-deficient mice was replaced by backcrossing. For example, PD-1-deficient mice developed lupus-like glomerulonephritis and arthritis, dilated cardiomyopathy and gastritis, subacute type I diabetes, and lethal myocarditis on the C57BL/6, BALB/c, nonobese diabetic (NOD), and MRL backgrounds, respectively (Nishimura et al. 1996, 2001; Wang et al. 2005, 2010). Studies of mouse models of autoimmunity further emphasize important immunoregulatory functions for PD-1 and its ligands. In the NOD mouse model of autoimmune diabetes, PD-L1 is upregulated in the pancreas on islet cells (Liang et al. 2003). Administration of anti-PD-1 or anti-PD-L1 to prediabetic NOD mice leads to rapid and exacerbated diabetes, which is associated with accelerated insulitis and proinflammatory cytokine production by T cells (Ansari et al. 2003). Compared to the blockade of CTLA-4, which exaggerates diabetes only in the neonates, PD-1/PD-L1 blockade exaggerates diabetes both in neonates and in older mice, indicating that PD-1–PD-L1 interactions regulate both the initiation and the progression of autoimmune diabetes in NOD mice. In the experimental autoimmune encephalomyelitis (EAE) model, PD-1 and its ligand also suppress EAE. The administration of anti-PD-1 during the induction of EAE accelerates the onset and increases the severity of EAE with increased frequency of IFN-γ-producing myelin oligodendrocyte glycoprotein (MOG)-reactive T cells and more MOG-specific antibodies in serum (Salama et al. 2003). Interestingly, the blockade of PD-L1 but not PD-L2 exacerbates EAE in BALB/c and SJL/J mice, whereas only PD-L2 blockade

markedly worsen EAE in C57BL/6 mice (Salama et al. 2003; Zhu et al. 2006). This strain-specific effect of antibody-mediated blockade of PD-1 ligand cannot be explained by expression of PD-L1 or PD-L2 because their expressions vary little in lymphoid APCs and spinal cord tissues among different strains (Zhu et al. 2006). However, adoptive transfer studies demonstrate the critical role of PD-L1 on T cells and host tissues in restraining myelin-reactive pathogenic effector T cells in EAE (Latchman et al. 2004).

The involvement of PD-1 in human autoimmune diseases has also become evident. Prokunina et al. reported that the allele A of a single nucleotide polymorphisms (SNPs) named PD1.3 (PD1.3A) in intron 4 of PD-1 gene was associated with the development of systemic lupus erythematosus (SLE) in Europeans (relative risk = 2.6) and Mexicans (relative risk = 3.5) but not African Americans (Prokunina et al. 2002). To date, the PD1.3 and several other SNPs on PD-1 gene have been reported to link with the development of various autoimmune diseases including type I diabetes, progressive multiple sclerosis (MS), rheumatoid arthritis, Graves disease, and ankylosing spondylitis (Nielsen et al. 2003; Kroner et al. 2005; Lee et al. 2006a; Okazaki and Honjo 2007). The PD1.3 locates on the binding site for the runt-related transcription factor 1 (RUNX1) and PD1.3A interferes the binding of RUNX1 resulting in the impaired induction of PD-1 (Bertsias et al. 2009). This polymorphism may alter PD-1 mRNA stability or expression level and is associated with reduced PD-1-mediated inhibition of IFN-γ production in MS patients (Kroner et al. 2005). Studies of human with autoimmune diseases also suggest important regulatory function of PD-1 and its ligands. Patients with MS treated with IFN-β, the principle immune-modulatory agent for the treatment of MS, *in vivo* for 6 months have eightfold more PD-L1 mRNA transcript than before treatment, suggesting that part of the anti-inflammatory effect of IFN-β treatment is due to PD-L1 expression (Schreiner et al. 2004). Autoantibodies to PD-L1 have been found in patients with rheumatoid arthritis and correlate with the progression of disease, indicating that autoantibodies can block the inhibitory function of the PD-1-PD-L pathway and thus contribute to the development of autoimmune disease (Dong et al. 2003).

Based on the important role of PD-1-PD-L pathway in autoimmunity, this pathway has become a new therapeutic target for ameliorating autoimmune disease by increasing the expression of PD-L or triggering PD-1. Even though these approaches are just beginning in animal models, the results appear promising. Hirata et al. genetically modified mouse embryonic stem (ES) cells to express surface PD-L1 and MOG in MHC class II (Hirata et al. 2005). They next differentiated the cells to DCs and transferred the cells into mice with EAE. These genetically modified DCs overexpressing PD-L1 and MOG dramatically ameliorated clinical EAE and reduced severity of central nervous system inflammation. Furthermore, Ding et al. tried to suppress lupus-like syndrome in BXSB mice by delivering recombinant adenovirus expressing full-length PD-L1 gene (rAd.PD-L1) (Ding et al. 2006). Intravenous injection of rAd.PD-L1 partially prevented the development of nephritis as evidenced by the lower frequency of proteinuria, reduced amount of serum anti-dsDNA IgG, and better renal pathology. Further

analyses may enable us to establish new therapeutic strategies for autoimmune disease by manipulating the PD-1-PD-L pathway.

5.2 PD-1 in Chronic Viral Infection

During chronic viral infection, virus-specific CD8 T cells become unresponsive to viral antigens and persist in a nonfunctional exhausted state (Wherry and Ahmed 2004). These exhausted CD8 T cells are characterized by a hierarchical and progressive loss of function with cytotoxicity and IL-2 production lost first, followed by TNF-α and IFN-γ cytokine production (Wherry and Ahmed 2004). Since CD8 T-cell exhaustion was characterized in the murine lymphocyte choriomeningitis virus (LCMV), such a functional impairment has been a common feature in human chronic viral infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) (Wherry et al. 2003; Klenerman and Hill 2005; Shin and Wherry 2007). These functional defects in responding T cells are probably a primary reason for failure of immunological control of these persisting pathogens. We initially have shown that PD-1 is highly expressed on exhausted CD8 T cells in LCMV system, and that PD-1-PD-L pathway plays a major role in regulating T cells exhaustion during this infection (Barber et al. 2006). PD-1 is transiently expressed by virus-specific CD8 T cells after acute LCMV infection and rapidly downregulated, whereas CD8 T cells retain high PD-1 expression in lymphoid and nonlymphoid tissues throughout chronic LCMV infection. Subsequently, several groups have shown that PD-1 is highly expressed on simian immunodeficiency virus (SIV)-specific (Velu et al. 2007), HIV-specific (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006), HBV-specific (Boettler et al. 2006; Boni et al. 2007), and HCV-specific (Urbani et al. 2006; Kasprowicz et al. 2008) T cells. The level of PD-1 expression per cell is important in regulating T-cell exhaustion during chronic viral infections (Freeman et al. 2006). The percentage of HIV-specific CD8 T cells expressing PD-1 correlates with viral load, declining CD4 counts, and decreased capacity of CD8 T cell to proliferate (Day et al. 2006; D'Souza et al. 2007). HCV-specific CD8 T cells during persistent infection also display impaired ability of T cells to proliferate and produce cytokines, which correlate with PD-1 expression level (Golden-Mason et al. 2007; Nakamoto et al. 2008). So, PD-1 may serve as a useful marker on virus-specific CD8 T cells to indicate the degree of T-cell exhaustion and disease severity.

The mechanisms of PD-1 regulation in exhausted T cells are still poorly defined. In a longitudinal study of HIV-infected subjects, PD-1 expression declined on T cells specific for epitopes that had undergone mutational escape, whereas PD-1 expression was highly increased over time on those specific for conserved epitope (Streeck et al. 2008). These data indicate that continued antigen-specific TCR stimulation plays an important role in modulating PD-1 expression in HIV infection. In addition, viral protein is known to contribute to TCR-independent upregulation of PD-1. The accessory Nef protein of HIV was shown to upregulate PD-1

through a p38 mitogen-activated protein kinase-dependent mechanism during infection (Muthumani et al. 2008). Furthermore, HCV-core protein binding to the complement receptor gC1q is responsible for inducing the expression of PD-1 on T cells (Yao et al. 2007).

The key role of PD-1-PD-L pathway in CD8 T-cell exhaustion during chronic viral infections drive development of strategies to manipulate the interaction of PD-1 and its ligands for the reversal of exhausted CD8 T cells and viral control. In mice, blocking PD-1 pathway with anti-PD-L1 antibody restored cytokine production, augmented the generation of LCMV-specific T cells and, most importantly, led to a dramatic reduction in viral load (Barber et al. 2006). During SIV infection in nonhuman primate, PD-1 blockade using anti-PD-1 antibody resulted in rapid expansion of virus-specific CD8 T cell with improved functionality, which was associated with significant reduction in plasma viral load (Velu et al. 2009). Furthermore, blocking PD-L1 with a monoclonal antibody led to increased HIV-specific T-cell proliferation, and production of TNF-α, IFN-γ, and granzyme B (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006). However, given that blocking or eliminating PD-1 or its ligands can accelerate autoimmune disease, we must better understand the immunoregulatory roles of PD-1-PD-L pathway to determine how to modulate this pathway to effectively activate virus-specific T cells while minimizing the risk of immunopathology.

5.3 PD-1 in Antitumor Immunity

There are accumulating evidences that tumors exploit PD-1-dependent immune suppression for immune evasion. The expression of PD-L1 and PD-L2 has been found on a wide variety of solid tumors and hematologic malignancies. In addition, PD-1 expression on tumor infiltrating lymphocytes has been reported, suggesting that these T cells are functionally exhausted. Strikingly, a strong correlation between PD-Ls expression on tumor cells and unfavorable prognosis has been demonstrated for various cancers including kidney, ovarian, esophageal, bladder, gastric, and pancreatic cancers and melanoma (Thompson et al. 2004; Ohigashi et al. 2005; Wu et al. 2006; Hamanishi et al. 2007; Nakanishi et al. 2007; Nomi et al. 2007; Hino et al. 2010). Thompson et al. have analyzed the expression of PD-L1 on renal cell carcinoma and found that patients with high tumor and/or lymphocyte PD-L1 levels were 4.5 times more likely to die of their cancer than patients exhibiting low levels of PD-L1 expression (Thompson et al. 2004). Hamanishi et al. reported that patients with tumors positive for both PD-L1 and PD-L2 showed dramatically lower survival rate than patients with tumors negative for both of these ligands (46% vs. 83% for 5-year survival) (Hamanishi et al. 2007).

To date, many groups reported that PD-Ls on tumor cells suppressed antitumor immunity by inhibiting T-cell activation and lysis of tumor cells, or inducing apoptosis of tumor-specific T cells (Iwai et al. 2002; Curiel et al. 2003; Hirano et al. 2005). PD-Ls on tumor-associated DCs also suppressed the activation of

antitumor T cells. Accordingly, the efficacy of PD-1 blockade in tumor eradication has been demonstrated in various experimental systems. The blocking strategies used include blocking antibodies against PD-1 and PD-L1, DNA vaccination of the extracellular region of PD-1, genetic ablation of PD-1 gene, RNA interference, and recombinant protein of the extracellular region of PD-1 and PD-L1 (Iwai et al. 2002, 2005; Curiel et al. 2003; Blank et al. 2004; He et al. 2004; Hirano et al. 2005; Terawaki et al. 2007; Borkner et al. 2010).

Currently, two monoclonal antibodies against PD-1 are in clinical trials for cancer and hepatitis C virus infection. ONO-4538/MDX-1106 is a fully human IgG4 monoclonal antibody against human PD-1. Phase I clinical trial of ONO-4538/MDX-1106 has been performed on 39 patients with nonsmall-cell lung cancer, renal cell carcinoma, colorectal cancer, melanoma, and prostate cancer (Brahmer et al. 2010). ONO-4538/MDX-1106 was well tolerated although one serious adverse event, inflammatory colitis was observed. One patient with colorectal cancer had a complete response and two patients with renal cell carcinoma and melanoma had partial responses (>30% regression) (Fig. 4). In addition,

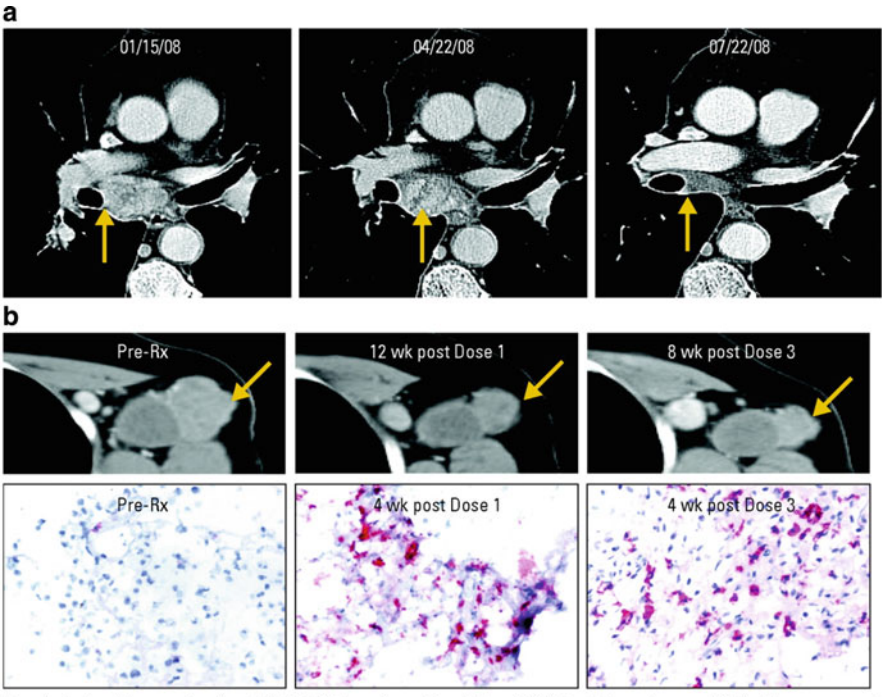

Fig. 4 Tumor regression in patient with metastatic renal cell carcinoma (RCC) and melanoma after repeated dosing with anti-PD-1 monoclonal antibody (MDX-1106). (**a**) Patient with RCC with regression of metastases in mediastinal lymph nodes after receiving three doses of MDX-1106. (**b**) Patient with melanoma experienced a partial response after receiving 11 doses of MDX-1106. Biopsies of a regressing axillary lymph node metastasis infiltrated with CD8 T cells. Figures are taken from the original paper (Brahmer et al. 2010) with permission

significant lesional tumor regressions not meeting PR criteria were observed in two patients with melanoma and nonsmall-cell lung cancer. CT-011, which was originally generated as a monoclonal antibody against B lymphoblastoid cells and developed as a drug for cancer for its lymphocyte-activating and tumor-regressing activities, turned out to recognize PD-1 (Berger et al. 2008). Phase I clinical trial of CT-011 has been performed on six patients (follicular B-cell lymphoma, chronic lymphocytic leukemia, Hodgkin's lymphoma, multiple myeloma, and acute myeloid leukemia) and no severe adverse events were observed. Clinical benefit was observed in 33% of the patients with one patient with follicular B-cell lymphoma showed complete remission. One minimal response was observed in an acute myeloid leukemia patient. The other four patients have shown stable disease for >35 weeks.

Further clinical studies are expected to reveal the efficacy of PD-1 blocking antibodies for the treatment of cancer. A low-molecular compound that can block PD-1 signal more efficiently may also appear based on the crystal structure of PD-1/PD-Ls. In addition, combinatorial treatments of PD-1 blockade and other immunotherapies including cytokine therapies, vaccination with tumor-associated antigens, blockade of other immunoregulatory molecules, infusion of activated DCs, and depletion of Tregs may help further improve therapeutic potential against tumors.

6 Conclusions

Since PD-1 was initially identified in 1992, accumulating evidences suggest that PD-1 and its ligands are key regulators in T cells activation and tolerance followed by induction and maintenance of peripheral tolerance. Subsequently, it became clear that PD-1 pathway plays crucial roles in the regulation of autoimmunity, transplantation immunity, infectious immunity, and tumor immunity. This biological significance of PD-1 and its ligands sheds light on the development of therapeutic strategies against clinical incurable diseases by manipulating the PD-1 pathway. Indeed, many groups are trying to develop not only PD-1 antagonists for the treatment of cancer and infectious disease but also PD-1 agonist for the treatment of autoimmune disease and transplantation rejection (Fig. 5). However, it still remains unclear how the expressions of PD-1 and its ligands are spatially and temporally regulated and what are the molecular mechanisms of signaling through PD-1 and its ligands. It is important to understand how PD-1 pathway mediates its inhibitory pathways. Recent observations have revealed that other inhibitory receptors, such as 2B4, LAG-3, CTLA-4, PirB, GP49, and CD160, were co-expressed on exhausted CD8 T cells during chronic infections (Blackburn et al. 2009). There is considerable diversity in the number and type of inhibitory receptors that can be expressed by T cells during virus infection, and these diverse inhibitory pathways appear to cooperate with PD-1 pathway in regulating T-cell function. Thus, further studies are required to address whether these diverse inhibitory receptors as well as

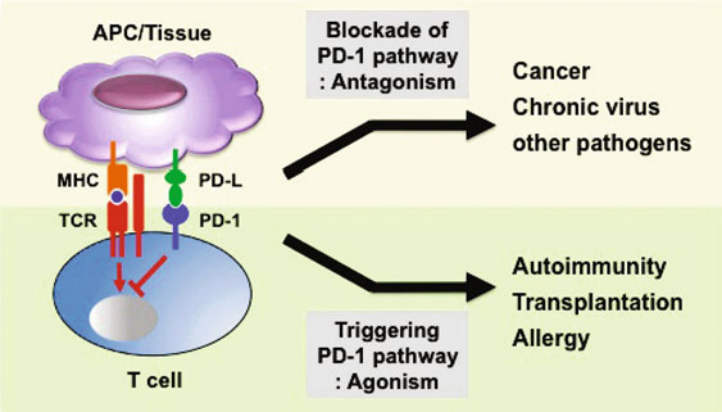

Fig. 5 Biological significance of PD-1-PD-L pathway and its clinical application. Blockade of PD-1 pathway with antagonist antibody, such as anti-PD-1 or anti-PD-L, augment immune responses and may be useful in the treatment of cancer and infectious disease. Triggering PD-1 pathway with agonist antibody or by delivery of PD-L gene/protein suppress adverse immune responses and can be used for the treatment of autoimmune disease, allergy, and transplant rejection

PD-1 are also involved in the coregulation of autoimmunity and tumor immunity. Such studies will not only provide a better mechanistic understanding of the PD-1 pathway in regulating T cell responses but will also facilitate precise manipulation of this pathway therapeutically.

References

Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T (1996) Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol 8:765–772

Ansari MJ, Salama AD, Chitnis T, Smith RN, Yagita H, Akiba H, Yamazaki T, Azuma M, Iwai H, Khoury SJ, Auchincloss H Jr, Sayegh MH (2003) The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. J Exp Med 198:63–69

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

Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, Koren-Michowitz M, Shimoni A, Nagler A (2008) Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res 14:3044–3051

Bertsias GK, Nakou M, Choulaki C, Raptopoulou A, Papadimitraki E, Goulielmos G, Kritikos H, Sidiropoulos P, Tzardi M, Kardassis D, Mamalaki C, Boumpas DT (2009) Genetic, immunologic, and immunohistochemical analysis of the programmed death 1/programmed death ligand 1 pathway in human systemic lupus erythematosus. Arthritis Rheum 60:207–218

Bishop KD, Harris JE, Mordes JP, Greiner DL, Rossini AA, Czech MP, Phillips NE (2009) Depletion of the programmed death-1 receptor completely reverses established clonal anergy in CD4(+) T lymphocytes via an interleukin-2-dependent mechanism. Cell Immunol 256:86–91

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

Blank C, Brown I, Marks R, Nishimura H, Honjo T, Gajewski TF (2003) Absence of programmed death receptor 1 alters thymic development and enhances generation of CD4/CD8 double-negative TCR-transgenic T cells. J Immunol 171:4574–4581

Blank C, Brown I, Peterson AC, Spiotto M, Iwai Y, Honjo T, Gajewski TF (2004) PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res 64:1140–1145

Blattman JN, Wherry EJ, Ha SJ, van der Most RG, Ahmed R (2009) Impact of epitope escape on PD-1 expression and CD8 T-cell exhaustion during chronic infection. J Virol 83:4386–4394

Boettler T, Panther E, Bengsch B, Nazarova N, Spangenberg HC, Blum HE, Thimme R (2006) Expression of the interleukin-7 receptor alpha chain (CD127) on virus-specific CD8+ T cells identifies functionally and phenotypically defined memory T cells during acute resolving hepatitis B virus infection. J Virol 80:3532–3540

Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, Laccabue D, Zerbini A, Cavalli A, Missale G, Bertoletti A, Ferrari C (2007) Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol 81:4215–4225

Borkner L, Kaiser A, van de Kasteele W, Andreesen R, Mackensen A, Haanen JB, Schumacher TN, Blank C (2010) RNA interference targeting programmed death receptor-1 improves immune functions of tumor-specific T cells. Cancer Immunol Immunother 59(8):1173–1183

Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I, Topalian SL (2010) Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 28:3167–3175

Brown JA, Dorfman DM, Ma FR, Sullivan EL, Munoz O, Wood CR, Greenfield EA, Freeman GJ (2003) Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol 170:1257–1266

Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ (2007) Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity 27:111–122

Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL (2004) SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol 173:945–954

Chikuma S, Terawaki S, Hayashi T, Nabeshima R, Yoshida T, Shibayama S, Okazaki T, Honjo T (2009) PD-1-mediated suppression of IL-2 production induces CD8+ T cell anergy in vivo. J Immunol 182:6682–6689

Cho HY, Lee SW, Seo SK, Choi IW, Choi I (2008) Interferon-sensitive response element (ISRE) is mainly responsible for IFN-alpha-induced upregulation of programmed death-1 (PD-1) in macrophages. Biochim Biophys Acta 1779:811–819

Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, Krzysiek R, Knutson KL, Daniel B, Zimmermann MC, David O, Burow M, Gordon A, Dhurandhar N, Myers L, Berggren R, Hemminki A, Alvarez RD, Emilie D, Curiel DT, Chen L, Zou W (2003) Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med 9:562–567

Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, Mncube Z, Duraiswamy J, Zhu B, Eichbaum Q, Altfeld M, Wherry EJ, Coovadia HM, Goulder PJ, Klenerman P, Ahmed R, Freeman GJ, Walker BD (2006) PD-1

expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443:350–354

Ding H, Wu X, Wu J, Yagita H, He Y, Zhang J, Ren J, Gao W (2006) Delivering PD-1 inhibitory signal concomitant with blocking ICOS co-stimulation suppresses lupus-like syndrome in autoimmune BXSB mice. Clin Immunol 118:258–267

Dong H, Zhu G, Tamada K, Chen L (1999) B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 5:1365–1369

Dong H, Strome SE, Matteson EL, Moder KG, Flies DB, Zhu G, Tamura H, Driscoll CL, Chen L (2003) Costimulating aberrant T cell responses by B7-H1 autoantibodies in rheumatoid arthritis. J Clin Invest 111:363–370

D'Souza M, Fontenot AP, Mack DG, Lozupone C, Dillon S, Meditz A, Wilson CC, Connick E, Palmer BE (2007) Programmed death 1 expression on HIV-specific CD4+ T cells is driven by viral replication and associated with T cell dysfunction. J Immunol 179:1979–1987

Eppihimer MJ, Gunn J, Freeman GJ, Greenfield EA, Chernova T, Erickson J, Leonard JP (2002) Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. Microcirculation 9:133–145

Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 206:3015–3029

Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 192:1027–1034

Freeman GJ, Wherry EJ, Ahmed R, Sharpe AH (2006) Reinvigorating exhausted HIV-specific T cells via PD-1-PD-1 ligand blockade. J Exp Med 203:2223–2227

Golden-Mason L, Palmer B, Klarquist J, Mengshol JA, Castelblanco N, Rosen HR (2007) Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. J Virol 81:9249–9258

Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, Noelle RJ, Coyle A, Mellor AL, Khoury SJ, Sayegh MH (2005) A critical role for the programmed death ligand 1 in fetomaternal tolerance. J Exp Med 202:231–237

Habicht A, Kewalaramani R, Vu MD, Demirci G, Blazar BR, Sayegh MH, Li XC (2007) Striking dichotomy of PD-L1 and PD-L2 pathways in regulating alloreactive CD4(+) and CD8(+) T cells in vivo. Am J Transplant 7:2683–2692

Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H, Takakura K, Minato N, Honjo T, Fujii S (2007) Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci USA 104:3360–3365

He YF, Zhang GM, Wang XH, Zhang H, Yuan Y, Li D, Feng ZH (2004) Blocking programmed death-1 ligand-PD-1 interactions by local gene therapy results in enhancement of antitumor effect of secondary lymphoid tissue chemokine. J Immunol 173:4919–4928

Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T, Okazaki T, Tokura Y (2010) Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 116:1757–1766

Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, Tamada K, Chen L (2005) Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res 65:1089–1096

Hirata S, Senju S, Matsuyoshi H, Fukuma D, Uemura Y, Nishimura Y (2005) Prevention of experimental autoimmune encephalomyelitis by transfer of embryonic stem cell-derived dendritic cells expressing myelin oligodendrocyte glycoprotein peptide along with TRAIL or programmed death-1 ligand. J Immunol 174:1888–1897

Holets LM, Hunt JS, Petroff MG (2006) Trophoblast CD274 (B7-H1) is differentially expressed across gestation: influence of oxygen concentration. Biol Reprod 74:352–358

Hori J, Wang M, Miyashita M, Tanemoto K, Takahashi H, Takemori T, Okumura K, Yagita H, Azuma M (2006) B7-H1-induced apoptosis as a mechanism of immune privilege of corneal allografts. J Immunol 177:5928–5935

Ishida Y, Agata Y, Shibahara K, Honjo T (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 11:3887–3895

Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N (2002) Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA 99:12293–12297

Iwai Y, Terawaki S, Honjo T (2005) PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. Int Immunol 17:133–144

Kanai T, Totsuka T, Uraushihara K, Makita S, Nakamura T, Koganei K, Fukushima T, Akiba H, Yagita H, Okumura K, Machida U, Iwai H, Azuma M, Chen L, Watanabe M (2003) Blockade of B7-H1 suppresses the development of chronic intestinal inflammation. J Immunol 171:4156–4163

Kasprowicz V, Schulze Zur Wiesch J, Kuntzen T, Nolan BE, Longworth S, Berical A, Blum J, McMahon C, Reyor LL, Elias N, Kwok WW, McGovern BG, Freeman G, Chung RT, Klenerman P, Lewis-Ximenez L, Walker BD, Allen TM, Kim AY, Lauer GM (2008) High level of PD-1 expression on hepatitis C virus (HCV)-specific CD8+ and CD4+ T cells during acute HCV infection, irrespective of clinical outcome. J Virol 82:3154–3160

Keir ME, Latchman YE, Freeman GJ, Sharpe AH (2005) Programmed death-1 (PD-1):PD-ligand 1 interactions inhibit TCR-mediated positive selection of thymocytes. J Immunol 175:7372–7379

Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, Koulmanda M, Freeman GJ, Sayegh MH, Sharpe AH (2006) Tissue expression of PD-L1 mediates peripheral T cell tolerance. J Exp Med 203:883–895

Keir ME, Butte MJ, Freeman GJ, Sharpe AH (2008) PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26:677–704

Klenerman P, Hill A (2005) T cells and viral persistence: lessons from diverse infections. Nat Immunol 6:873–879

Kroner A, Mehling M, Hemmer B, Rieckmann P, Toyka KV, Maurer M, Wiendl H (2005) A PD-1 polymorphism is associated with disease progression in multiple sclerosis. Ann Neurol 58:50–57

Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, Greenfield EA, Bourque K, Boussiotis VA, Carter LL, Carreno BM, Malenkovich N, Nishimura H, Okazaki T, Honjo T, Sharpe AH, Freeman GJ (2001) PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2:261–268

Latchman YE, Liang SC, Wu Y, Chernova T, Sobel RA, Klemm M, Kuchroo VK, Freeman GJ, Sharpe AH (2004) PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. Proc Natl Acad Sci USA 101:10691–10696

Lazar-Molnar E, Yan Q, Cao E, Ramagopal U, Nathenson SG, Almo SC (2008) Crystal structure of the complex between programmed death-1 (PD-1) and its ligand PD-L2. Proc Natl Acad Sci USA 105:10483–10488

Lee SH, Lee YA, Woo DH, Song R, Park EK, Ryu MH, Kim YH, Kim KS, Hong SJ, Yoo MC, Yang HI (2006a) Association of the programmed cell death 1 (PDCD1) gene polymorphism with ankylosing spondylitis in the Korean population. Arthritis Res Ther 8:R163

Lee SJ, Jang BC, Lee SW, Yang YI, Suh SI, Park YM, Oh S, Shin JG, Yao S, Chen L, Choi IH (2006b) Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274). FEBS Lett 580:755–762

Liang SC, Latchman YE, Buhlmann JE, Tomczak MF, Horwitz BH, Freeman GJ, Sharpe AH (2003) Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol 33:2706–2716

Lin DY, Tanaka Y, Iwasaki M, Gittis AG, Su HP, Mikami B, Okazaki T, Honjo T, Minato N, Garboczi DN (2008) The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors. Proc Natl Acad Sci USA 105:3011–3016

Liu J, Hamrouni A, Wolowiec D, Coiteux V, Kuliczkowski K, Hetuin D, Saudemont A, Quesnel B (2007) Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. Blood 110:296–304

Long EO (1999) Regulation of immune responses through inhibitory receptors. Annu Rev Immunol 17:875–904

Matsumoto K, Inoue H, Nakano T, Tsuda M, Yoshiura Y, Fukuyama S, Tsushima F, Hoshino T, Aizawa H, Akiba H, Pardoll D, Hara N, Yagita H, Azuma M, Nakanishi Y (2004) B7-DC regulates asthmatic response by an IFN-gamma-dependent mechanism. J Immunol 172:2530–2541

Meng Q, Yang P, Li B, Zhou H, Huang X, Zhu L, Ren Y, Kijlstra A (2006) CD4+PD-1+ T cells acting as regulatory cells during the induction of anterior chamber-associated immune deviation. Invest Ophthalmol Vis Sci 47:4444–4452

Moll M, Kuylenstierna C, Gonzalez VD, Andersson SK, Bosnjak L, Sonnerborg A, Quigley MF, Sandberg JK (2009) Severe functional impairment and elevated PD-1 expression in CD1d-restricted NKT cells retained during chronic HIV-1 infection. Eur J Immunol 39:902–911

Muthumani K, Choo AY, Shedlock DJ, Laddy DJ, Sundaram SG, Hirao L, Wu L, Thieu KP, Chung CW, Lankaraman KM, Tebas P, Silvestri G, Weiner DB (2008) Human immunodeficiency virus type 1 Nef induces programmed death 1 expression through a p38 mitogen-activated protein kinase-dependent mechanism. J Virol 82:11536–11544

Nakae S, Suto H, Iikura M, Kakurai M, Sedgwick JD, Tsai M, Galli SJ (2006) Mast cells enhance T cell activation: importance of mast cell costimulatory molecules and secreted TNF. J Immunol 176:2238–2248

Nakamoto N, Kaplan DE, Coleclough J, Li Y, Valiga ME, Kaminski M, Shaked A, Olthoff K, Gostick E, Price DA, Freeman GJ, Wherry EJ, Chang KM (2008) Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. Gastroenterology 134:1927–1937

Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S (2007) Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. Cancer Immunol Immunother 56:1173–1182

Nielsen C, Hansen D, Husby S, Jacobsen BB, Lillevang ST (2003) Association of a putative regulatory polymorphism in the PD-1 gene with susceptibility to type 1 diabetes. Tissue Antigens 62:492–497

Nishimura H, Agata Y, Kawasaki A, Sato M, Imamura S, Minato N, Yagita H, Nakano T, Honjo T (1996) Developmentally regulated expression of the PD-1 protein on the surface of double-negative (CD4-CD8-) thymocytes. Int Immunol 8:773–780

Nishimura H, Nose M, Hiai H, Minato N, Honjo T (1999) Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 11:141–151

Nishimura H, Honjo T, Minato N (2000) Facilitation of beta selection and modification of positive selection in the thymus of PD-1-deficient mice. J Exp Med 191:891–898

Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, Honjo T (2001) Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science 291:319–322

Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, Nakamura S, Enomoto K, Yagita H, Azuma M, Nakajima Y (2007) Clinical significance and therapeutic potential of the

programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 13:2151–2157

Oestreich KJ, Yoon H, Ahmed R, Boss JM (2008) NFATc1 regulates PD-1 expression upon T cell activation. J Immunol 181:4832–4839

Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, Mizuno T, Yoriki R, Kashizuka H, Yane K, Tsushima F, Otsuki N, Yagita H, Azuma M, Nakajima Y (2005) Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res 11:2947–2953

Okazaki T, Honjo T (2007) PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 19:813–824

Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T (2001) PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci USA 98:13866–13871

Okazaki T, Tanaka Y, Nishio R, Mitsuiye T, Mizoguchi A, Wang J, Ishida M, Hiai H, Matsumori A, Minato N, Honjo T (2003) Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. Nat Med 9:1477–1483

Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, Linsley PS, Thompson CB, Riley JL (2005) CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 25:9543–9553

Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, Cachola KE, Murray JC, Tihan T, Jensen MC, Mischel PS, Stokoe D, Pieper RO (2007) Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 13:84–88

Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, Precopio ML, Schacker T, Roederer M, Douek DC, Koup RA (2006) PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. J Exp Med 203:2281–2292

Prokunina L, Castillejo-Lopez C, Oberg F, Gunnarsson I, Berg L, Magnusson V, Brookes AJ, Tentler D, Kristjansdottir H, Grondal G, Bolstad AI, Svenungsson E, Lundberg I, Sturfelt G, Jonssen A, Truedsson L, Lima G, Alcocer-Varela J, Jonsson R, Gyllensten UB, Harley JB, Alarcon-Segovia D, Steinsson K, Alarcon-Riquelme ME (2002) A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 32:666–669

Salama AD, Chitnis T, Imitola J, Ansari MJ, Akiba H, Tushima F, Azuma M, Yagita H, Sayegh MH, Khoury SJ (2003) Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. J Exp Med 198:71–78

Schreiner B, Mitsdoerffer M, Kieseier BC, Chen L, Hartung HP, Weller M, Wiendl H (2004) Interferon-beta enhances monocyte and dendritic cell expression of B7-H1 (PD-L1), a strong inhibitor of autologous T-cell activation: relevance for the immune modulatory effect in multiple sclerosis. J Neuroimmunol 155:172–182

Selenko-Gebauer N, Majdic O, Szekeres A, Hofler G, Guthann E, Korthauer U, Zlabinger G, Steinberger P, Pickl WF, Stockinger H, Knapp W, Stockl J (2003) B7-H1 (programmed death-1 ligand) on dendritic cells is involved in the induction and maintenance of T cell anergy. J Immunol 170:3637–3644

Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, Qiu Y, Jussif JM, Carter LL, Wood CR, Chaudhary D (2004) PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett 574:37–41

Shin H, Wherry EJ (2007) CD8 T cell dysfunction during chronic viral infection. Curr Opin Immunol 19:408–415

Sidorenko SP, Clark EA (2003) The dual-function CD150 receptor subfamily: the viral attraction. Nat Immunol 4:19–24

Streeck H, Brumme ZL, Anastario M, Cohen KW, Jolin JS, Meier A, Brumme CJ, Rosenberg ES, Alter G, Allen TM, Walker BD, Altfeld M (2008) Antigen load and viral sequence diversification determine the functional profile of HIV-1-specific CD8+ T cells. PLoS Med 5:e100

Sugita S, Usui Y, Horie S, Futagami Y, Yamada Y, Ma J, Kezuka T, Hamada H, Usui T, Mochizuki M, Yamagami S (2009) Human corneal endothelial cells expressing programmed death-ligand 1 (PD-L1) suppress PD-1+ T helper 1 cells by a contact-dependent mechanism. Invest Ophthalmol Vis Sci 50:263–272

Terawaki S, Tanaka Y, Nagakura T, Hayashi T, Shibayama S, Muroi K, Okazaki T, Mikami B, Garboczi DN, Honjo T, Minato N (2007) Specific and high-affinity binding of tetramerized PD-L1 extracellular domain to PD-1-expressing cells: possible application to enhance T cell function. Int Immunol 19:881–890

Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, Krejci KG, Lobo JR, Sengupta S, Chen L, Zincke H, Blute ML, Strome SE, Leibovich BC, Kwon ED (2004) Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci USA 101:17174–17179

Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, Boulassel MR, Delwart E, Sepulveda H, Balderas RS, Routy JP, Haddad EK, Sekaly RP (2006) Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med 12:1198–1202

Tseng SY, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, Shalabi A, Shin T, Pardoll DM, Tsuchiya H (2001) B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. J Exp Med 193:839–846

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

Velu V, Kannanganat S, Ibegbu C, Chennareddi L, Villinger F, Freeman GJ, Ahmed R, Amara RR (2007) Elevated expression levels of inhibitory receptor programmed death 1 on simian immunodeficiency virus-specific CD8 T cells during chronic infection but not after vaccination. J Virol 81:5819–5828

Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, Vanderford TH, Chennareddi L, Silvestri G, Freeman GJ, Ahmed R, Amara RR (2009) Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature 458:206–210

Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T (2005) Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. Proc Natl Acad Sci USA 102:11823–11828

Wang J, Okazaki IM, Yoshida T, Chikuma S, Kato Y, Nakaki F, Hiai H, Honjo T, Okazaki T (2010) PD-1 deficiency results in the development of fatal myocarditis in MRL mice. Int Immunol 22:443–452

Watson MP, George AJ, Larkin DF (2006) Differential effects of costimulatory pathway modulation on corneal allograft survival. Invest Ophthalmol Vis Sci 47:3417–3422

Wherry EJ, Ahmed R (2004) Memory CD8 T-cell differentiation during viral infection. J Virol 78:5535–5545

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

Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N (2006) Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem 108:19–24

Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, Shin T, Tsuchiya H, Pardoll DM, Okumura K, Azuma M, Yagita H (2002) Expression of programmed death 1 ligands by murine T cells and APC. J Immunol 169:5538–5545

Yao ZQ, King E, Prayther D, Yin D, Moorman J (2007) T cell dysfunction by hepatitis C virus core protein involves PD-1/PDL-1 signaling. Viral Immunol 20:276–287

Zhang X, Schwartz JC, Guo X, Bhatia S, Cao E, Lorenz M, Cammer M, Chen L, Zhang ZY, Edidin MA, Nathenson SG, Almo SC (2004) Structural and functional analysis of the costimulatory receptor programmed death-1. Immunity 20:337–347

Zhong X, Tumang JR, Gao W, Bai C, Rothstein TL (2007) PD-L2 expression extends beyond dendritic cells/macrophages to B1 cells enriched for V(H)11/V(H)12 and phosphatidylcholine binding. Eur J Immunol 37:2405–2410

Zhu B, Guleria I, Khosroshahi A, Chitnis T, Imitola J, Azuma M, Yagita H, Sayegh MH, Khoury SJ (2006) Differential role of programmed death-ligand 1 [corrected] and programmed death-ligand 2 [corrected] in regulating the susceptibility and chronic progression of experimental autoimmune encephalomyelitis. J Immunol 176:3480–3489

The Role of IL-10 in Regulating Immunity to Persistent Viral Infections

Elizabeth B. Wilson and David G. Brooks

Contents

Abstract The immune system has evolved multipronged responses that are critical to effectively defend the body from invading pathogens and to clear infection. However, the same weapons employed to eradicate infection can have caustic effects on normal bystander cells. Therefore, tight regulation is vital and the host must balance engendering correct and sufficient immune responses to pathogens while limiting errant and excessive immunopathology. To accomplish this task, a complex network of positive and negative immune signals are delivered, which in most instances successfully eliminate the pathogen. However, in response to some viral infections, immune function is rapidly suppressed leading to viral persistence.

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Immune suppression is a critical obstacle to the control of many persistent viral infections such as HIV, hepatitis C, and hepatitis B virus, which together affect more than 500 million individuals worldwide. Thus, the ability to therapeutically enhance immunity is a potentially powerful approach to resolve persistent infections. The host-derived cytokine IL-10 is a key player in the establishment and perpetuation of viral persistence. This chapter discusses the role of IL-10 in viral persistence and explores the exciting prospect of therapeutically blocking IL-10 to increase antiviral immunity and vaccine efficacy.

1 Introduction

1.1 Immune Dynamics During Acute and Persistent Virus Infection: The Failure of Antiviral Immunity

Viral invasion is rapidly detected through the triggering of pattern recognition receptors such as toll-like receptors (TLRs) and the retinoic acid-inducible gene 1-like helicases (RIG-I). The presence of viral genome triggers TLRs and RIG-I to stimulate production of type I interferon (i.e., IFNα and β) as well as other immunomodulatory proteins (Koyama et al. 2008; Schlee and Hartmann 2010). Type I interferons control early viral replication by directly fostering apoptosis and hindering proliferation of virally infected cells, as well as strongly stimulating cytotoxic natural killer (NK) cells (Biron et al. 1999; Gartel et al. 1996; Tanaka et al. 1998). In addition to orchestrating the innate inflammatory environment, interferons play important roles in directing adaptive immune responses to effectively combat viral assault (Goodbourn et al. 2000).

Successful resolution of viral infection and the subsequent establishment of lasting immunological memory hinge upon the effecter cells of the adaptive immune system. The interplay of CD4 and CD8 T cell, and B cell responses ultimately dictates the outcome of infection. Naïve CD4 and CD8 T cell responses are primed by dendritic cells (DC). DC direct the type of T cell responses generated based on the cytokine environment and general millieau in which antigen is encountered (Banchereau and Steinman 1998). Effective antiviral $CD8^+$ T cytolytic lymphocytes (CTL) responses are primed by DC via cognate interactions of the T cell receptor (TCR) with peptide/major histocompatibility class I (MHC I) complexes resulting in cellular activation and a clonal expansion of antigen specific cells. CTL produce multiple inflammatory cytokines, such as IFNγ and TNFα, and have the ability to lyse infected cells. Concurrently, $CD4^+$ T cells are activated by DC and differentiate into distinct T helper (Th) subsets that shape ensuing CD8 T and B cell responses (Fahey and Brooks 2010). Classically speaking, $CD4^+$ Th1 responses are tailored to combat intracellular infections via production of IFNγ, TNFα, IL-2, and the perpetuation of the inflammatory environment. Levels of costimulatory molecules (i.e., CD80/86 and CD40) and the cytokine environment

(notably IL12 and interferons) promote Th1 differentiation (Constant and Bottomly 1997). At the same time, and dependent on CD4 T cell help, B cells are activated and produce antibody to further neutralize virus. After the peak of this acute response, virus-specific T cells undergo significant contraction and further differentiation into stable memory populations (Kaech et al. 2002). Ultimately, it is the summation of all these events that lead to successful viral clearance and memory differentiation to prevent reinfection.

In most situations, this concerted effort of innate and adaptive responses is effective in eliminating the pathogen. However, in some cases, the acute resolution of infection is incomplete and viral persistence results. Herpes simplex virus, human cytomegalovirus (HCMV), and Epstein–Barr virus (EBV), along with γ2-herpes virus in mice, are hallmark examples of infections that develop lifelong viral persistence by "hiding" from the immune response. This presence of latent/reactivating infection is associated with functional T cell responses that control viral replication upon reemergence. In contrast, human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) infections in humans, and lymphocytic choriomeningitis virus (LCMV) infection in rodents, establish persistent infections characterized by sustained high levels of viral replication and immunosuppression (Klenerman and Hill 2005). In response to these persistent infections, virus-specific CD4 and CD8 T cells are physically deleted or persist in an attenuated (termed exhausted) developmental program unable to proliferate to viral antigens or produce important antiviral and immunostimulatory cytokines (e.g., IFNγ, TNFα, IL-2) (Brooks et al. 2006a; Gallimore et al. 1998; Wherry et al. 2003, 2007; Zajac et al. 1998). The physical deletion of high affinity CTL and the low amount of remaining virus-specific CD8 T cells, in conjunction with the loss of cytokine production, the inability to proliferate to viral antigen and attenuated CD4 Th cell and B cell responses all culminate in the failure to purge infection. The exhausted state is characterized by a unique transcriptional profile featuring up-regulation of the transcription factor Blimp1 and inhibitory cell surface molecules, such as programmed death receptor 1 (PD1), along with down modulation of cytokine and TCR signaling molecules (Agnellini et al. 2007; Shin et al. 2009; Wherry et al. 2007). Thus, T cell exhaustion is a unique T cell developmental program, still active and exerting some control over virus replication (Battegay et al. 1994; Elsaesser et al. 2009; Frohlich et al. 2009; Matloubian et al. 1994; Yi et al. 2009), but distinct from the productive T cell responses during acute infection or the anergic/tolerant responses to self-proteins (Wherry et al. 2007).

Although potentially counter-intuitive, induction of this exhausted state is an important mechanism by which excessive immunopathology and death are avoided in the face of persistent antigen load. Our laboratory and others have demonstrated that host immunosuppressive factors potentiate the exhausted phenotype, even at the expense of facilitating viral persistence (Barber et al. 2006; Brooks et al. 2006b; Ejrnaes et al. 2006; Thimme et al. 2001) The benefits of this immune strategy are apparent in persistent LCMV infection, where rapid mortality occurs when T cell responses are therapeutically augmented to prevent exhaustion during the initial response (Barber et al. 2006; Yi et al. 2009). Excitingly, however, there are

conditions in which early augmentation to prevent T cell exhaustion can be productive in clearing persistent infections without producing fatal immunopathology (Brooks et al. 2006b; Ejrnaes et al. 2006; Tinoco et al. 2009).

The immunoregulatory cytokine IL-10 has been shown to be a key host factor in inducing and maintaining T cell exhaustion and facilitating viral persistence (Bachmann et al. 2007; Brooks et al. 2006b; Ejrnaes et al. 2006; Humphreys et al. 2007; Moore et al. 2001). This review focuses on the potential of therapeutically manipulating IL-10 to safely promote clearance of viral infection without disturbing normal immune homeostasis and inadvertently inducing immunopathology.

2 The Impact of Host-Based Regulatory Mechanisms in T Cell Exhaustion

2.1 Discovery That Host-Based Suppressive Factors Inhibit Clearance of Persistent Viral Infection

Both general and virus-specific immune suppression has been well-established during persistent viral infection; however, the mechanisms that govern this phenomenon have only recently been brought to light. Many persisting viruses (e.g., HCMV, HIV, HCV) encode proteins that actively suppress immunity either by direct inhibition of T cell responses and/or by down-regulating antigen recognition molecules (Klenerman and Hill 2005; Slobedman et al. 2009). Other persistent viruses (e.g., LCMV) do not encode suppressive factors yet their infection still rapidly leads to a suppressive state (Ahmed et al. 1984). This suggests that the inability to rapidly control infection triggers a suppressive program within the host.

The seminal discovery by Rafi Ahmed and colleagues that blockade of the host-encoded protein programmed death (PD) ligand 1 (PD-L1) restored function to exhausted CD8 T cells and enhanced control of persistent LCMV infection, led to the realization that the host itself potentiates immune suppression during viral persistence (Barber et al. 2005). The suppressive role of PD-1/PD-L1 interaction was promptly demonstrated to suppress CD8 T cell responses to a variety of diverse persistent infections in vitro including the RNA viruses HIV (a retrovirus) and HCV (a flavivirus) and the DNA virus, HBV (a hepadnavirus) as well as in vivo against SIV (a non-human primate retrovirus) (Boni et al. 2007; Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006; Urbani et al. 2006b; Velu et al. 2008). The diversity of these viruses with respect to replication strategies, target organs, and infected cell types highlights the conserved role of PD-1/PD-L1 mediated immunosuppression and establish that the host is a powerful inhibitor of T cell immunity during persistent infection.

2.2 *Multiple Regulatory Factors Suppress T Cell Responses During Viral Persistence*

Shortly following the identification of PD-1 mediated immunosuppresion during viral persistence, the dominant role of IL-10 in attenuating effector T cell responses to initiate persistent infection was established (Brooks et al. 2006b; Ejrnaes et al. 2006). Infection of mice with a persistent, but not an acutely cleared variant of LCMV leads to sustained expression of IL-10 by multiple immune cell subsets and functional exhaustion of CD4 and CD8 T cells (Brooks et al. 2006b; Ejrnaes et al. 2006). However, when IL-10 activity was neutralized, either using mice genetically deficient in IL-10 expression or antibodies that block the IL-10 receptor (IL-10R), immune function was sustained and the otherwise persistent virus was rapidly cleared. Persistent LCMV replicated to high titers in wild-type and IL-10 deficient mice 5 days postinfection and prior to the onset of T cell responses. By day 9, T cells did not lose function in IL-10-deficient mice, and they were able to clear persistent LCMV whereas viral titers remained high in wild-type mice (Brooks et al. 2006b). CD8 T cells were required for this clearance since depletion of CD8 T cells in IL-10-deficient mice prior to infection led to LCMV persistence (E. Wilson and D. Brooks, unpublished observation). This was the first identification that a single factor was responsible for derailing the immune response to permit viral persistence and importantly that sustaining T cell immunity could facilitate the clearance of an otherwise persistent infection.

Subsequently, multiple immunoregulatory factors were identified to limit T cell responses during viral persistence, including TGFβ, Tim3, CTLA4, CD27/CD70 (Jones et al. 2008; Kaufmann et al. 2007; Matter et al. 2006; Tinoco et al. 2009). Exhausted CD8 T cells simultaneously express multiple negative regulatory factors during persistent infection (Blackburn et al. 2009) and these factors can simultaneously, but via different pathways, limit T cell activity (Blackburn et al. 2009; Brooks et al. 2008a). Further, a single suppressive factor can differentially affect distinct T cell populations. For example, blockade of CTLA4 during persistent LCMV infection did not impact CD8 T cell responses in vivo (Barber et al. 2005), whereas it did enhance HIV-specific CD4 T cell responses in vitro (Kaufmann et al. 2007). Similarly, IL-10 directly limits CD4 T cell responses, but not CD8 T cell responses, to an acute LCMV infection (Brooks et al. 2010). In addition to T cells, IL-10 and other suppressive factors modulate multiple immune subsets including B cells, DC, macrophages, and NK cells to contribute to enhanced virus control. The diversity in suppressive mechanisms provides the potential opportunity to individually manipulate T cell responses (particularly in combination with therapeutic vaccines) to produce the optimal effector response required to control a specific viral infection (see discussion later). Antibody therapies that block multiple suppressive pathways additively increase antiviral T cell activity (Blackburn et al. 2009; Brooks et al. 2008a). Thus, although increased production of suppressive factors by the immune system itself ultimately leads to the demise and failure of antiviral immunity, these factors can be inhibited for therapeutic benefit.

2.3 *Differential Impact of Reversing T Cell Exhaustion During Viral Persistence*

Understanding the mechanisms and coordination of the multitude of suppressive factors involved in immunoregulation is crucial to the design of effective antiviral therapies. Therapeutic strategies that target host-based factors to restore immune function are less susceptible to resistance via viral mutation as they do not target a specific viral protein. Thus, blockade of host-based negative regulatory factors could be effective against diverse persistent viruses that induce T cell exhaustion without engendering viral resistance.

Although extremely promising, the efficacy of blocking suppressive factors to enhance antiviral immunity in humans remains unclear. However, recent evidence blocking PD-1 in SIV-infected rhesus macaques suggests that these blockade strategies may be effective (Velu et al. 2008). Short-term PD-1 blockade (four treatments over 10 days) provided long-term restoration of T cell responses that correlated with enhanced SIV control. Interestingly, memory B cell responses and SIV-specific antibody production were also increased following PD-1 blockade. The reason for the increased B cell responses was not elucidated and could be due to either direct or indirect mechanisms (e.g., enhanced CD4 T cell help to B cells), but does indicate the exciting prospect that targeting a single molecule may simultaneously enhance multiple arms of the immune response culminating in virus control.

In total these studies indicate the incredible effect of blocking regulatory factors to enhance T cell function during viral persistence; however, this is not without potential negative impact. These dominant regulatory factors and pathways are instilled to prevent errant or unrestricted immune responses. Even in the absence of overt infection, deficiencies in these factors can lead to the massive expansion of effector-like T cells and a variety of autoimmune disorders (Hedrich and Bream 2010; Moore et al. 2001). In response to an infection, the inability to attenuate T cell responses can lead to severe immunopathology and death. For example, persistent LCMV infection is fatal in PD-L1 knockout mice (Barber et al. 2005) and while IL-10-deficient mice survive and clear persistent LCMV infection (Brooks et al. 2006b; Ejrnaes et al. 2006), they are more susceptible to death in response to higher doses of persistent LCMV when compared with IL-10 sufficient hosts (D.G. Brooks, unpublished observations). Further, treatment with the immunostimulatory cytokine IL-21 during the early phase of persistent LCMV infection dramatically elevated virus-specific CD8 T cell responses and mortality (Yi et al. 2009). Thus, although detrimental to virus clearance, it is likely that the increased expression of negative regulatory factors and T cell exhaustion is a conserved and rapid mechanism to prevent lethal immunopathology when the host "senses" that virus replication has out-competed the immune response to it. In some instances, enhanced immunopathology is observed in the absence of IL-10 regulation without an effect on viral replication, as is the case during a neurotropic model of mouse hepatitis virus infection (Lin et al. 1998). On the other hand, once chronic infection has been

established and T cell numbers have contracted, reversing exhaustion appears to be well handled in animal models of LCMV and SIV infection (Barber et al. 2005; Brooks et al. 2008a, b; Velu et al. 2008). The relationship between T cell exhaustion (i.e., attenuating T cell responses), excessive immunopathology, and host survival must be carefully considered when optimizing therapies targeting host suppressive factors, particularly if instituted early during persistent virus infection.

Despite freeing virus-specific T cells to fight infection, blockade of regulatory factors may simultaneously unleash the regulation of self-specific immune cells or "tolerant" immune cells in multiple organs and in the case of IL-10 blockade, particularly in the gut (Kuhn et al. 1993). Such a result could have the unintended consequence of triggering autoimmunity or immune responses to ingested food or endogenous enteric bacterial microbiota. It should be noted that overt autoimmunity was observed neither in our studies when IL-10R blockade was implemented during the chronic phase of LCMV infection (D.G. Brooks, unpublished observation) nor following PD-1 blockade in SIV-infected macaques (Velu et al. 2008). However, these studies utilized short-term treatment regimens and longer term therapy or different individuals' dependence on a particular pathway to maintain immune homeostasis that could affect negative responses. Thus, although therapies that block negative regulators of immune function clearly hold tremendous antiviral potential, the possible consequences should be carefully evaluated.

3 The Impact of IL-10 Toward Viral Persistence

3.1 The Diverse Roles of IL-10 During Viral Infection

IL-10 was initially known as cytokine synthesis inhibitory factor (CSIF) and was first identified as a CD4 produced Th2 cytokine with the ability to indirectly repress Th1 responses (Fiorentino et al. 1989; Moore et al. 2001). It is now evident that multiple cell types including DC, B cells, macrophages, CD4 T cells, CD8 T cells, NK cells as well as innate and adaptive regulatory T cells can produce IL-10 (Mege et al. 2006; Moore et al. 2001).

The IL-10 receptor (IL-10R) is a class II cytokine family member composed of two subunits: IL-10R1 is the unique ligand-binding subunit and IL-10R2 is the signaling subunit that is shared with other family member cytokines (IL-22, IL-26, IL-28, and IL-29) (Donnelly et al. 2004). Dimerization of the receptor by IL-10 results in signaling through STAT3 and activation of gene expression (Kotenko et al. 1997; Liu et al. 1994; Moore et al. 2001; Spencer et al. 1998). Specificity of IL-10 responsiveness is dictated both by the expression of IL-10R1 and availability of the cytokine (Brooks et al. 2006b, 2010). The signaling subunit (IL-10R2) is constitutively expressed by most cells, while IL-10R1 is differentially regulated by activation in a cell type specific manner in hematopoietic cells and is inducible on non-hematopoietic cells (Donnelly et al. 2004; Moore et al. 2001).

Figure 1 illustrates some of the important targets of IL-10 signaling in persistent viral infections.

IL-10 aborts T cell responses when present during priming and can inhibit ongoing T cell activity to viral infections (Brooks et al. 2006b; Ejrnaes et al. 2006; Groux et al. 1996; Steinbrink et al. 1997). It acts directly on antigen-presenting cells to decrease stimulatory molecule expression (i.e., MHC class I and class II, B7-1, B7-2), alter cytokine production, and prevent maturation ultimately dampening T cell activation (Carbonneil et al. 2004; Fiorentino et al. 1991; Moore et al. 2001; Steinbrink et al. 1997). In addition to these indirect effects, IL-10 can also act directly on T cells to limit proliferation, functional differentiation, and effector activity (Brooks et al. 2010; Maynard and Weaver 2008). Although controversial, emerging data also indicate that genetic polymorphisms in the IL-10 promoter that result in lower IL-10 production are associated with clearance of HCV infection and enhanced viral control during chronic HCV, HBV, HIV, and EBV infections further supporting the important role of this cytokine in host

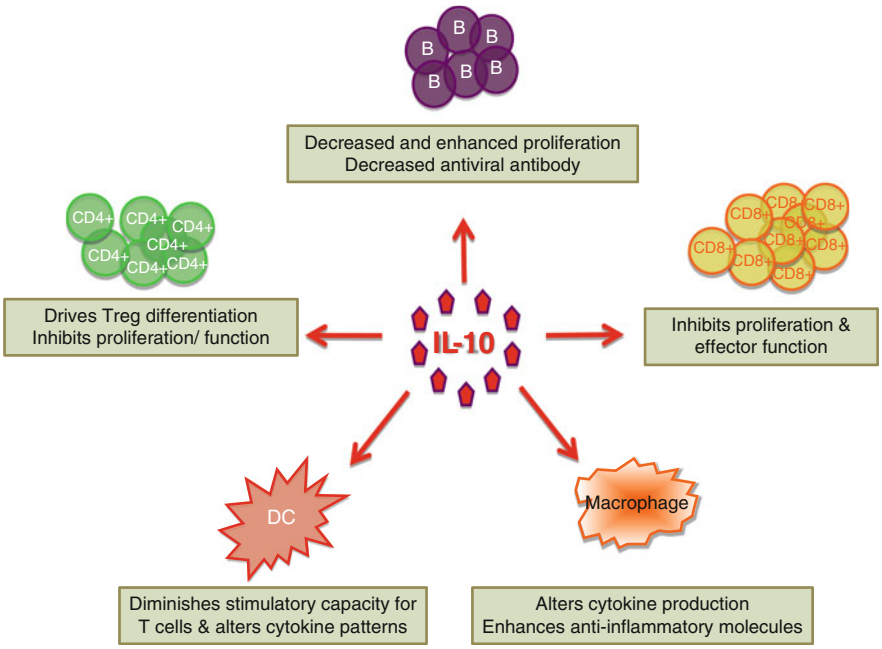

Fig. 1 Effects of IL-10 on distinct cellular targets during persistent viral infections: IL-10-mediated immunosuppression in response to viral infection occurs via both direct and indirect modulation of APC function, T and B cell activity. This figure denotes some of the important functional consequences of IL-10 targeting of distinct immune subsets. Note that IL-10 likely acts on multiple other immune and non-immune cells during viral persistence and the overall influence of IL-10 is likely a summation of all these effects. Additional mechanisms of IL-10-mediated immune modulation have been described in bacterial and autoimmune models, and investigating these pathways in viral persistence will be informative

immunosuppression (Cheong et al. 2006; Helminen et al. 1999; Paladino et al. 2006; Shin et al. 2000, 2003).

Counter to its negative regulatory functions, IL-10 can positively stimulate NK cells, in some instances CD8 T cells and induce B cell proliferation and antibody production (Foulds et al. 2006; Kang and Allen 2005; Moore et al. 2001). Thus, although generally immunosuppressive, IL-10 can function through a variety of mechanisms (likely simultaneously) to fine-tune the pathogen-specific immune response. In total, these data emphasize the diverse functions (many of which may be pathogen specific) that IL-10 plays to regulate multiple immune parameters.

IL-10 plays a major role in limiting autoimmune disease under steady-state conditions by controlling circulating, self- or gut microbiota-reactive T cells. Consistent with this role, IL-10 knockout (KO) mice develop inflammatory bowel disease (IBD)/colitis, approximately 8 weeks after birth (Davidson et al. 1996; Kuhn et al. 1993). Both CD4 T cells as well as resident enteric bacteria were required for the emergence of colitis indicating that under steady-state conditions IL-10 serves to suppress immune activation against the endogenous gut microbiota (Kuhn et al. 1993; Sellon et al. 1998). Although autoimmunity was not generally observed in other organs of IL-10 deficient mice, it is likely that the cytokine also negatively regulates immunity in other tissue compartments. Specific incidence of IBD and colitis demonstrate the key importance of IL-10 in immune homeostasis to gut antigens.

3.2 Sources, Mechanisms, and Targets of IL-10 During Persistent Viral Infection

Sources: As discussed earlier, IL-10 can be produced by a variety of cell types to regulate their own and other cells functions. During persistent viral infection, multiple cell types have been identified to produce IL-10, generally with immunosuppressive effects (Belkaid and Tarbell 2009; Rouse et al. 2006). The dominant IL-10 producing cell type varies with different viral infections likely reflecting inherent differences in the pathogen-specific response as well as tissue-specific immune regulation. Although individual IL-10 expressing cells may exert some level of suppression, it is likely that during persistent viral infections, the suppressive state mediated by IL-10 is maintained by multiple cells types that individually limit a variety of immune parameters, with the final effect being the inability to clear infection.

CD4 T cells were the first identified and are probably the most recognized IL-10 producing cell type. Upon activation and in response to the antigenic environment, CD4 T cells differentiate into multiple Th subsets. These different subsets have both unique and overlapping qualities with multiple Th subsets capable of producing IL-10 (Maynard and Weaver 2008). Initially identified as a Th2 cytokine (Fiorentino et al. 1989), IL-10 can also be produced by Th1, Th17, T follicular

helper and both natural (Foxp3+) and induced (Foxp3-) T regulatory (Treg) cells (Maynard and Weaver 2008). In general, IL-10 production by CD4 T cells during viral persistence is associated with the inducible Tr1 Treg population consisting of both virus-specific and nonspecific cells (Rouse et al. 2006). The mechanism of Tr1 cell emergence in persistent infection is not known. They may arise nonspecifically as a bystander effect of general T cell activation, they may be preferentially induced in an effort to limit excessive immunopathology, or they could have deliberately evolved to arise in instances of prolonged immune activation. Finally, a variety of IL-10 expressing CD4 Th cells may be lumped into the Tr1 category with "regulation" as only one of their potentially diverse functions.

The actual impact of Treg produced IL-10 in limiting virus-specific responses during persistent viral infection remains largely unclear. IL-10-mediated Treg activity during viral persistence is observed following infection of mice with Friend virus (FV). IL-10 expressing CD4 Treg cells are activated following FV infection and limit antiviral CD8 T cell responses in vivo facilitating increased persistent virus replication (Dittmer et al. 2004). However, inhibition of Treg activity alone did not enhance control of virus replication, which required the adoptive transfer of large amounts of FV-specific CD8 T cells that could now function in the absence of IL-10 signals. Interestingly, FV-induced immunosuppression was IL-10-dependent. However, IL-10 was not the Treg effector mechanism required to suppress CD8 T cells (Dittmer et al. 2002, 2004). IL-10 present during CD4 T cell priming can induce anergy, but also programs the development of additional IL-10 producing CD4 T cells (Groux et al. 1996, 1997). In such a manner, Treg produced IL-10 could promote the differentiation of more Treg cells as opposed to directly suppressing antiviral function, the latter being performed by other Treg-produced inhibitory mechanims. Thus, IL-10 would be important for suppressing T cell responses, but would not itself be the direct effector mechanism.

Early following persistent LCMV infection, virus-specific IL-10 producing CD4 T cells are observed, but IL-10 protein expression rapidly decreases in conjunction with other Th1 cytokines (Brooks et al. 2005). IL-10 producing CD4 T cells are observed throughout persistent LCMV infection; however, they are relegated to the non-virus-specific CD4 T cell subset (Brooks et al. 2006b; D. Brooks, unpublished observation). Further, the amount of non-LCMV-specific IL-10 producing CD4 T cells in the spleen is similar during acute and persistent LCMV infection (E. Wilson and D. Brooks, unpublished observation). It will ultimately be interesting and important to determine whether these non-LCMV-specific CD4 T cells regulate the virus-specific immune response and the outcome of deleting these cells toward clearance of persistent LCMV infection.

In addition to CD4 T cells, IL-10 producing CD8 T cells, monocytes/macrophages, dendritic cells, B cells, and NK cells are observed during persistent viral infections (Couper et al. 2008). During persistent LCMV infection, IL-10 is produced by multiple cell types including NK cells, DC, and B cells (Brooks et al. 2006b) all of which likely contribute to immune regulation. MCMV persistence in the salivary gland of infected mice is dependent on IL-10 producing CD4 T cells (Humphreys et al. 2007), whereas B cell produced IL-10 suppresses CD8 T cells in

the spleen during MCMV infection (Madan et al. 2009). Thus, different cell types utilize IL-10 in a compartmentalized fashion to suppress distinct facets of immunity in the same host during persistent infection. A multicell-mediated IL-10 response is also observed during HIV infection (Brockman et al. 2009; Yang et al. 2009), with monocyte/macrophages often comprising the largest IL-10 producing subset in the peripheral blood (Hagiwara et al. 1995; Kumar et al. 1998; Said et al. 2010). However, whether IL-10 producing cells differ in PBMC and tissue during HIV infection, and if so, which cells produce IL-10 in various tissue compartments, remains to be determined.

It will ultimately be critical to establish whether persistent viral infections are the result of IL-10 production by a single cell type with other IL-10 producing cells playing an auxiliary role or whether IL-10 production by multiple cell types is necessary. In the case of the former, it will be important to define what cell type produces the "relevant" IL-10 and how it aborts immunity. In the latter case, how each cell type suppresses individual immune components will need to be determined. Answers to these questions are critical from both a biologic standpoint to define the pathogenesis of persistent infection as well as from a therapeutic standpoint to modulate IL-10 expression by cells inhibiting antiviral activity while leaving other IL-10 producing cells intact to prevent autoimmunity and immunopathology.

Perhaps most strongly corroborating the importance of IL-10 toward viral persistence, several persistent viruses encode their own IL-10 homologs, including EBV, HCMV, and some poxviruses to modulate the immune response and facilitate replication, spread, and/or persistence (Slobedman et al. 2009). The first viral IL-10 (vIL-10) homolog to be identified was encoded by EBV with ebvIL-10 exhibiting ~70% amino acid sequence identity with human IL-10 (hIL-10) (Moore et al. 1990). EBV infects B cells leading to latent infection and in some cases B cell transformation. Both hIL-10 and ebvIL-10 have similar immunosuppressive activity and stimulated B cell proliferation, differentiation, and antibody production (Slobedman et al. 2009). However, ebvIL-10 had ~1,000-fold lower affinity for the cellular IL-10R and failed to promote MHC class II upregulation by B cells or to inhibit IL-2 production by CD4 T cells (Liu et al. 1997; Slobedman et al. 2009). Thus, in addition to its suppressive role permitting immune escape, an important function of ebvIL-10 may be to target B cell proliferation and differentiation thereby increasing the amount, permissiveness, and/or transformation of infected cells without affecting the immune-stimulatory capacity. Similarly, HCMV encodes an IL-10 homolog with 27% identity to hIL-10 (Kotenko et al. 2000; Lockridge et al. 2000). hIL-10 and cmvIL-10 exhibit similar immunosuppressive and stimulatory characteristics: inhibiting LPS-induced DC maturation, cytokine production, and upregulation of multiple T cell costimulatory molecules (Chang et al. 2004; Raftery et al. 2004). cmvIL-10 also inhibited type I interferon production by pDC, a major source of type I interferon during viral infection (Chang et al. 2009). As discussed, type I interferons stimulate the virus-specific immune response and trigger a general antiviral state, but they also potently block HCMV infection. As a result, cmvIL-10 may enhance the spread of HCMV while

simultaneously suppressing the early immune response. Interestingly, in vivo infection of mouse DC by murine CMV (MCMV) induced many of these same immunosuppressive effects despite not encoding an IL-10 homolog (Andrews et al. 2001). Thus, in addition to the direct affect of HCMV encoded IL-10, HCMV replication in DC in vivo may itself trigger an immunosuppressive program. During viral latency, HCMV produces a shorter differentially spliced IL-10 variant sharing some of the immunosuppressive qualities of the cmvIL-10 produced during productive infection (e.g., down-regulation of MHC class II on monocytes); however, this homolog did not suppress DC maturation, costimulatory molecule induction, or induce proliferation of a B cell line (Jenkins et al. 2004, 2008; Spencer et al. 2008). The decreased expression of MHC II inhibited CD4 T cell identification of latently infected cells allowing HCMV to evade immune recognition without affecting other immune functions that may compromise infection (Cheung et al. 2009). Thus, in its lifecycle, HCMV utilizes different IL-10 mediated suppressive mechanisms at different stages to persist.

Induction: One constant among persistent viruses is elevated expression of IL-10 and its direct correlation with viral replication (Brockman et al. 2009; Brooks et al. 2010; Cacciarelli et al. 1996; Yang et al. 2009). In addition to stimulatory factors, virus replication inherently triggers counter-regulatory measures to ultimately contain the immune response. Many signals inherent to immune activation induce IL-10 expression, but the precise "sensors" of prolonged/heightened virus replication during persistent infection are yet to be determined. Pathogen-specific IL-10 induction is likely achieved through the integration of multiple virus- and host-derived mechanisms and therefore will likely be dictated in a conserved and in a pathogen-specific manner. It is possible that the same mechanisms responsible for the initial recognition of viral infection and induction of IL-10 continue to function throughout persistent infection. On the other hand (but certainly excluding the latter), prolonged/elevated viral levels may trigger subsequent factors that serve to continually stimulate IL-10 production.

As discussed in the introduction, the innate immune system initially senses viral infection via pattern recognition receptors (including multiple TLRs) leading to type I interferon production and activation of the immune response (Koyama et al. 2008; Schlee and Hartmann 2010). However, TLR signaling also induces counter-regulatory molecules, including IL-10 (Saraiva and O'Garra 2010). Components of HCV, CMV, EBV, and LCMV all bind to TLR2 and TLR2, which in turn can induce IL-10 expression via recruitment of the signaling adaptor MyD88 and activation of ERK pathways (Ariza et al. 2009; Compton et al. 2003; Dolganiuc et al. 2006; Zhou et al. 2008). In humans, HIV glycoprotein binding to a mannose C-type lectin receptor (likely DC-SIGN) on the surface of monocyte derived-DC led to IL-10 expression (Shan et al. 2007). In addition to stimulating IL-10, HIV, and LCMV, infections also lead to dysregulated type I interferon production promoting a suppressive environment and further dampening the antiviral response (Taylor et al. 1999; Zuniga et al. 2008). This is also true for HCV, where interaction of the core protein with TLR2 results not only in upregulation of IL-10 expression but also in decreased expression of type I interferon by Kupffer cells and pDC

(Dolganiuc et al. 2006; Tu et al. 2010). A second HCV protein, NS3, concurrently upregulated IL-10 and down-regulated IL-12 expression by macrophages and DC, leading to diminished T cell stimulatory capacity in vitro (Dolganiuc et al. 2003; Eisen-Vandervelde et al. 2004). Therefore, increased/prolonged levels of antigen may continue to trigger these same innate receptors throughout infection leading to sustained IL-10 expression while simultaneously down-modulating stimulatory factors and potentiating the immunosuppressive environment.

Continued viral infection also stimulates the de novo expression of factors that potentially modulate IL-10 expression. PD-1/PD-L1 interaction suppresses antiviral T cell activity during persistent viral infection, and has also been shown to increase IL-10 expression (Dong et al. 1999). A recent study (Said et al. 2010) demonstrated that peripheral blood monocytes from HIV viremic individuals express high levels of PD-1. Stimulation of PD-1 with antibody or PD-L1 transfected cells induced IL-10 expression capable of limiting CD4 T cell proliferation in vitro. These data demonstrate that PD-1 stimulation can activate IL-10 expression and suppression of antiviral immunity. Interestingly, during persistent LCMV infection, we observed similar levels of IL-10 RNA expression in wild-type and PD-L1 KO mice (Brooks et al. 2008a), indicating that PD-L1 and IL-10 largely interact via different pathways. Functioning through different suppressive pathways was also consistent with the ability of dual IL-10 and PD-L1 blockade to additively increase exhausted T cell function compared with either IL-10R or PD-L1 blockade alone (Brooks et al. 2008a). The difference between these studies may relate to the fact that although most of the monocytes express PD-1, very few produced IL-10 upon PD-1 triggering. As a result, in PD-L1 KO mice, the amount of IL-10 triggered by PD-1 on monocytes may not substantially impact the overall level of IL-10 expression. On the other hand, although only a small fraction of monocytes were stimulated to produce IL-10 by PD-1stimulation, these cells may be functionally distinct from other monocyte subsets and, therefore, may have an enhanced ability to affect T cell immunity while not contributing significantly to global IL-10 production. Thus, it remains to be determined whether the population of IL-10 producing monocytes in vivo impact CD4 T cell responses similarly to that observed in vitro.

We and others also recently identified the important and progressive role of IL-21 in sustaining CD8 T cell responses during prolonged periods of virus replication (Elsaesser et al. 2009; Frohlich et al. 2009; Yi et al. 2009). For us, these experiments were initiated in our effort to define the mechanism(s) that induce IL-10 during LCMV persistence and based on the known role of IL-21 in stimulating IL-10 expression (Spolski et al. 2009). However, no change in IL-10 expression was observed in mice lacking IL-21R expression (Frohlich et al. 2009; D.G. Brooks, unpublished observation). Further, we have not observed changes in IL-10 RNA or serum protein expression during persistent LCMV infection in mice deficient in factors that stimulate IL-10 in other models of disease (E. Wilson and D. Brooks, unpublished observations), including IL-27 and Galectin-1 (Ilarregui et al. 2009), TLR2 (Sing et al. 2002), and MyD88 (Boonstra et al. 2006). In total the discrepancy between IL-10 inducing factors in other disease models compared with persistent

virus infection again indicates that multiple regulatory mechanisms can be instituted to suppress immunity in a pathogen/disease-specific manner.

Another mechanism of IL-10 induction may not result specifically from alterations in factors produced, but instead changes in antigen-presenting cell subsets. One of the defining characteristics of persistent LCMV variants is their ability to bind with high affinity to their cellular receptor α-dystroglycan enabling efficient infection of dendritic cells (Cao et al. 1998; Smelt et al. 2001). Because of infection, DC become targets for CTL lysis and the loss of DC was associated with the ensuing immunosuppression (Borrow et al. 1995; Sevilla et al. 2004). In particular, the CD8α + DC subset is depleted during persistent LCMV infection and the remaining CD8α-DC were shown to increase IL-10 production by virus-specific CD4 T cells, which might in turn suppress antiviral CD8 T cell responses (Ejrnaes et al. 2006). The preferential killing of mature DC during HIV infection by NK cells in an IL-10-dependent fashion would similarly increase the frequency of immature DC and potentially augment T cell responses (Alter et al. 2010). Further, MCMV disruption of APC function leads to insufficient T cell activation, but the decreased levels of MHC may also prevent DC interaction with T cells, thereby effectively shifting the APC subsets that prime/sustain T cells. In reality, it is likely a culmination of all these events (and more yet to be discovered) that account for IL-10-mediated immune suppression to persistent viral infection. The elucidation of how IL-10 suppresses the immune response to facilitate persistence is actively being investigated in our laboratory, and we anticipate that the results of these studies will lead to important insight into how the host "senses" the level of virus replication then progressively translates those signals into immune suppression.

Targets: Since IL-10 has been shown to attenuate numerous important biological functions in persistent virus infections, it is likely that many relevant cellular targets of IL-10 exist. The sum of all the IL-10 induced events (in conjunction with those induced by other suppressive factors) act in concert to orchestrate immune suppression and facilitate viral persistence. Therapeutically, the targeting of multiple cell types by IL-10 means that alleviating IL-10-mediated immunosuppression would enhance several immune parameters compromised by persistent infection.

In HIV-infected individuals, IL-10 produced by PBMC inhibits CD4 and CD8 T cell proliferation and cytokine production and blockade of IL-10 efficiently restores these functions in vitro (Brockman et al. 2009; Clerici et al. 1994; Landay et al. 1996; Said et al. 2010). Interaction of HIV with DC stimulates IL-10 production resulting in multiple functional defects (Alter et al. 2010; Shan et al. 2007). Interestingly, immature and mature DC respond differently to HIV-induced IL-10 upregulation. Immature DC exhibits an aberrant resistance to NK cell-mediated cytolysis, whereas mature DCs are targeted and destroyed by DC (Alter et al. 2010). This APC switch results in an over represented presence of "toleregenic" DC during HIV infection that may fail to sustain T cell responses and/or ineffectively prime de novo T cell responses against evolving HIV mutants. There is also evidence that IL-10 augments B cell responses during HIV infection, inducing B cell exhaustion that may hinder antibody production (Moir et al. 2008). In some circumstances, IL-10 is a positive regulator of CD8 T cells (Foulds et al. 2006; Groux et al. 1998; Kang and

Allen 2005; Santin et al. 2000). In these situations, IL-2 is required for the stimulatory effect of IL-10 on CD8 T cells (Groux et al. 1998; Santin et al. 2000). However, IL-2 production by CD4 and CD8 T cells is rapidly lost during persistent infections (Brooks et al. 2005; Clerici et al. 1996; Klenerman and Hill 2005; Petrovas et al. 2006; Semmo et al. 2005; Wherry et al. 2003; Younes et al. 2003). Thus, the presence of IL-10 without IL-2 may lead to suppressive instead of stimulatory CD8 T cell programming and highlights the important interplay between stimulatory and suppressive factors that fine-tune the immune response to affect the outcome of infection.

We and others have clearly established the dominant role of IL-10 in facilitating LCMV persistence, and based on its translatability to human persistent viral infections, it is likely that LCMV will be an important system to address IL-10-induced immunosuppression. IL-10 is produced by multiple APC subsets during persistent LCMV infection (Brooks et al. 2006b). Although priming of virus-specific CD4 and CD8 T cells is initially effective during persistent infection (Brooks et al. 2006a), the subsequent interactions with APC (i.e., occurring after the initial priming events) may attenuate ongoing T cell responses. CD4 T cell help is critical during persistent LCMV infection to sustain antiviral immune responses (Battegay et al. 1994; Matloubian et al. 1994). IL-10 directly targets CD4 T cells during an acute LCMV infection (Brooks et al. 2010) and similar diminution/alteration of the CD4 response by IL-10 during viral persistence may attenuate help, further exasperating the debilitated immune response. IL-10 may also act directly on virus-specific CD8 T cells, B cells, and/or NK cells to attenuate their function and facilitate viral persistence. Identification of the relevant targets of IL-10 in vivo is currently underway and should yield important insight into the mechanisms that abort immune responses to facilitate viral persistence.

4 Targeting Immunosuppression: Potential Therapeutic Applications for Blocking IL-10

The initial finding that IL-10R blockade prevented T cell exhaustion and facilitated immune-mediated eradication of an otherwise persistent LCMV infection (Brooks et al. 2006b; Ejrnaes et al. 2006) was the first example that single factor could alone suppress antiviral immunity to prevent virus clearance. In addition to early blockade of IL-10 to prevent T cell exhaustion and LCMV persistence, late blockade of IL-10 activity also enhanced T cell responses leading to control of an established persistent infection (Brooks et al. 2008a, b). These findings indicate that IL-10 suppresses and can be targeted to restore antiviral immunity at multiple stages throughout persistent infection (Fig. 2). Similarly, IL-10R blockade prevents MCMV persistence, although in the latter case the enhanced antiviral effects were accompanied by increased immunopathology (Brooks et al. 2006b; Campbell et al. 2008; Ejrnaes et al. 2006; Humphreys et al. 2007; Oakley et al. 2008).

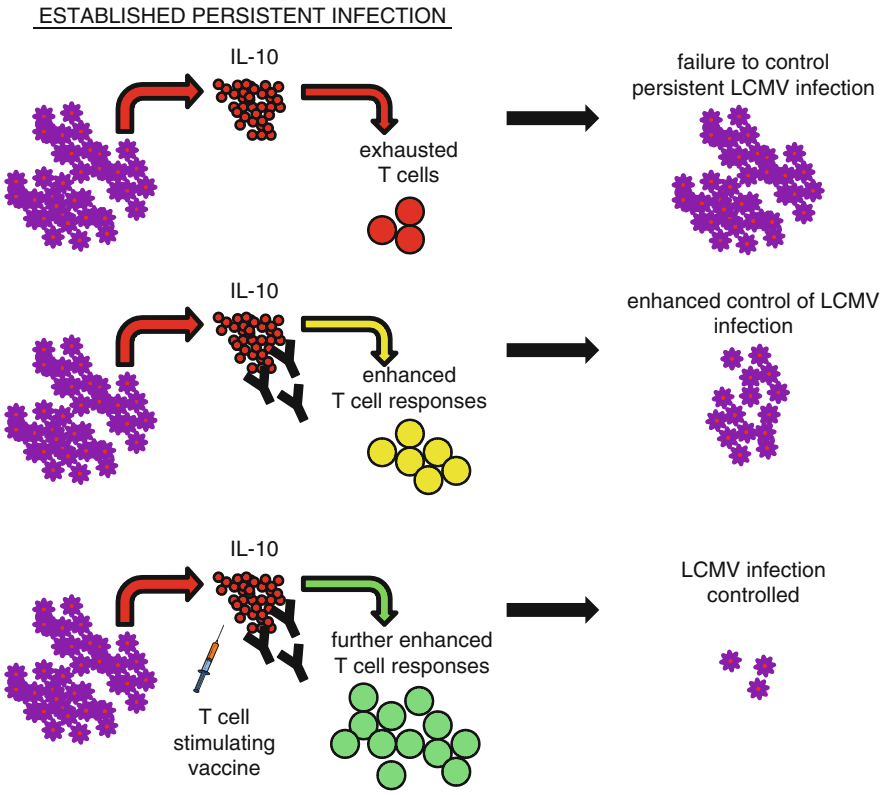

Fig. 2 Blocking IL-10 to enhance antiviral immunity and vaccine efficacy: Overcoming IL-10-mediated immunosuppression represents an exciting strategy to enhance antiviral T cell responses both alone and in combination with other immunotherapies. Antibody blockade of IL-10 activity alone boosts T cell function and enhances control of an established persistent LCMV infection. Further, by alleviating IL-10-mediated suppression, viral-specific T cells become reactive to otherwise ineffective therapeutic vaccines facilitating markedly enhanced T cell responses and control of persistent infection. The upregulation of IL-10 during HIV and HCV infections suggests that similar strategies may also effectively enhance antiviral immunity and control infection

Elevated serum levels of IL-10 are observed during many persistent virus infections in humans and, similar to LCMV infection, it correlates with diminished T cell activity and increased viral replication (Brady et al. 2003; Cacciarelli et al. 1996; Clerici et al. 1996; Dolganiuc et al. 2003; Marin-Serrano et al. 2006; Orsilles et al. 2006; Rico et al. 2001). Blockade of IL-10 in vitro restores function to HIV-specific and HCV-specific T cells (Cacciarelli et al. 1996; Clerici et al. 1994; Landay et al. 1996; Rigopoulou et al. 2005). Consistent with the correlation between HIV replication and IL-10 expression, antibody blockade of IL-10 only increased T cell function when cells were isolated from productively infected individuals and had minimal impact when cells originating from patients with effectively suppressed levels of HIV replication were analyzed (Brockman et al. 2009).

However, in a separate study, IL-10 blockade was shown to efficiently boost T cell responses even during effective anti-HIV therapy (in which HIV replication and IL-10 expression were low) (Yang et al. 2009). In total, these multiple studies demonstrate that blocking IL-10 during HIV infection can enhance antiviral T cell responses, but importantly, they indicate that IL-10 differentially affects different individuals and does so at distinct phases of infection.

The tight relationship between IL-10 and virus titers suggests that IL-10 may serve as a rheostat to constantly modulate immunity in relation to changing levels of virus replication. In agreement with this function, the experiments in which IL-10 was blocked to enhance HIV and HCV-specific responses were performed after cell isolation techniques that eliminated in vivo produced IL-10 (Brockman et al. 2009; Rigopoulou et al. 2005). These findings indicate that de novo IL-10 production continually suppress T cell responses, suggesting that T cells are teetering on a fine line between exhaustion and productive immunity. Once function is diminished, blockade of no single factor restores T cell activity to that observed during an acute infection. However, therapies that alleviate some level of suppression appear to propel T cells across that fine line to better fight infection.

Recent research has clearly established that multiple negative immune-regulatory mechanisms are invoked to suppress T cell responses during viral persistence (Blackburn et al. 2009). We recently demonstrated that at least two of these factors (i.e., IL-10 and PD-L1) operate through distinct pathways to suppress immunity (Brooks et al. 2008a). As a result, dual antibody blockade of IL-10 and PD-L1 during LCMV persistence significantly enhanced antiviral T cell responses compared with blockade of either factor alone and rapidly controlled systemic viral replication. Similarly, simultaneous blockade of PD-L1 and another inhibitory receptor Lag-3 further enhanced T cell responses despite Lag-3 blockade alone having only minimal effect (Blackburn et al. 2009; Richter et al. 2010). This is particularly important because it indicates that there are layers of immunosuppression and that some negative regulatory pathways are dominant over others during persistent infection. However, once the dominant suppression is relieved other factors that appear to have limited function may become relevant and serve as targets to additionally enhance antiviral immunity.

Because of the varied lifecycles of persistent viruses, it will ultimately be important to determine how IL-10 blockade (as well as blockade of other negative regulatory factors) impacts control of different viral infections. HIV rapidly establishes a long-lived latent reservoir that is able to rekindle infection after prolonged periods of virus control (Chun et al. 1997; Finzi et al. 1997; Wong et al. 1997). During latent infection, HIV remains hidden from immune recognition and as a result would not be targeted by therapies that amplify immune function (Brooks et al. 2003). However, in cases such as HCV infection, where a latent viral reservoir is not established and therapeutic elimination of infection can be achieved, overcoming exhaustion and boosting immunity by blocking IL-10 activity could further control HCV replication. Thus, on the one hand, blockade of IL-10 (or other regulatory factors) may facilitate control (perhaps even long-term control) over HIV infection, but may not be able to completely eradicate infection because of

immune-resistant latent reservoirs. On the other hand, a similar blockade of IL-10 during HCV infection may ultimately facilitate long-term clearance of infection due to the absence of a long-lived latent reservoir.

IL-10 also regulates immunity to acute viral infections, limiting the magnitude of the ensuing response, the production of effector cytokines and consequently immunopathology, but generally without substantially impacting viral titers or clearance kinetics. Infection of mice with Influenza often leads to severe immunopathology, morbidity, and mortality, and IL-10 limits these negative effects (Sun et al. 2009). Following influenza infection of mice, a large population of lung infiltrating CD4 and particularly CD8 T cells produce IL-10 (Sun et al. 2009). Blockade of IL-10 enhanced IFNγ production leading to increased immunopathology and mortality without impacting virus clearance. These data suggest that in response to unknown cues, virus-specific CD8 T cells are capable of producing IL-10 to curb their own responses. IL-10 expression also restricted CD4 T cell responses during influenza resulting in decreased antibody titers, whereas lack of IL-10 expression led to increased influenza-specific antibody production and enhanced survival (Sun and Metzger 2008; Sun et al. 2010). Interestingly, IL-10-mediated immunosuppression was linked to heightened susceptibility to secondary bacterial infection following influenza infection (van der Sluijs et al. 2004), although many factors likely contribute (Sun and Metzger 2008). IL-10 is rapidly upregulated following acute LCMV-Armstrong infection, and although not to the extent observed following persistent LCMV infection, it negatively regulated what is generally considered the "optimal" antiviral immune response (Brooks et al. 2010). Interestingly, blockade of IL-10 activity directly enhanced both the quality and the quantity of virus-specific CD4 T cell responses without affecting virus-specific CD8 T cells, illustrating the differential regulation of T cell subsets following infection. Minor decreases in virus titers were observed in acute LCMV infected, IL-10 deficient mice, although both wild-type and IL-10-deficient mice cleared virus with a comparable kinetic. Excitingly, this promiscuity of IL-10 further substantiates the ability to restore many diverse effector mechanisms by blocking a single molecule. The increased expression and inhibitory activity of IL-10 following acute viral infection suggest that IL-10 blockade may be an effective adjuvant to prophylactic (i.e., preventative) vaccines to further enhance immunity (Brooks et al. 2010; Darrah et al. 2010). This would be particularly true for vaccines in which heightened CD4 T cell responses would be beneficial, such as HCV wherein the strength of the CD4 T cell response is an important determinant of clearance (Gerlach et al. 1999; Grakoui et al. 2003; Thimme et al. 2001; Urbani et al. 2006a).

Unlike prophylactic vaccines that aim to engender immune memory de novo, therapeutic vaccines (i.e., vaccines delivered during an established viral infection) must rebuild a debilitated immune response to now overcome the infection that it could not initially control. Along this line, vaccine agents that are immunogenic when administered to antigen naïve individuals often fail to efficiently stimulate immunity when provided prophylactically (Autran et al. 2004). Additionally, many prophylactic vaccines rely on stimulating antibody production, whereas therapeutic

vaccines will likely have to restore/stimulate antiviral T cell responses as well as other immune parameters that are often refractory to further stimulation (Brooks et al. 2008b; Ha et al. 2008; Wherry et al. 2005; Zuniga et al. 2008). The finding that IL-10 actively inhibited T cell responses during persistent infection led us to hypothesize that one reason therapeutic vaccination strategies have thus far failed to resurrect/sustain T cell responses and control persistent infection is because they do not alleviate the immunosuppressive environment. Consequently, even if T cell responses could be restored, they would rapidly again succumb to the same constraints that had previously limited their responsiveness. Consistent with this mechanism, we demonstrated that antibody blockade of IL-10 during an established persistent viral infection permitted an otherwise ineffective DNA vaccine now highly efficient at stimulating CD4 and CD8 T cell responses leading to accelerated clearance of the persistent infection (Fig. 2) (Brooks et al. 2008b). In conjunction, Rafi Ahmed's group demonstrated that during persistent LCMV infection PD-L1 blockade similarly enhanced therapeutic vaccination with a live-replicating vaccine vector (Ha et al. 2008). Together, our findings established the immunosuppressive environment as an important factor inhibiting vaccination attempts to restore antiviral T cell function during persistent viral infection and suggested that blockade of negative immune-regulatory molecules may ultimately prove a powerful strategy to aid therapeutic vaccination and purge an established persistent viral infection.

5 Past, Present, and Future

The initial discovery of IL-10 as an inhibitor of Th1 differentiation has rapidly diversified such that now IL-10 is widely considered a "master-regulator" of host immunity. The conserved nature of IL-10-mediated suppression among evolutionarily distinct species and the ability to boost immune function by blocking a single factor, despite the presence of other very powerful negative immune regulators, is quite astounding and speaks clearly to the significance of this pathway. However, many important discoveries remain to be made concerning IL-10-mediated suppression and how best to manipulate it for therapeutic benefit. The promiscuity of IL-10 production and function suggests that its blockade could amplify multiple antiviral mechanisms to control persistent virus replication. By understanding how IL-10 regulates distinct components of the immune response, it may be possible to block IL-10 production by or function on certain cells and unleash antiviral T cells while maintaining regulation of those cells prevent immunopathology. Ultimately, blockade of immune-regulatory factors holds great promise as an approach to restore immunity and purge established persistent viral infections. The ability of IL-10 and other inhibitory factors to operate at distinct levels of immune function and on different cell subsets indicates the possibility of combinatorial blockade cocktails to specifically enhance desired immune cell subsets and evoke different immune responses; thus, paving the way into an age of rationale vaccine design.

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References

Agnellini P, Wolint P, Rehr M, Cahenzli J, Karrer U, Oxenius A (2007) Impaired NFAT nuclear translocation results in split exhaustion of virus-specific CD8+ T cell functions during chronic viral infection. Proc Natl Acad Sci U S A 104:4565–4570

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

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

Andrews DM, Andoniou CE, Granucci F, Ricciardi-Castagnoli P, Degli-Esposti MA (2001) Infection of dendritic cells by murine cytomegalovirus induces functional paralysis. Nat Immunol 2:1077–1084

Ariza ME, Glaser R, Kaumaya PT, Jones C, Williams MV (2009) The EBV-encoded dUTPase activates NF-kappa B through the TLR2 and MyD88-dependent signaling pathway. J Immunol 182:851–859

Autran B, Carcelain G, Combadiere B, Debre P (2004) Therapeutic vaccines for chronic infections. Science 305:205–208

Bachmann MF, Wolint P, Walton S, Schwarz K, Oxenius A (2007) Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections. Eur J Immunol 37:1502–1512

Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252

Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R (2005) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439:682–687

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

Battegay M, Moskophidis D, Rahemtulla A, Hengartner H, Mak TW, Zinkernagel RM (1994) Enhanced establishment of a virus carrier state in adult CD4+ T-cell-deficient mice. J Virol 68:4700–4704

Belkaid Y, Tarbell K (2009) Regulatory T cells in the control of host-microorganism interactions (*). Annu Rev Immunol 27:551–589

Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 17:189–220

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

Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, Laccabue D, Zerbini A, Cavalli A, Missale G et al (2007) Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol 81:4215–4225

Boonstra A, Rajsbaum R, Holman M, Marques R, Asselin-Paturel C, Pereira JP, Bates EE, Akira S, Vieira P, Liu YJ 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 177:7551–7558
Borrow P, Evans CF, Oldstone MB (1995) Virus-induced immunosuppression: immune system-mediated destruction of virus-infected dendritic cells results in generalized immune suppression. J Virol 69:1059–1070
Brady MT, MacDonald AJ, Rowan AG, Mills KH (2003) Hepatitis C virus non-structural protein 4 suppresses Th1 responses by stimulating IL-10 production from monocytes. Eur J Immunol 33:3448–3457
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 upregulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. Blood 114:346–356
Brooks DG, Hamer DH, Arlen PA, Gao L, Bristol G, Kitchen CM, Berger EA, Zack JA (2003) Molecular characterization, reactivation, and depletion of latent HIV. Immunity 19:413–423
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, 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, 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
Cacciarelli TV, Martinez OM, Gish RG, Villanueva JC, Krams SM (1996) Immunoregulatory cytokines in chronic hepatitis C virus infection: pre- and posttreatment with interferon alfa. Hepatology 24:6–9
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
Cao W, Henry MD, Borrow P, Yamada H, Elder JH, Ravkov EV, Nichol ST, Compans RW, Campbell KP, Oldstone MB (1998) Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. Science 282:2079–2081
Carbonneil C, Donkova-Petrini V, Aouba A, Weiss L (2004) Defective dendritic cell function in HIV-infected patients receiving effective highly active antiretroviral therapy: neutralization of IL-10 production and depletion of CD4+ CD25+ T cells restore high levels of HIV-specific CD4+ T cell responses induced by dendritic cells generated in the presence of IFN-alpha. J Immunol 172:7832–7840
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
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
Cheong JY, Cho SW, Hwang IL, Yoon SK, Lee JH, Park CS, Lee JE, Hahm KB, Kim JH (2006) Association between chronic hepatitis B virus infection and interleukin-10, tumor necrosis factor-alpha gene promoter polymorphisms. J Gastroenterol Hepatol 21:1163–1169
Cheung AK, Gottlieb DJ, Plachter B, Pepperl-Klindworth S, Avdic S, Cunningham AL, Abendroth A, Slobedman B (2009) The role of the human cytomegalovirus UL111A gene in

down-regulating CD4+ T-cell recognition of latently infected cells: implications for virus elimination during latency. Blood 114:4128–4137
Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS (1997) Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci U S A 94:13193–13197
Clerici M, Wynn TA, Berzofsky JA, Blatt SP, Hendrix CW, Sher A, Coffman RL, Shearer GM (1994) Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. J Clin Invest 93:768–775
Clerici M, Balotta C, Salvaggio A, Riva C, Trabattoni D, Papagno L, Berlusconi A, Rusconi S, Villa ML, Moroni M, Galli M (1996) Human immunodeficiency virus (HIV) phenotype and interleukin-2/ interleukin-10 ratio are associated markers of protection and progression in HIV infection. Blood 88:574–579
Compton T, Kurt-Jones EA, Boehme KW, Belko J, Latz E, Golenbock DT, Finberg RW (2003) Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. J Virol 77:4588–4596
Constant SL, Bottomly K (1997) Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. Annu Rev Immunol 15:297–322
Couper KN, Blount DG, Riley EM (2008) IL-10: the master regulator of immunity to infection. J Immunol 180:5771–5777
Darrah PA, Hegde ST, Patel DT, Lindsay RW, Chen L, Roederer M, Seder RA (2010) IL-10 production differentially influences the magnitude, quality, and protective capacity of Th1 responses depending on the vaccine platform. J Exp Med 207:1421–1433
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
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
Dittmer U, Race B, Peterson KE, Stromnes IM, Messer RJ, Hasenkrug KJ (2002) Essential roles for CD8+ T cells and gamma interferon in protection of mice against retrovirus-induced immunosuppression. J Virol 76:450–454
Dittmer U, He H, Messer RJ, Schimmer S, Olbrich AR, Ohlen C, Greenberg PD, Stromnes IM, Iwashiro M, Sakaguchi S et al (2004) Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. Immunity 20:293–303
Dolganiuc A, Kodys K, Kopasz A, Marshall C, Do T, Romics L Jr, Mandrekar P, Zapp M, Szabo G (2003) Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation. J Immunol 170: 5615–5624
Dolganiuc A, Chang S, Kodys K, Mandrekar P, Bakis G, Cormier M, Szabo G (2006) Hepatitis C virus (HCV) core protein-induced, monocyte-mediated mechanisms of reduced IFN-alpha and plasmacytoid dendritic cell loss in chronic HCV infection. J Immunol 177:6758–6768
Dong H, Zhu G, Tamada K, Chen L (1999) B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 5:1365–1369
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
Eisen-Vandervelde AL, Waggoner SN, Yao ZQ, Cale EM, Hahn CS, Hahn YS (2004) Hepatitis C virus core selectively suppresses interleukin-12 synthesis in human macrophages by interfering with AP-1 activation. J Biol Chem 279:43479–43486
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
Elsaesser H, Sauer K, Brooks DG (2009) IL-21 is required to control chronic viral infection. Science 324:1569–1572

Fahey LM, Brooks DG (2010) Opposing positive and negative regulation of T cell activity during viral persistence. Curr Opin Immunol 22:348–354

Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R et al (1997) Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278:1295–1300

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

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

Foulds KE, Rotte MJ, Seder RA (2006) IL-10 is required for optimal CD8 T cell memory following Listeria monocytogenes infection. J Immunol 177:2565–2574

Frohlich A, Kisielow J, Schmitz I, Freigang S, Shamshiev AT, Weber J, Marsland BJ, Oxenius A, Kopf M (2009) IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science 324:1576–1580

Gallimore A, Glithero A, Godkin A, Tissot AC, Pluckthun A, Elliott T, Hengartner H, Zinkernagel R (1998) Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. J Exp Med 187:1383–1393

Gartel AL, Serfas MS, Tyner AL (1996) p21–negative regulator of the cell cycle. Proc Soc Exp Biol Med 213:138–149

Gerlach JT, Diepolder HM, Jung MC, Gruener NH, Schraut WW, Zachoval R, Hoffmann R, Schirren CA, Santantonio T, Pape GR (1999) Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. Gastroenterology 117:933–941

Goodbourn S, Didcock L, Randall RE (2000) Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J Gen Virol 81:2341–2364

Grakoui A, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghrayeb J, Murthy KK, Rice CM, Walker CM (2003) HCV persistence and immune evasion in the absence of memory T cell help. Science 302:659–662

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, 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

Groux H, Bigler M, de Vries JE, Roncarolo MG (1998) Inhibitory and stimulatory effects of IL-10 on human CD8+ T cells. J Immunol 160:3188–3193

Ha SJ, Mueller SN, Wherry EJ, Barber DL, Aubert RD, Sharpe AH, Freeman GJ, Ahmed R (2008) Enhancing therapeutic vaccination by blocking PD-1-mediated inhibitory signals during chronic infection. J Exp Med 205:543–555

Hagiwara E, Abbasi F, Mor G, Ishigatsubo Y, Klinman DM (1995) Phenotype and frequency of cells secreting IL-2, IL-4, IL-6, IL-10, IFN and TNF-alpha in human peripheral blood. Cytokine 7:815–822

Hedrich CM, Bream JH (2010) Cell type-specific regulation of IL-10 expression in inflammation and disease. Immunol Res 47:185–206

Helminen M, Lahdenpohja N, Hurme M (1999) Polymorphism of the interleukin-10 gene is associated with susceptibility to Epstein-Barr virus infection. J Infect Dis 180:496–499

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

Ilarregui JM, Croci DO, Bianco GA, Toscano MA, Salatino M, Vermeulen ME, Geffner JR, Rabinovich GA (2009) Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. Nat Immunol 10:981–991

Jenkins C, Abendroth A, Slobedman B (2004) A novel viral transcript with homology to human interleukin-10 is expressed during latent human cytomegalovirus infection. J Virol 78:1440–1447

Jenkins C, Garcia W, Godwin MJ, Spencer JV, Stern JL, Abendroth A, Slobedman B (2008) 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

Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, Wong JC, Satkunarajah M, Schweneker M, Chapman JM et al (2008) Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. J Exp Med 205:2763–2779

Kaech SM, Hemby S, Kersh E, Ahmed R (2002) Molecular and functional profiling of memory CD8 T cell differentiation. Cell 111:837–851

Kang SS, Allen PM (2005) Priming in the presence of IL-10 results in direct enhancement of CD8+ T cell primary responses and inhibition of secondary responses. J Immunol 174: 5382–5389

Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, Palmer S, Brockman M, Rathod A, Piechocka-Trocha A et al (2007) Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nat Immunol 8:1246–1254

Klenerman P, Hill A (2005) T cells and viral persistence: lessons from diverse infections. Nat Immunol 6:873–879

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 U S A 97:1695–1700

Koyama S, Ishii KJ, Coban C, Akira S (2008) Innate immune response to viral infection. Cytokine 43:336–341

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

Kumar A, Angel JB, Daftarian MP, Parato K, Cameron WD, Filion L, Diaz-Mitoma F (1998) Differential production of IL-10 by T cells and monocytes of HIV-infected individuals: association of IL-10 production with CD28-mediated immune responsiveness. Clin Exp Immunol 114:78–86

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

Lin MT, Hinton DR, Parra B, Stohlman SA, van der Veen RC (1998) The role of IL-10 in mouse hepatitis virus-induced demyelinating encephalomyelitis. Virology 245:270–280

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

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

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

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

Marin-Serrano E, Rodriguez-Ramos C, Diaz F, Martin-Herrera L, Giron-Gonzalez JA (2006) Modulation of the anti-inflammatory interleukin 10 and of proapoptotic IL-18 in patients with chronic hepatitis C treated with interferon alpha and ribavirin. J Viral Hepat 13:230–234

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
Matter M, Odermatt B, Yagita H, Nuoffer JM, Ochsenbein AF (2006) Elimination of chronic viral infection by blocking CD27 signaling. J Exp Med 203:2145–2155
Maynard CL, Weaver CT (2008) Diversity in the contribution of interleukin-10 to T-cell-mediated immune regulation. Immunol Rev 226:219–233
Mege JL, Meghari S, Honstettre A, Capo C, Raoult D (2006) The two faces of interleukin 10 in human infectious diseases. Lancet Infect Dis 6:557–569
Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, Roby G, Kottilil S, Arthos J, Proschan MA et al (2008) Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med 205:1797–1805
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
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
Oakley OR, Garvy BA, Humphreys S, Qureshi MH, Pomeroy C (2008) Increased weight loss with reduced viral replication in interleukin-10 knock-out mice infected with murine cytomegalovirus. Clin Exp Immunol 151:155–164
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 114:55–60
Paladino N, Fainboim H, Theiler G, Schroder T, Munoz AE, Flores AC, Galdame O, Fainboim L (2006) Gender susceptibility to chronic hepatitis C virus infection associated with interleukin 10 promoter polymorphism. J Virol 80:9144–9150
Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, Precopio ML, Schacker T, Roederer M, Douek DC, Koup RA (2006) PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. J Exp Med 203:2281–2292
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
Richter K, Agnellini P, Oxenius A (2010) On the role of the inhibitory receptor LAG-3 in acute and chronic LCMV infection. Int Immunol 22:13–23
Rico MA, Quiroga JA, Subira D, Castanon S, Esteban JM, Pardo M, Carreno V (2001) Hepatitis B virus-specific T-cell proliferation and cytokine secretion in chronic hepatitis B e antibody-positive patients treated with ribavirin and interferon alpha. Hepatology 33:295–300
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
Rouse BT, Sarangi PP, Suvas S (2006) Regulatory T cells in virus infections. Immunol Rev 212:272–286
Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, Hill BJ, Noto A, Ancuta P, Peretz Y et al (2010) Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nat Med 16:452–459
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
Saraiva M, O'Garra A (2010) The regulation of IL-10 production by immune cells. Nat Rev Immunol 10:170–181
Schlee M, Hartmann G (2010) The chase for the RIG-I ligand – recent advances. Mol Ther 18:1254–1262
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

Semmo N, Day CL, Ward SM, Lucas M, Harcourt G, Loughry A, Klenerman P (2005) Preferential loss of IL-2-secreting CD4+ T helper cells in chronic HCV infection. Hepatology 41:1019–1028

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

Shan M, Klasse PJ, Banerjee K, Dey AK, Iyer SP, Dionisio R, Charles D, Campbell-Gardener L, Olson WC, Sanders RW, Moore JP (2007) HIV-1 gp120 mannoses induce immunosuppressive responses from dendritic cells. PLoS Pathog 3:e169

Shin HD, Winkler C, Stephens JC, Bream J, Young H, Goedert JJ, O'Brien TR, Vlahov D, Buchbinder S, Giorgi J et al (2000) Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. Proc Natl Acad Sci U S A 97:14467–14472

Shin HD, Park BL, Kim LH, Jung JH, Kim JY, Yoon JH, Kim YJ, Lee HS (2003) Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. Hum Mol Genet 12:901–906

Shin H, Blackburn SD, Intlekofer AM, Kao C, Angelosanto JM, Reiner SL, Wherry EJ (2009) A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. Immunity 31:309–320

Sing A, Roggenkamp A, Geiger AM, Heesemann J (2002) Yersinia enterocolitica evasion of the host innate immune response by V antigen-induced IL-10 production of macrophages is abrogated in IL-10-deficient mice. J Immunol 168:1315–1321

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

Smelt SC, Borrow P, Kunz S, Cao W, Tishon A, Lewicki H, Campbell KP, Oldstone MB (2001) Differences in affinity of binding of lymphocytic choriomeningitis virus strains to the cellular receptor alpha-dystroglycan correlate with viral tropism and disease kinetics. J Virol 75:448–457

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

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

Spolski R, Kim HP, Zhu W, Levy DE, Leonard WJ (2009) IL-21 mediates suppressive effects via its induction of IL-10. J Immunol 182:2859–2867

Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH (1997) Induction of tolerance by IL-10-treated dendritic cells. J Immunol 159:4772–4780

Sun K, Metzger DW (2008) Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. Nat Med 14:558–564

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 K, Torres L, Metzger DW (2010) A detrimental effect of interleukin-10 on protective pulmonary humoral immunity during primary influenza A virus infection. J Virol 84: 5007–5014

Tanaka N, Sato M, Lamphier MS, Nozawa H, Oda E, Noguchi S, Schreiber RD, Tsujimoto Y, Taniguchi T (1998) Type I interferons are essential mediators of apoptotic death in virally infected cells. Genes Cells 3:29–37

Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM (1999) Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science 285:107–110

Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV (2001) Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 194:1395–1406

Tinoco R, Alcalde V, Yang Y, Sauer K, Zuniga EI (2009) Cell-intrinsic transforming growth factor-beta signaling mediates virus-specific CD8+ T cell deletion and viral persistence in vivo. Immunity 31:145–157

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

Tu Z, Pierce RH, Kurtis J, Kuroki Y, Crispe IN, Orloff MS (2010) Hepatitis C virus core protein subverts the antiviral activities of human Kupffer cells. Gastroenterology 138:305–314

Urbani S, Amadei B, Fisicaro P, Tola D, Orlandini A, Sacchelli L, Mori C, Missale G, Ferrari C (2006a) Outcome of acute hepatitis C is related to virus-specific CD4 function and maturation of antiviral memory CD8 responses. Hepatology 44:126–139

Urbani S, Amadei B, Tola D, Massari M, Schivazappa S, Missale G, Ferrari C (2006b) PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. J Virol 80:11398–11403

van der Sluijs KF, van Elden LJ, Nijhuis M, Schuurman R, Pater JM, Florquin S, Goldman M, Jansen HM, Lutter R, van der Poll T (2004) IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. J Immunol 172:7603–7609

Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, Vanderford TH, Chennareddi L, Silvestri G, Freeman GJ et al (2008) Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature 458:206–210

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, Blattman JN, Ahmed R (2005) Low CD8 T-cell proliferative potential and high viral load limit the effectiveness of therapeutic vaccination. J Virol 79:8960–8968

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

Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, Richman DD (1997) Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 278:1291–1295

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

Yi JS, Du M, Zajac AJ (2009) A vital role for interleukin-21 in the control of a chronic viral infection. Science 324:1572–1576

Younes SA, Yassine-Diab B, Dumont AR, Boulassel MR, Grossman Z, Routy JP, Sekaly RP (2003) HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. J Exp Med 198:1909–1922

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

Zhou S, Halle A, Kurt-Jones EA, Cerny AM, Porpiglia E, Rogers M, Golenbock DT, Finberg RW (2008) Lymphocytic choriomeningitis virus (LCMV) infection of CNS glial cells results in TLR2-MyD88/Mal-dependent inflammatory responses. J Neuroimmunol 194:70–82

Zuniga EI, Liou LY, Mack L, Mendoza M, Oldstone MB (2008) Persistent virus infection inhibits type I interferon production by plasmacytoid dendritic cells to facilitate opportunistic infections. Cell Host Microbe 4:374–386

Inhibitory Ly49 Receptors on Mouse Natural Killer Cells

Mark T. Orr and Lewis L. Lanier

Contents

Abstract The Ly49 receptors, which are expressed in a stochastic manner on subsets of murine natural killer (NK) cells, T cells, and other cells, are encoded by the *Klra* gene family and include receptors with either inhibitory or activating function. All of the inhibitory Ly49 receptors are characterized by an immunoreceptor tyrosine-based inhibitory motif in their cytoplasmic domain, which upon phosphorylation recruits tyrosine or lipid phosphatases to dampen signals transmitted through other activating receptors. Most of the inhibitory Ly49 receptors recognize polymorphic epitopes on major histocompatibility complex (MHC) class I proteins as ligands. Here, we review the polymorphism, ligand specificity, and signaling capacity of the inhibitory Ly49 receptors and discuss how these molecules regulate NK cell development and function.

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1 Introduction

The Ly49 receptors are type II C-type lectin-like glycoproteins encoded by a polygenic and polymorphic gene family designated *Klra*. The family includes genes encoding both inhibitory receptors that contain a single immunoreceptor tyrosine-based inhibitory motif (ITIM) and activating receptors that lack an intracellular signaling motif but instead non-covalently associate with the DAP10 adapter and/or the immunoreceptor tyrosine-based activating motif (ITAM)-containing adapter DAP12 (Orr et al. 2009; Smith et al. 1998). Expression of the activating receptors is restricted to natural killer (NK) cells, whereas inhibitory receptor family members are expressed predominantly by NK cells, but also by subsets of NKT cells, $CD4^+$ T cells, $CD8^+$ T cells, and myeloid cells (Vivier and Anfossi 2004). Although the Ly49 receptors are structurally related to the C-type lectins, they lack a Ca^{2+}-dependent carbohydrate recognition domain and do not bind to carbohydrate ligands. Instead, Ly49 receptors bind to major histocompatibility complex (MHC) class I and MHC class I-like proteins. Along with the MHC class I molecules, the Ly49 family is the fastest evolving gene family in rodents. Other species, including rats, cows, and horses, have multiple *Ly49* genes in their genomes, whereas cats, dogs, pigs, and orangutans possess only a single *Ly49* gene and in humans the only Ly49 locus, *KLRA1*, is a pseudogene (Gagnier et al. 2003). In humans, the functional counterpart of the Ly49 family is the killer cell immunoglobulin-like receptor (KIR) gene family, which encodes activating and inhibitory receptors that bind HLA class I molecules as ligands.

2 Ly49 Haplotypes and Ligands

The *Ly49* (*Klra*) gene family resides within the NK complex (NKC) on mouse chromosome 6 (Yokoyama and Plougastel 2003). Four haplotypes of the Ly49 family have been determined in inbred mice (Fig. 1) (Carlyle et al. 2008). Since MHC class I molecules are the predominant ligands for inhibitory Ly49 receptors, it is important to consider both the Ly49 receptor haplotype and MHC class I haplotype when studying NK cell functions. A minimal structure is conserved among all four Ly49 receptor haplotypes, three pairs of framework inhibitory receptors (*a* and *c*, *g* and *i*, and *e* and *q*) interspersed with a variable number of genes encoding inhibitory or activating receptors, as well as pseudogenes, most of which resemble activating receptor genes (Carlyle et al. 2008). Additionally, the *Klra2* (Ly49b) gene is retained in all strains, but lies outside of the *Ly49* gene cluster. The BALB/c ($H\text{-}2^d$) Ly49 haplotype, shared with AKR ($H\text{-}2^k$), DBA/2 ($H\text{-}2^d$), C3H/He ($H\text{-}2^k$), CBA/J ($H\text{-}2^k$), and A/J ($H\text{-}2^a$) stains of mice, contains only the additional *Ly49l* activating receptor gene and the *Ly49y* pseudogene (Proteau et al. 2004). C57BL/6 ($H\text{-}2^b$) mice contain fifteen *Ly49* genes, including genes encoding the six framework inhibitory receptors, two additional inhibitory

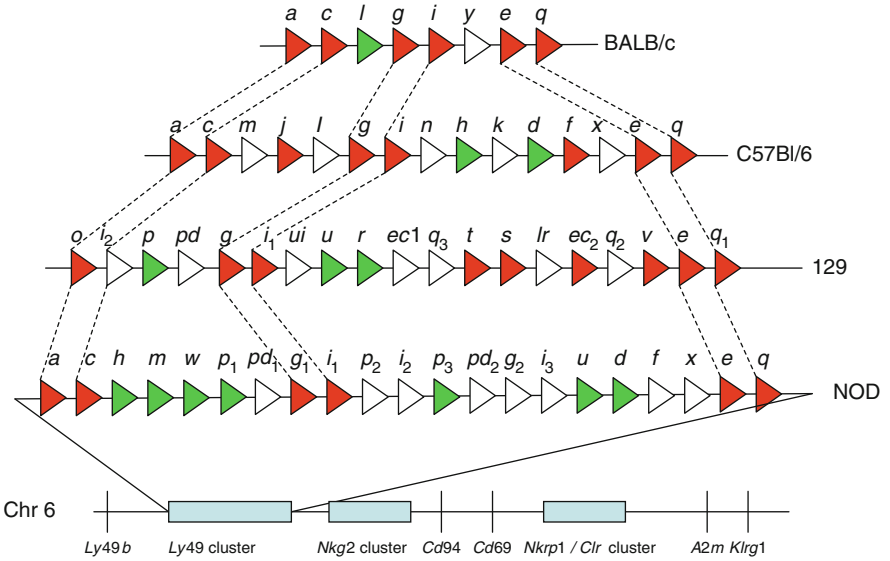

Fig. 1 Schematic of the Ly49 locus in different haplotypes. Activating Ly49 family members are in *green*, inhibitory Ly49 family members are in *red*, and pseudogenes are in *white*. The locus is drawn telomeric to centromeric with the direction of transcription indicated. The conserved framework inhibitory receptors are connected via *dashed lines*

receptors (*Ly49f* and *j*), two activating receptors (*Ly49d* and *h*), and five pseudogenes (Brown et al. 1997; Depatie et al. 2000; McQueen et al. 1998, 1999; Smith et al. 1994; Takei et al. 1997; Wilhelm et al. 2002; Wong et al. 1991). The 129 (H-2^b) NKC shared with C57L/J (H-2^b), FVB/N (H-2^q), SJL/J (H-2^{s2}), and Ma/My (H-2^k) contains nineteen genes, three of which encode activating receptors (*Ly49r*, *u,* and *p*), three unique inhibitory receptors (*Ly49v*, *s*, and *t*), and seven pseudogenes (Makrigiannis et al. 2001, 2002). The framework *a* and *c* genes have been replaced with *o* and *i2* in the 129 (H-2^b) haplotype. The recently elucidated NOD/LtJ (H-2^{g7}) haplotype is the most diverse with seven genes encoding activating receptors (*Ly49h, m, w, p1, p3, u,* and *d*) and eight pseudogenes, in addition to the six framework inhibitory receptor genes (Belanger et al. 2008). Gene duplication and conversion are the major mechanisms generating the diversity of the Ly49 receptor repertoire, with the activating receptor genes being more recent in evolution and arising from inhibitory receptor genes (Abi-Rached and Parham 2005; Hao and Nei 2004). The extracellular domain of the activating Ly49D receptor is quite similar to that of the inhibitory Ly49A receptor, suggesting a common origin (Mehta et al. 2001a). The *Klra16* gene encoding Ly49P likely arose by a gene conversion event involving the exons encoding the transmembrane and intracellular domains of *Klra4* (Ly49d) and the extracellular domain of *Klra1* (Ly49a) (Makrigiannis et al. 1999). *Klra12* (Ly49l) and *Klra8* (Ly49h) likely arose from gene conversion involving exons derived from *Klra4* (Ly49d) and *Klra7* (Ly49g) or *Klra9*

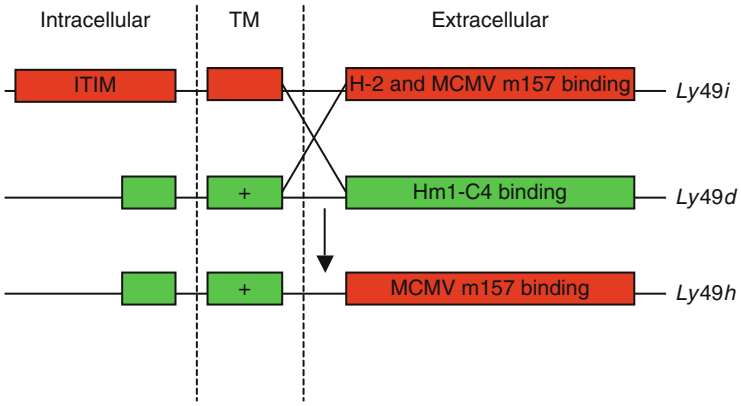

Fig. 2 Generation of new Ly49 receptors by gene duplication and conversion. New Ly49 receptor family members may arise by recombination events between existing family members. *Ly49h* may have arisen by a duplication of *Ly49i* and a subsequent recombination between the exons encoding the extracellular MCMV m157 recognition domain and the exons encoding the intracellular and positively charged transmembrane (TM) domains of *Ly49d*

(Ly49i), respectively (Fig. 2). The close proximity within the genome and high-degree of sequence similarity in the *Klra* genes facilitates their very plastic and dynamic reorganization to generate genes with new ligand specificities and functions.

Klra genes encoding inhibitory Ly49 receptors are expressed largely in a stochastic, mono-allelic fashion on subsets of NK cells, although some individual NK cell clones demonstrate bi-allelic expression (Held and Kunz 1998; Held and Raulet 1997; Held et al. 1995). In contrast, the genes encoding activating Ly49 receptors are frequently expressed in a bi-allelic manner (Rouhi et al. 2009). In the steady-state Ly49 expression by individual mature NK cells is stable, but under certain environmental conditions new Ly49 receptors can be induced on mature NK cells (Dorfman and Raulet 1998); however, loss of Ly49 receptor expression on individual mature NK cell clones has not been observed (Orr et al. 2010).

The Ly49 receptors are expressed as disulfide-linked homodimers on the cell surface and recognize proteins encoded by various H-2 alleles and genes, as well as xenogeneic MHC class I and at least one virally encoded MHC class I-like molecule (Table 1). Recognition of MHC class I molecules requires β2-microglobulin and a peptide to be bound within the MHC class I groove. The identity of the peptide presented may sometimes affect Ly49 binding, although the details of this remain controversial. For example, replacement of all non-anchor residues in a peptide binding to H-2D^{d} had no impact on recognition by the Ly49A^{B6} receptor, but Ly49C^{B6} was sensitive to changes in non-anchor peptide residues (Correa and Raulet 1995; Franksson et al. 1999; Hanke et al. 1999; Michaelsson et al. 2000). From a biological perspective, there is no evidence that the presence of "foreign" peptides

Table 1 Ly49 receptors and their known receptors

Common name	Gene name	Strain haplotype	Function	Ligands	Ligand excluded
Ly49A	*Klra1*	B6	Inhibiting	H-2D^{d}, D^{k}, D^{p}, H-2$^{f, q, r, s, v}$	H-2K^{d}, K^{k}, Ld, H-2^{b}
Ly49B	*Klra2*	B6	Inhibiting		H-2$^{b, d, k, f, q, r, s, v}$
Ly49C	*Klra3*	B6	Inhibiting	H-2K^{b}, K^{d}, D^{d}, D^{k}, H-2$^{f, q, r, s, v}$	H-2D^{b}, L^{d}
Ly49D	*Klra4*	B6	Activating	H-2D^{d}, Hm1-C4	
Ly49E	*Klra5*	B6	Inhibiting	Urokinase plasminogen activator	H-2$^{b, d, k, f, q, r, s, v}$
Ly49F	*Klra6*	B6	Inhibiting	H-2K^{d}	H-2D^{d}, L^{d}, H-2$^{b, k, f, q, r, s, v}$
Ly49G2	*Klra7*	B6	Inhibiting	H-2D^{d}	H-2K^{d}, L^{d}, H-2$^{b, k, f, q, r, s, v}$
Ly49H	*Klra8*	B6	Activating	MCMV m157	
Ly49I	*Klra9*	B6	Inhibiting	H-2K^{b}, D^{d}, K^{d}, K^{k}, H-2$^{q, r, s, v}$	MCMV m157, H0-L^{d}, D^{b}, H-2^{f}
Ly49J	*Klra10*	B6	Inhibiting		
Ly49Q	*Klra17*	B6	Inhibiting	H-2K^{b}	H-2$^{a, d, k, q}$
Ly49B	*Klra2*	129	Inhibiting		
Ly49E	*Klra5*	129	Inhibiting		
Ly49G2	*Klra7*	129	Inhibiting	H-2D^{d}, D^{k}, K^{d}, D^{b}	H-2K^{b}, K^{k}, L^{d}
Ly49I	*Klra9*	129	Inhibiting	H-2D^{k}, K^{b}, K^{d}, K^{d} MCMV m157	H-2D^{b}, D^{d}, L^{d}
Ly49I2	*Klra10*	129	Inhibiting		
Ly49O	*Klra15*	129	Inhibiting	H-2D^{b}, D^{d}, D^{k}, L^{d}	H-2K^{b}, K^{d}, K^{k}
Ly49P	*Klra16*	129	Activating	H-2D^{k} in conjunction with MCMV m04	
Ly49Q	*Klra17*	129	Inhibiting		
Ly49R	*Klra18*	129	Activating		
Ly49S	*Klra19*	129	Inhibiting		H-2$^{b, d, k}$
Ly49T	*Klra20*	129	Inhibiting		H-2$^{b, d, k}$
Ly49U	*Klra21*	129	Activating		
Ly49V	*Klra22*	129	Inhibiting	H-2D^{b}, D^{d}, D^{k}, K^{b}, K^{d}, K^{k}, L^{d}	
Ly49A	*Klra1*	BALB/c	Inhibiting	H-2D^{d}	
Ly49B	*Klra2*	BALB/c	Inhibiting		
Ly49C	*Klra3*	BALB/c	Inhibiting	H-2K^{b}, K^{d}, D^{d} D^{k}, H-2$^{f, q, r, s, v}$	H-2D^{b}, L^{d}
Ly49E	*Klra5*	BALB/c	Inhibiting		
Ly49G2	*Klra7*	BALB/c	Inhibiting	H-2D^{d}, D^{k}	
Ly49I	*Klra9*	BALB/c	Inhibiting		
Ly49L	*Klra12*	BALB/c	Activating		
Ly49Q	*Klra17*	BALB/c	Inhibiting		
Ly49A	*Klra1*	NOD	Inhibiting		
Ly49B	*Klra2*	NOD	Inhibiting		
Ly49C	*Klra3*	NOD	Inhibiting		
Ly49D	*Klra4*	NOD	Activating		
Ly49E	*Klra5*	NOD	Inhibiting		
Ly49G2	*Klra7*	NOD	Inhibiting		
Ly49H	*Klra8*	NOD	Activating		
Ly49I	*Klra9*	NOD	Inhibiting		
Ly49M	*Klra13*	NOD	Activating		

(*continued*)

Table 1 (continued)

Common name	Gene name	Strain haplotype	Function	Ligands	Ligand excluded
Ly49P1		NOD	Activating		
Ly49P3	*Klra16*	NOD	Activating		
Ly49Q	*Klra17*	NOD	Inhibiting		
Ly4U	*Klra21*	NOD	Activating		
Ly4W	*Krla23*	NOD	Activating		

in the H-2 groove would be preferentially recognized over "self" peptides by Ly49 receptors.

Affinities for MHC class I as measured by tetramer binding and cell adhesion vary with the Ly49 receptor and allele. Proteins encoded by different alleles of H-2 bind with different strength to the same Ly49 receptor, resulting in a wide array of specificities and affinities between Ly49 receptors and MHC class I molecules. The MHC class I specificity for the C57BL/6 inhibitory receptors Ly49A, C, I, and G2 have been the most extensively studied. The Ly49A receptor encoded by the C57BL/6 allele of the *Klra1* gene (designated Ly49A^{B6}) binds H-2D^{d}, D^{k}, and D^{p}, as well as H-2 from f, q, r, s, and v haplotypes, but not H-2^{b}, K^{d}, K^{k}, or L^{d} (Takei et al. 1997; Hanke et al. 1999; Daniels et al. 1994; Kane 1994; Karlhofer et al. 1992; Olsson-Alheim et al. 1999). Ly49A^{B6} has the highest affinity for H-2^{d} with a K_D of ~10 μM, followed in order of decreasing affinity by H-2^{r}, H-2^{k}, H-2^{q}, and H-2^{s} (Jonsson et al. 2010; Natarajan et al. 1999). The allele encoding the Ly49A receptor in BALB/c mice (Ly49A^{BALB}), which varies from Ly49A^{B6} by four amino acids, also binds H-2D^{d}, but with lower affinity than the Ly49A^{B6} receptor (Mehta et al. 2001b). Ly49C^{B6} and Ly49C^{BALB}, which also differ by four amino acids, both bind H-2K^{b}, K^{d}, D^{d}, and D^{k} in addition to H-2 from the f, q, r, s, and v haplotypes, but not H-2D^{b} or H-2L^{d} (Hanke et al. 1999; Brennan et al. 1996; Lian et al. 1999; Raulet et al. 1997). Ly49I^{B6} binds H-2K^{b}, D^{d}, K^{d}, and K^{k}, as well as H-2 from q, r, s, and v haplotypes, but not H-2^{f}, H-2L^{d}, or H-2D^{b} (Hanke et al. 1999). Ly49I^{129} (the allele of *Klra9* in 129/J mice) recognizes the virally encoded m157 MHC class I-like molecule encoded by the Smith strain of mouse cytomegalovirus (MCMV), which is also recognized by the activating Ly49H^{B6}, but not by Ly49I^{B6} or Ly49I^{BALB} (Arase et al. 2002; Smith et al. 2002). Ly49G2^{B6} binds H-2D^{d}, but not H-2K^{d}, H-2L^{d}, or H-2 from b, k, f, q, r, s or v haplotypes (Hanke et al. 1999; Johansson et al. 1998; Mason et al. 1995). Ly49G2^{BALB} binds H-2D^{b} with higher affinity than Ly49G2^{B6}, and also binds H-2D^{k} (Silver et al. 2002). Importantly, neither Ly49A^{B6} nor Ly49G2^{B6} have any measurable affinity for H-2^{b} proteins and thus do not recognize self-MHC class I in C57BL/6 mice.

Whereas the interactions between several inhibitory Ly49 receptors expressed on NK cells and classical MHC class I molecules have been extensively documented and their biological consequences well defined, the ligands of other members of the Ly49 family that are expressed on other cell types are not well established. Ly49F^{B6} binds weakly to H-2K^{d}, but not H-2D^{d}, H-2L^{d}, or H-2 from b, k, f, q, r, s,

or v haplotypes (Hanke et al. 1999). Ly49Q, which is expressed on myeloid cells, but not NK cells or T cells, binds H-2K^{b}, but not H-2 from a, d, k, or q haplotypes (Sasawatari et al. 2010; Tai et al. 2007, 2008; Toyama-Sorimachi et al. 2004). Similarly, Ly49B, which is expressed by macrophages, does not bind H-2 molecules of the b, d, k, f, q, r, s, or v haplotypes (Hanke et al. 1999; Gays et al. 2006). Many transcripts of *Klra10* (Ly49j) lack a transmembrane and thus may encode for an intracellular protein and the specificity of Ly49J has not been determined (McQueen et al. 1999). Ly49E is expressed on some γδ T cells and fetal NK cells, but is rarely expressed on adult NK cells (Fraser et al. 2002; Van Beneden et al. 2001, 2002). Ly49E, which does not bind H-2 molecules of the b, d, k, f, q, r, s, or v haplotypes, recognizes cells expressing the urokinase plasminogen activator protein, although an interaction between Ly49E and urokinase plasminogen activator protein has not been shown directly (Van Den Broeck et al. 2008). The functional importance of this interaction remains unknown.

The ligand specificities of the Ly49 receptors expressed by 129/J mice have also been investigated using H-2^{b}, H-2^{d}, and H-2^{k} tetrameric reagents (Makrigiannis et al. 2001). Ly49V^{129} bound all H-2 tetramers tested: D^{b}, D^{d}, D^{k}, K^{b}, K^{d}, K^{k}, and L^{d}. Despite being 96% identical at the amino acid level to Ly49G2^{B6}, Ly49G2^{129} displays much broader reactivity to H-2 alleles than Ly49G2^{B6}, binding H2-D^{d}, D^{k}, K^{d}, and D^{b}. Ly49I^{129} displayed a similar affinity for H-2 as Ly49I^{B6} (96% identity), binding H2-D^{k}, K^{b}, K^{d}, and K^{d}. Ly49O^{129} shares the highest identity with Ly49A^{B6} (93%) and recognizes H2-D^{b}, D^{d}, D^{k}, and L^{d}. The inhibitory receptors Ly49S^{129} and Ly49T^{129} did not bind any of the tetramers tested. Because of the different *Ly49* gene content and differing Ly49 receptor affinities for H-2 alleles in C57BL/6 mice compared with 129/J mice, the use of 129/J embryonic stem cells to ablate alleles within or near the *Klra* loci may lead to difficulties in interpreting results if the gene-deficient mice generated with 129/J embryonic stem cells are backcrossed onto the C57BL/6 background because they will typically retain the 129/J NKC genomic region. The affinities of the Ly49 receptors encoded by genes of the NOD/LtJ genetic background have not been reported.

Some activating members of the Ly49 receptor family also bind MHC class I and MHC class I-like molecules. The activating receptor Ly49D binds the Hm1-C4 MHC class I molecule from hamsters, accounting for Ly49D-mediated lysis of CHO cells by NK cells from C57BL/6 mice (Merck et al. 2009). NK cells expressing Ly49D recognize target cells expressing H-2D^{d} but not other MHC class I molecules, although direct binding of Ly49D to H-2D^{d} has not been reported (George et al. 1999a, b). Ly49H^{B6} directly recognizes the MCMV-encoded m157 protein expressed on the surface of infected cells, but does not bind to any H-2 ligand (Arase et al. 2002; Smith et al. 2002; Adams et al. 2007). Ly49P$^{Ma/My}$ recognition of MCMV-infected cells is dependent on the expression of the viral m04 protein and H-2D^{k}, but not H-2K^{k} or other H-2 alleles (Desrosiers et al. 2005; Kielczewska et al. 2009). MCMV m04 binds to MHC class I molecules and traffics to the cell surface and may modify the conformation of H-2D^{k} allowing recognition by Ly49P (Hengel et al. 1999).

3 Inhibitory Signaling Events

All inhibitory receptors in the Ly49 family express in their cytoplasmic domains an ITIM, which is characterized by the signature sequence, (I/L/V/S)xYxx (L/V) (where x represents any amino acid, and slashes separate alternative amino acids that may occupy a given position). The mechanisms by which NK cell inhibitory receptors abrogate NK cell activation have been best worked out for the human inhibitory KIR family. These signaling pathways are thought to be similar for inhibitory Ly49 receptors, although this may not always be the case (Lanier 2008; Long 2008; MacFarlane and Campbell 2006). When the inhibitory receptors on NK cells bind to their MHC class I ligands on potential target cells, the ITIMs are phosphorylated by Src family kinases including Lck (Fig. 3) (Binstadt et al. 1996). The SH2-domain-containing protein tyrosine phosphatases 1 and 2 (SHP-1 and SHP-2) are recruited to the phosphorylated ITIMs at the immunological synapse (Daws et al. 1999; Eriksson et al. 1999a; Fassett et al. 2001; Fry et al. 1996; Mason et al. 1997; Nakamura et al. 1997; Olcese et al. 1996; Vyas et al. 2004; Vyas et al. 2001, 2002; Burshtyn et al. 1996). SHP-1 and -2 are normally in an inactive conformation with the SH2 domain bound to the catalytic domain. Binding of phosphorylated ITIMs releases the SH2 domain, allowing SHP-1 and -2 to become catalytically active (Hof et al. 1998; Tonks and Neel 1996). SHP-1 and 2 dephosphorylate different substrates, and thus likely have differing, non-redundant roles in NK cell inhibition (Mishra et al. 2002; Yang et al. 1998). The motheaten viable mutation of SHP-1 is sufficient to abrogate Ly49A, Ly49C, and Ly49I inhibition of NK cell activation; thus, SHP-1 may be the primary mediator of inhibition by Ly49 receptors (Orr et al. 2010; Nakamura et al. 1997). Although a number of proteins including Src family kinases, PLCγ, ZAP70, Vav, SLP76, LAT, Grb2, and PI3K are dephosphorylated when inhibitory receptors are triggered, it is unclear whether all of these are direct substrates of the recruited phosphatases or represent downstream abrogation of activation (Binstadt et al. 1996, 1998; Palmieri et al. 1999; Stebbins et al. 2003; Valiante et al. 1996). However, Vav1 is a direct target of SHP-1 and is a critical target of dephosphorylation upon inhibitory receptor ligation (Stebbins et al. 2003). Vav1 is phosphorylated upon NK cell activating receptor triggering and is necessary for cytoskeletal rearrangements, secretion of cytotoxic granules, and release of effector cytokines and chemokines including IFN-γ, TNF, MIP1α, and RANTES (Long 2008). The SH2-domain-containing inositol polyphosphate 5′ phosphatase-1 (SHIP-1) is also recruited to the phosphorylated ITIMs of Ly49 receptors, but not human KIRs (Daws et al. 1999; Gupta et al. 1997; Wang et al. 2002). SHIP-1 dephosphorylates PI-3,4,5-P_3 to PI-3,4-P_2, thus abrogating Ca^{2+}-dependent signaling. Over-expression of SHIP-1 inhibits CD16-mediated antibody-dependent cellular cytotoxicity (Galandrini et al. 2001).

In human NK cells, HLA class I binding to inhibitory KIRs induces phosphorylation of Crk, and disruption of the Crk-cCbl-C3G-p130CAS complex (Peterson and Long 2008). Whether a similar mechanism of inhibitory signaling is active upon inhibitory Ly49 triggering remains to be determined. β-arrestin has also been

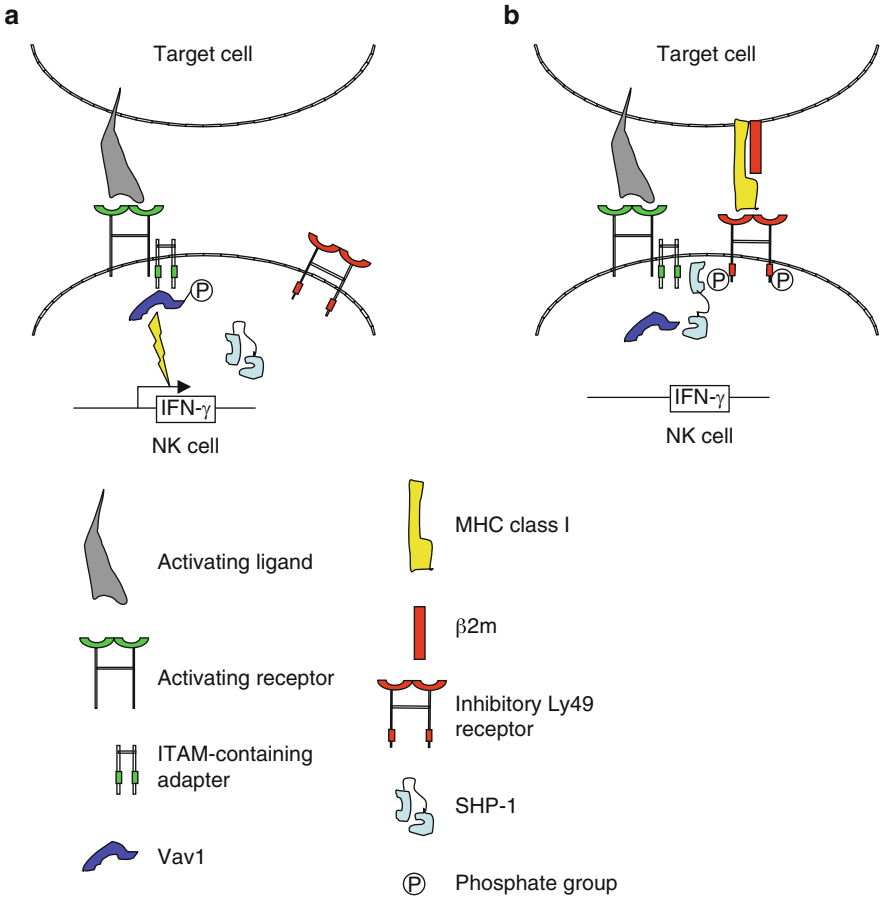

Fig. 3 Signaling by inhibitory Ly49 receptors. (**a**) Upon engagement with cognate ligands on target cells activating receptors on NK cells signal via ITAM-containing adapter proteins or DAP10, an adapter protein containing an YINM motif, to phosphorylate Vav1, resulting in NK cell activation. (**b**) Engagement with MHC class I on target cells recruits inhibitory Ly49 receptors on the NK cell to the immunological synapse and results in phosphorylation of the ITIM domains. SHP-1, normally in a closed, inactive state, binds to the phosphorylated ITIM domains via the SH2 domain, freeing the phosphatase domain to dephosphorylate Vav1 and dampen NK cell activation

implicated in the recruitment of SHP-1 and 2 to the phosphorylated ITIMs of KIR (Yu et al. 2008). Whether β-arrestin is necessary for inhibitory Ly49 function remains to be determined; however, NK cells from β-arrestin-deficient mice displayed increased cytotoxicity toward NK susceptible and resistant targets and these mice controlled MCMV infection better than wildtype mice in an NK cell-dependent manner (Yu et al. 2008).

The strength of the inhibitory signal varies directly with the affinity of the inhibitory receptor for the MHC class I ligand. For example, the affinity of

Ly49A for H-2D^d is much stronger than for H-2D^k, and Ly49A ligation of H-2D^d is more inhibitory than ligation of H-2D^k (Hanke et al. 1999; Jonsson et al. 2010). Simultaneous engagement of multiple inhibitory Ly49 receptors increases the strength of inhibition (Hanke and Raulet 2001). Interestingly, simultaneous engagement of inhibitory Ly49 receptors by MHC class I by one potential target cell does not prevent the same NK cell from being activated by and lysing a second target cell that does not engage an inhibitory receptor (Eriksson et al. 1999b). This implies that the inhibitory signaling events must be restricted to one subcellular location near the cells surface of the NK cell, while allowing another site on the cell surface interacting with another target cell to mediate NK cell activation unimpaired.

In addition to binding MHC class I in *trans* on other cells, Ly49 receptors also interact in *cis* with MHC class I on the same NK cell surface membrane. This *cis* interaction impedes the binding of some antibodies against the Ly49 receptors leading to an apparent "down-regulation" of these receptors by *cis* interactions (Scarpellino et al. 2007). These *cis* interactions sequester inhibitory receptors away from the immunologic synapse, reducing the inhibitory capacity of Ly49 receptors that bind to self-MHC class I (Chalifour et al. 2009; Doucey et al. 2004). Additionally, *cis* interactions require Ly49 receptors to adopt a different conformation from the one used to bind MHC class I in *trans* and may result in different intracellular signaling events that do not result in NK cell inhibition (Doucey et al. 2004; Back et al. 2009).

4 Educational Impact of Ly49 Receptors on NK Cells

Ly49 receptors are expressed in a variegated, overlapping manner resulting in many subsets of NK cells that express different constellations of inhibitory Ly49 receptors or in some cases no Ly49 receptors on some NK cells (Raulet et al. 1997; Kubota et al. 1999). For example, in C57BL/6 mice 30–50% of mature NK cells lack Ly49C and Ly49I and thus do not recognize self-MHC class I via Ly49 inhibitory receptors. Although these cells are phenotypically indistinguishable from cells that express Ly49C and/or Ly49I, they are less responsive to triggering through their activating receptors (Fernandez et al. 2005). NK cells lacking Ly49C and Ly49I are also impaired in their ability to acutely reject MHC class I-deficient bone marrow (Fernandez et al. 2005). Similarly, responsiveness of Ly49A^{B6} single-positive NK cells correlated with expression of H-2D^d, which is a ligand of Ly49A, but not H-2^b, which is not ligated by Ly49A^{B6} (Kim et al. 2005). NK cells from MHC class I-deficient mice are hyporesponsive to activation through multiple activating receptors and fail to reject MHC class I-deficient bone marrow (Fernandez et al. 2005; Kim et al. 2005; Hoglund et al. 1991). Responsiveness can be restored to the Ly49C subset of NK cells by from MHC class I deficient mice transgenic expression of H-2K^b in MHC class I-deficient mice (Kim et al. 2005). Thus, the interaction between self-MHC class I and inhibitory Ly49 receptors engenders NK

cell responsiveness and has been termed "licensing" or "arming" of NK cells by inhibitory Ly49 receptors (Brodin et al. 2009a).

Expression of multiple self-reactive inhibitory receptors increases the responsive capacity of NK cells, such that NK cells expressing both Ly49C and Ly49I are more responsive than either Ly49C or Ly49I single-positive NK cells (Brodin et al. 2009b; Joncker et al. 2009). The affinity for self-MHC class I also affects the functional responsiveness of the NK cell. For example, in Ly49A^{+} NK cells the high affinity H-2D^{d} ligand engenders more responsive capacity than low affinity ligands such as H-2^{s} (Jonsson et al. 2010). Expression of the inhibitory CD94-NKG2A inhibitory receptor that binds the MHC class Ib molecule Qa-1 also enhances NK cell responsiveness (Fernandez et al. 2005). Mutations to the ITIM domain of Ly49 inhibitory receptors abrogate NK cell licensing or arming, indicating that licensing depends on signaling by the inhibitory Ly49 receptor (Kim et al. 2005). Licensing occurs independently of SHP-1 and SHIP signaling, suggesting other signaling pathways must be engaged downstream of ITIM phosphorylation to engender responsiveness (Orr et al. 2010; Kim et al. 2005). The hyporesponsiveness of NK cells from mice lacking surface expression of MHC class I has been used to explain why NK cells from mice lacking β2-microglobulin, TAP-1, or H-2K and H-2D heavy chains do not exert overt autoimmunity (Hoglund et al. 1998; Liao et al. 1991; Ljunggren et al. 1994), and why NK cells from *B2m*$^{-/-}$ mice fail to lyse *B2m*$^{-/-}$ T cell blasts (Hoglund et al. 1991).

In mixed bone marrow chimeras containing MHC class I-sufficient and deficient hematopoetic cells, NK cells of both genotypes are hyporesponsive against MHC class I-deficient target cells and the chimerism is stable (Wu and Raulet 1997). This suggests that licensing is not mediated by *cis* interactions with MHC class I expressed on the NK cells, as has been recently suggested (Chalifour et al. 2009). A transgenic mouse model expressing H-2D^{d} on only a subset of cells (mosaic expression) also renders NK cells hypofunctional due to the presence of a large number of cells not expressing the H-2D^{d} transgene (Johansson et al. 1997). These results suggest that the hyporesponsiveness is dominant and inducible by lack of MHC class I interactions with inhibitory Ly49 receptors. MHC class I-deficient bone marrow is rejected by NK cells in MHC class I-sufficient recipients, suggesting that bone marrow cells express at least one activating ligand that if not opposed by MHC class I engagement of inhibitory receptors is sufficient to activate NK cells. Thus, chronic exposure to activating ligands unopposed by inhibitory receptors for self-MHC class I may "disarm" NK cells including Ly49C^{-} and Ly49I^{-} NK cells in C57BL/6 mice or all NK cells in MHC class I-deficient mice (Gasser and Raulet 2006). This would be consistent with the hyporesponsiveness of NK cells in the mixed bone marrow chimeric mice containing MHC class I-sufficient and deficient hematopoetic cells. It is unclear if lack of expression by MHC class I by a particular cell type drives disarming of NK cells or whether it is simply an overwhelming number of MHC class I-deficient cells that express one or more activating ligands. Responsiveness of disarmed or unlicensed NK cells can be restored in a variety of ways including culture in IL-2, stimulation with high doses of IL-12 and IL-18, or in vivo by infection with *Listeria monocytogenes* or MCMV (Orr et al. 2010;

Fernandez et al. 2005; Kim et al. 2005; Sun and Lanier 2008; Yokoyama and Kim 2006).

5 Inhibitory Ly49 Receptors and Viral Infections

Inhibitory receptors regulate NK cell responses at the level of detection of alterations in MHC class I expression. $CD8^+$ T cells are activated by T cell receptor engagement of cognate MHC class I:peptide ligands. Inhibition of MHC class I expression is a common immune evasion strategy employed by many viruses, including herpesviruses, adenovirus, and HIV (Tortorella et al. 2000). Loss of MHC class I on transformed cells is also a frequent event during malignancy (Marincola et al. 1994). Both viral and transformation-induced loss of MHC class I renders target cells invisible to recognition and clearance by $CD8^+$ cytotoxic T cells. However, the loss of self-MHC class I, termed "missing self", removes the inhibitory signals provided by Ly49 receptors on NK cells thereby allowing NK cells to detect and eliminate infected or transformed cells that express one or more ligands for activating NK cell receptors (Hoglund et al. 1991; Liao et al. 1991; Bix et al. 1991; Karre et al. 1986; Lanier 2005).

To date there remains little in vivo evidence addressing the significance of missing self-recognition to NK cell control of viral infection. MCMV is the most well studied example of viral control by NK cells in mouse models (Orr et al. 2010; Arase et al. 2002; Bukowski et al. 1983; Dokun et al. 2001; Sun et al. 2009). Although MCMV encodes two proteins that impede expression of MHC class I on the surface of infected cells, there are no reports of this enhancing NK cell control of infection in vivo (Doom and Hill 2008). Conversely, NK cell activating receptors play a critical role in the activation of NK cells and elimination of MCMV-infected cells. $Ly49H^{B6}$ ligation by the MCMV-encoded m157 glycoprotein that is expressed on the cell surface of infected cells activates NK cells and is necessary for NK cell control of MCMV infection (Arase et al. 2002; Smith et al. 2002). During MCMV infection $Ly49H^+$ NK cells proliferate extensively after recognition of the cognate ligand MCMV-m157 (Dokun et al. 2001). Ly49C and/or Ly49I receptors restrain this Ly49H-driven proliferation by interacting with self-MHC class I via inhibitory signaling through SHP-1 (Orr et al. 2010). Consequently, licensed $Ly49C/I^+$ $Ly49H^+$ NK cells make very little contribution to viral control, rather it is the unlicensed or disarmed $Ly49C/I^-$ $Ly49H^+$ NK cells that control MCMV replication (Orr et al. 2010). Thus, in the case of MCMV infection where contact with the infected cells is required for NK cells to mediate immunity, Ly49C/I-mediated inhibition of NK cell functions overrides the responsive benefit gained by licensing, whereas the unlicensed or disarmed NK cells, likely activated by the inflammatory milieu associated with infection, are competent to respond to MCMV-infected cells, unimpeded by inhibitory Ly49 receptor signaling. It is possible that these competent NK cells that are not inhibited by self-MHC class I mediate collateral damage by attacking uninfected cells expressing activating ligands.

However, during MCMV infection Ly49H$^+$ NK cells upregulate the inhibitory receptor KLRG1, which binds cadherins expressed on host cells, thus non-MHC class I restricted inhibitory receptors may prevent auto-aggression by these cells (Sun et al. 2009; Grundemann et al. 2006; Ito et al. 2006; Robbins et al. 2004). The decrease in the frequency of NK cells in C57BL/6 mice expressing Ly49C and/or Ly49I that occurs during MCMV infection has also been observed during other infections including lymphocytic choriomeningitis virus, vaccinia, and mouse hepatitis virus, and is thus not unique to MCMV infection (Orr et al. 2010; Daniels et al. 2001).

6 Inhibitory Ly49 Receptors in Transplantation and Malignancy

In bone marrow transplantation, NK cells play an important role in preventing graft-versus-host disease (GVHD), while still conferring a beneficial graft-versus-leukemia (GVL) effect (Glass et al. 1996; Asai et al. 1998). GVHD results when donor allogeneic T cells included in the graft bone marrow are activated by recipient antigen-presenting cells (APCs) displaying recipient MHC antigens. GVHD can be prevented by depletion of donor T cells from the bone marrow graft, but this often results in leukemia relapse (Shlomchik et al. 1999). Co-transferred alloreactive donor NK cells are able to kill recipient APCs, thereby preventing GVHD and enhancing engraftment of the donor bone marrow (Ruggeri et al. 2002). Simultaneously, these NK cells kill residual allogeneic host leukemic cells, thus increasing disease-free survival. NK cells lacking inhibitory Ly49 receptors for host H-2 mediate both the GVL effects and killing of host APCs to prevent GVHD. Co-transfer of these uninhibited donor NK cells allows for transfer of 20 times more donor T cells, which speeds up the reconstitution of the immune system, limiting fatal infections after transplantation (Ruggeri et al. 2002). NK cell prevention of GVHD can also be enhanced by siRNA knockdown of inhibitory Ly49 receptors that recognize the recipient MHC class I (Cao et al. 2009). In the case of MHC class I-matched donor bone marrow transferred into lethally irradiated hosts, such as BALB/c (H-2^d) mice receiving B10.D2 (H-2^d) bone marrow, host APCs prime donor T cells against minor histocompatibility antigens, resulting in delayed GVHD. Adoptive transfer of Ly49C/I$^+$ Ly49G2$^-$ NK cells from B10.D2 mice limited GVHD, whereas transfer of equal numbers of Ly49C/I$^-$ Ly49G2$^+$ NK cells from B10.D2 had little effect because they are inhibited by recipient H-2^d (Lundqvist et al. 2007). Thus, although Ly49C ^{B6} and Ly49I^{B6} recognize H-2^d, H-2^d-mediated inhibition appears stronger for Ly49G2 than for Ly49C or Ly49I. Similarly, in a model of lung metastases, adoptively transferred Ly49C/I$^+$ Ly49G2$^-$ NK cells were more efficient than a similar number of Ly49C/I$^-$ Ly49G2$^+$ NK cells in preventing the growth of the renal carcinoma cell line RENCA, which expresses H-2^d, in BALB/c mice (Lundqvist et al. 2007).

"Missing self" recognition can be mimicked by blocking Ly49 interactions with MHC class I by using Ly49 specific antibodies. Blockade of Ly49C and/or Ly49I by using $F(ab')_2$ fragments of the 5E6 monoclonal antibody enhanced endogenous NK cell-mediated control of the H-2^b leukemic cell line C1498 in vivo in C57BL/6 mice (Koh et al. 2001). Moreover, adoptive transfer of IL-2-activated NK cells enhanced control of the C1498 tumor in vivo, and this was further enhanced by blocking the Ly49C and Ly49I receptors (Koh et al. 2001). C57BL/6 NK cells are also able to purge C1498 leukemic cells from bone marrow prior to infusion, resulting in an increase in leukemia-free survival of irradiated recipient mice. Blocking Ly49C and Ly49I during this conditioning period further reduced the leukemic burden, increasing survival of recipient mice without damage to the normal bone marrow cells (Koh et al. 2002). Co-culture of bone marrow containing leukemia cells with NK cells from H-2^d donors more efficiently eliminated leukemic cells than NK cells from H-2^b donors, resulting in increased leukemia-free survival of irradiated recipients. Again, this was further enhanced by blocking Ly49C and Ly49I during the ex vivo conditioning period (Koh et al. 2003). Although it is unclear why H-2^d NK cells were more efficacious than H-2^b NK cells, it is possible that Ly49C/I^- NK cells expressing Ly49A and/or Ly49G2 are licensed by the donor H-2^d, but not donor H-2^b, and are not inhibited by H-2^b on the leukemic cells and thus are more efficient at killing the leukemia targets.

In contrast to hematopoietic malignancies, NK cells are less efficient at controlling solid organ tumors (Yu et al. 1996). This may be due to a failure of NK cells to traffic efficiently to solid tumors, decreased expression of activating ligands by solid organ tumors, and/or other mechanisms that may inhibit NK cell function. The importance of tumor location is documented by the observation that Ly49G2 blockade using $F(ab')_2$ of the 4D11 monoclonal antibody enhanced rejection of the H-2^k T cell lymphoma line B2-Sp3 when transferred intravenously into AKR recipient mice ($Ly49G2^{AKR}$ is identical to $Ly49G2^{BALB}$ and ligates H-$2D^k$), whereas the same treatment did not enhance rejection when the same tumor was implanted subcutaneously into the flank (Barber et al. 2008). With respect to solid tumors, combining high-dose IL-2 therapy with blocking Ly49C and Ly49I by using $F(ab')_2$ fragments limited the growth of B16-F10 (H-2^b) melanomas, whereas either therapy alone had minimal effect on tumor growth (Vahlne et al. 2010). Despite resulting in tumor elimination, this combination therapy did not break tolerance to normal self, suggesting that either normal self inhibits NK cells by receptors other than Ly49C and Ly49I or that B16-F10 expresses activating ligands not found on normal cells. Also, long-term blocking of Ly49C and Ly49I with 5E6 $F(ab')_2$ did not render these NK cells "unlicensed" or "disarmed" (Vahlne et al. 2010). Collectively, these studies demonstrate that blocking the inhibitory receptors for MHC class I enable NK cells to attack and eliminate both hematopoietic and solid tumors, thereby providing a new therapeutic strategy for cancer immunotherapy. One such blocking human monoclonal antibody (1-7F9) reactive with several inhibitory human KIRs increased NK cell-mediated clearance of MHC class I-expressing leukemia in humanized mice and is currently in phase I clinical trials for cancer therapy (Romagne et al. 2009).

7 Concluding Remarks

The Ly49 family of inhibitory receptors plays a critical role in controlling the immune functions of NK cells, first by shaping the educational and tolerant state of NK cells in the steady-state and then by augmenting or inhibiting NK cell responses to both pathogens and tumors. Many outstanding questions remain. First, we need a fuller understanding of the MHC class I ligand repertoire and affinities for the Ly49 receptors in haplotypes other than C57BL/6. Second, the molecular mechanism controlling education via Ly49 receptors remains an unanswered question. Whether developing NK cells gain responsive capacity only after expressing a self-reactive inhibitory receptor (licensing or arming) or alternatively, whether NK cells are innately responsive and hyporesponsiveness is a result of chronic stimulation unchecked by inhibitory receptors (disarming) is presently unresolved. Moreover, studies are needed to address the in vivo significance of viral down-regulation of MHC class I on NK cell responses in vivo (the "missing self" or "reduced self" hypothesis), as well as to determine whether the Ly49 receptors are involved in immunity to viruses other than MCMV. Finally, the clinical use of induced missing self through lack of inhibitory ligands to treat leukemia or blockade of inhibitory receptors to treat both tumors and infections is an attractive application that requires further consideration.

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References

Abi-Rached L, Parham P (2005) Natural selection drives recurrent formation of activating killer cell immunoglobulin-like receptor and Ly49 from inhibitory homologues. J Exp Med 201:1319–1332

Adams EJ, Juo ZS, Venook RT et al (2007) Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors. Proc Natl Acad Sci U S A 104:10128–10133

Arase H, Mocarski ES, Campbell AE et al (2002) Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science 296:1323–1326

Asai O, Longo DL, Tian ZG et al (1998) Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural killer cells after allogeneic bone marrow transplantation. J Clin Invest 101:1835–1842

Back J, Malchiodi EL, Cho S et al (2009) Distinct conformations of Ly49 natural killer cell receptors mediate MHC class I recognition in trans and cis. Immunity 31:598–608

Barber MA, Zhang T, Gagne BA et al (2008) Ly49G2 receptor blockade reduces tumor burden in a leukemia model but not in a solid tumor model. Cancer Immunol Immunother 57:655–662

Belanger S, Tai LH, Anderson SK et al (2008) Ly49 cluster sequence analysis in a mouse model of diabetes: an expanded repertoire of activating receptors in the NOD genome. Genes Immun 9:509–521

Binstadt BA, Brumbaugh KM, Dick CJ et al (1996) Sequential involvement of Lck and SHP-1 with MHC-recognizing receptors on NK cells inhibits FcR-initiated tyrosine kinase activation. Immunity 5:629–638

Binstadt BA, Billadeau DD, Jevremovic D et al (1998) SLP-76 is a direct substrate of SHP-1 recruited to killer cell inhibitory receptors. J Biol Chem 273:27518–27523

Bix M, Liao NS, Zijlstra M et al (1991) Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice. Nature 349:329–331

Brennan J, Mahon G, Mager DL et al (1996) Recognition of class I major histocompatibility complex molecules by Ly-49: specificities and domain interactions. J Exp Med 183:1553–1559

Brodin P, Karre K, Hoglund P (2009a) NK cell education: not an on-off switch but a tunable rheostat. Trends Immunol 30:143–149

Brodin P, Lakshmikanth T, Johansson S et al (2009b) The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. Blood 113:2434–2441

Brown MG, Fulmek S, Matsumoto K et al (1997) A 2-Mb YAC contig and physical map of the natural killer gene complex on mouse chromosome 6. Genomics 42:16–25

Bukowski JF, Woda BA, Habu S et al (1983) Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo. J Immunol 131:1531–1538

Burshtyn DN, Scharenberg AM, Wagtmann N et al (1996) Recruitment of tyrosine phosphatase HCP by the killer cell inhibitor receptor. Immunity 4:77–85

Cao D, Hu L, Wang Y et al (2009) Suppression of graft-versus-host disease after adoptive infusion of alloreactive NK cells induced by silencing Ly49C gene in mice. Transpl Immunol 20:243–248

Carlyle JR, Mesci A, Fine JH et al (2008) Evolution of the Ly49 and Nkrp1 recognition systems. Semin Immunol 20:321–330

Chalifour A, Scarpellino L, Back J et al (2009) A role for cis interaction between the inhibitory Ly49A receptor and MHC class I for natural killer cell education. Immunity 30:337–347

Correa I, Raulet DH (1995) Binding of diverse peptides to MHC class I molecules inhibits target cell lysis by activated natural killer cells. Immunity 2:61–71

Daniels BF, Karlhofer FM, Seaman WE et al (1994) A natural killer cell receptor specific for a major histocompatibility complex class I molecule. J Exp Med 180:687–692

Daniels KA, Devora G, Lai WC et al (2001) Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. J Exp Med 194: 29–44

Daws MR, Eriksson M, Oberg L et al (1999) H-2Dd engagement of Ly49A leads directly to Ly49A phosphorylation and recruitment of SHP1. Immunology 97:656–664

Depatie C, Lee SH, Stafford A et al (2000) Sequence-ready BAC contig, physical, and transcriptional map of a 2-Mb region overlapping the mouse chromosome 6 host-resistance locus Cmv1. Genomics 66:161–174

Desrosiers MP, Kielczewska A, Loredo-Osti JC et al (2005) Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. Nat Genet 37:593–599

Dokun AO, Kim S, Smith HR et al (2001) Specific and nonspecific NK cell activation during virus infection. Nat Immunol 2:951–956

Doom CM, Hill AB (2008) MHC class I immune evasion in MCMV infection. Med Microbiol Immunol 197:191–204

Dorfman JR, Raulet DH (1998) Acquisition of Ly49 receptor expression by developing natural killer cells. J Exp Med 187:609–618

Doucey MA, Scarpellino L, Zimmer J et al (2004) Cis association of Ly49A with MHC class I restricts natural killer cell inhibition. Nat Immunol 5:328–336

Eriksson M, Ryan JC, Nakamura MC et al (1999a) Ly49A inhibitory receptors redistribute on natural killer cells during target cell interaction. Immunology 97:341–347

Eriksson M, Leitz G, Fallman E et al (1999b) Inhibitory receptors alter natural killer cell interactions with target cells yet allow simultaneous killing of susceptible targets. J Exp Med 190:1005–1012

Fassett MS, Davis DM, Valter MM et al (2001) Signaling at the inhibitory natural killer cell immune synapse regulates lipid raft polarization but not class I MHC clustering. Proc Natl Acad Sci U S A 98:14547–14552

Fernandez NC, Treiner E, Vance RE et al (2005) A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. Blood 105:4416–4423

Franksson L, Sundback J, Achour A et al (1999) Peptide dependency and selectivity of the NK cell inhibitory receptor Ly-49C. Eur J Immunol 29:2748–2758

Fraser KP, Gays F, Robinson JH et al (2002) NK cells developing in vitro from fetal mouse progenitors express at least one member of the Ly49 family that is acquired in a time-dependent and stochastic manner independently of CD94 and NKG2. Eur J Immunol 32:868–878

Fry AM, Lanier LL, Weiss A (1996) Phosphotyrosines in the killer cell inhibitory receptor motif of NKB1 are required for negative signaling and for association with protein tyrosine phosphatase 1C. J Exp Med 184:295–300

Gagnier L, Wilhelm BT, Mager DL (2003) Ly49 genes in non-rodent mammals. Immunogenetics 55:109–115

Galandrini R, Tassi I, Morrone S et al (2001) The adaptor protein shc is involved in the negative regulation of NK cell-mediated cytotoxicity. Eur J Immunol 31:2016–2025

Gasser S, Raulet DH (2006) Activation and self-tolerance of natural killer cells. Immunol Rev 214:130–142

Gays F, Aust JG, Reid DM et al (2006) Ly49B is expressed on multiple subpopulations of myeloid cells. J Immunol 177:5840–5851

George TC, Mason LH, Ortaldo JR et al (1999a) Positive recognition of MHC class I molecules by the Ly49D receptor of murine NK cells. J Immunol 162:2035–2043

George TC, Ortaldo JR, Lemieux S et al (1999b) Tolerance and alloreactivity of the Ly49D subset of murine NK cells. J Immunol 163:1859–1867

Glass B, Uharek L, Zeis M et al (1996) Graft-versus-leukaemia activity can be predicted by natural cytotoxicity against leukaemia cells. Br J Haematol 93:412–420

Grundemann C, Bauer M, Schweier O et al (2006) Cutting edge: identification of E-cadherin as a ligand for the murine killer cell lectin-like receptor G1. J Immunol 176:1311–1315

Gupta N, Scharenberg AM, Burshtyn DN et al (1997) Negative signaling pathways of the killer cell inhibitory receptor and Fc gamma RIIb1 require distinct phosphatases. J Exp Med 186:473–478

Hanke T, Raulet DH (2001) Cumulative inhibition of NK cells and T cells resulting from engagement of multiple inhibitory Ly49 receptors. J Immunol 166:3002–3007

Hanke T, Takizawa H, McMahon CW et al (1999) Direct assessment of MHC class I binding by seven Ly49 inhibitory NK cell receptors. Immunity 11:67–77

Hao L, Nei M (2004) Genomic organization and evolutionary analysis of Ly49 genes encoding the rodent natural killer cell receptors: rapid evolution by repeated gene duplication. Immunogenetics 56:343–354

Held W, Kunz B (1998) An allele-specific, stochastic gene expression process controls the expression of multiple Ly49 family genes and generates a diverse, MHC-specific NK cell receptor repertoire. Eur J Immunol 28:2407–2416

Held W, Raulet DH (1997) Expression of the Ly49A gene in murine natural killer cell clones is predominantly but not exclusively mono-allelic. Eur J Immunol 27:2876–2884

Held W, Roland J, Raulet DH (1995) Allelic exclusion of Ly49-family genes encoding class I MHC-specific receptors on NK cells. Nature 376:355–358

Hengel H, Reusch U, Gutermann A et al (1999) Cytomegaloviral control of MHC class I function in the mouse. Immunol Rev 168:167–176

Hof P, Pluskey S, Dhe-Paganon S et al (1998) Crystal structure of the tyrosine phosphatase SHP-2. Cell 92:441–450

Hoglund P, Ohlen C, Carbone E et al (1991) Recognition of beta 2-microglobulin-negative (beta 2 m-) T-cell blasts by natural killer cells from normal but not from beta 2 m- mice: nonresponsiveness controlled by beta 2 m- bone marrow in chimeric mice. Proc Natl Acad Sci U S A 88:10332–10336

Hoglund P, Glas R, Menard C et al (1998) Beta2-microglobulin-deficient NK cells show increased sensitivity to MHC class I-mediated inhibition, but self tolerance does not depend upon target cell expression of H-2Kb and Db heavy chains. Eur J Immunol 28:370–378

Ito M, Maruyama T, Saito N et al (2006) Killer cell lectin-like receptor G1 binds three members of the classical cadherin family to inhibit NK cell cytotoxicity. J Exp Med 203:289–295

Johansson MH, Bieberich C, Jay G et al (1997) Natural killer cell tolerance in mice with mosaic expression of major histocompatibility complex class I transgene. J Exp Med 186:353–364

Johansson MH, Hoglund E, Nakamura MC et al (1998) Alpha1/alpha2 domains of H-2D(d), but not H-2L(d), induce "missing self" reactivity in vivo–no effect of H-2L(d) on protection against NK cells expressing the inhibitory receptor Ly49G2. Eur J Immunol 28:4198–4206

Joncker NT, Fernandez NC, Treiner E et al (2009) NK cell responsiveness is tuned commensurate with the number of inhibitory receptors for self-MHC class I: the rheostat model. J Immunol 182:4572–4580

Jonsson AH, Yang L, Kim S et al (2010) Effects of MHC class I alleles on licensing of Ly49A + NK cells. J Immunol 184(7):3424–3432

Kane KP (1994) Ly-49 mediates EL4 lymphoma adhesion to isolated class I major histocompatibility complex molecules. J Exp Med 179:1011–1015

Karlhofer FM, Ribaudo RK, Yokoyama WM (1992) MHC class I alloantigen specificity of Ly-49+ IL-2-activated natural killer cells. Nature 358:66–70

Karre K, Ljunggren HG, Piontek G et al (1986) Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature 319:675–678

Kielczewska A, Pyzik M, Sun T et al (2009) Ly49P recognition of cytomegalovirus-infected cells expressing H2-Dk and CMV-encoded m04 correlates with the NK cell antiviral response. J Exp Med 206:515–523

Kim S, Poursine-Laurent J, Truscott SM et al (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 436:709–713

Koh CY, Blazar BR, George T et al (2001) Augmentation of antitumor effects by NK cell inhibitory receptor blockade in vitro and in vivo. Blood 97:3132–3137

Koh CY, Raziuddin A, Welniak LA et al (2002) NK inhibitory-receptor blockade for purging of leukemia: effects on hematopoietic reconstitution. Biol Blood Marrow Transplant 8:17–25

Koh CY, Ortaldo JR, Blazar BR et al (2003) NK-cell purging of leukemia: superior antitumor effects of NK cells H2 allogeneic to the tumor and augmentation with inhibitory receptor blockade. Blood 102:4067–4075

Kubota A, Kubota S, Lohwasser S et al (1999) Diversity of NK cell receptor repertoire in adult and neonatal mice. J Immunol 163:212–216

Lanier LL (2005) NK cell recognition. Annu Rev Immunol 23:225–274

Lanier LL (2008) Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 9:495–502

Lian RH, Li Y, Kubota S et al (1999) Recognition of class I MHC by NK receptor Ly-49C: identification of critical residues. J Immunol 162:7271–7276

Liao NS, Bix M, Zijlstra M et al (1991) MHC class I deficiency: susceptibility to natural killer (NK) cells and impaired NK activity. Science 253:199–202

Ljunggren HG, Van Kaer L, Ploegh HL et al (1994) Altered natural killer cell repertoire in Tap-1 mutant mice. Proc Natl Acad Sci U S A 91:6520–6524

Long EO (2008) Negative signaling by inhibitory receptors: the NK cell paradigm. Immunol Rev 224:70–84

Lundqvist A, McCoy JP, Samsel L et al (2007) Reduction of GVHD and enhanced antitumor effects after adoptive infusion of alloreactive Ly49-mismatched NK cells from MHC-matched donors. Blood 109:3603–3606

MacFarlane AW, Campbell KS (2006) Signal transduction in natural killer cells. Curr Top Microbiol Immunol 298:23–57

Makrigiannis AP, Gosselin P, Mason LH et al (1999) Cloning and characterization of a novel activating Ly49 closely related to Ly49A. J Immunol 163:4931–4938

Makrigiannis AP, Pau AT, Saleh A et al (2001) Class I MHC-binding characteristics of the 129/J Ly49 repertoire. J Immunol 166:5034–5043

Makrigiannis AP, Pau AT, Schwartzberg PL et al (2002) A BAC contig map of the Ly49 gene cluster in 129 mice reveals extensive differences in gene content relative to C57BL/6 mice. Genomics 79:437–444

Marincola FM, Shamamian P, Alexander RB et al (1994) Loss of HLA haplotype and B locus down-regulation in melanoma cell lines. J Immunol 153:1225–1237

Mason LH, Ortaldo JR, Young HA et al (1995) Cloning and functional characteristics of murine large granular lymphocyte-1: a member of the Ly-49 gene family (Ly-49G2). J Exp Med 182:293–303

Mason LH, Gosselin P, Anderson SK et al (1997) Differential tyrosine phosphorylation of inhibitory versus activating Ly-49 receptor proteins and their recruitment of SHP-1 phosphatase. J Immunol 159:4187–4196

McQueen KL, Freeman JD, Takei F et al (1998) Localization of five new Ly49 genes, including three closely related to Ly49c. Immunogenetics 48:174–183

McQueen KL, Lohwasser S, Takei F et al (1999) Expression analysis of new Ly49 genes: most transcripts of Ly49j lack the transmembrane domain. Immunogenetics 49:685–691

Mehta IK, Smith HR, Wang J et al (2001a) A "chimeric" C57l-derived Ly49 inhibitory receptor resembling the Ly49D activation receptor. Cell Immunol 209:29–41

Mehta IK, Wang J, Roland J et al (2001b) Ly49A allelic variation and MHC class I specificity. Immunogenetics 53:572–583

Merck E, Voyle RB, MacDonald HR (2009) Ly49D engagement on T lymphocytes induces TCR-independent activation and CD8 effector functions that control tumor growth. J Immunol 182:183–192

Michaelsson J, Achour A, Salcedo M et al (2000) Visualization of inhibitory Ly49 receptor specificity with soluble major histocompatibility complex class I tetramers. Eur J Immunol 30:300–307

Mishra AK, Zhang A, Niu T et al (2002) Substrate specificity of protein tyrosine phosphatase: differential behavior of SHP-1 and SHP-2 towards signal regulation protein SIRPalpha1. J Cell Biochem 84:840–846

Nakamura MC, Niemi EC, Fisher MJ et al (1997) Mouse Ly-49A interrupts early signaling events in natural killer cell cytotoxicity and functionally associates with the SHP-1 tyrosine phosphatase. J Exp Med 185:673–684

Natarajan K, Boyd LF, Schuck P et al (1999) Interaction of the NK cell inhibitory receptor Ly49A with H-2Dd: identification of a site distinct from the TCR site. Immunity 11:591–601

Olcese L, Lang P, Vely F et al (1996) Human and mouse killer-cell inhibitory receptors recruit PTP1C and PTP1D protein tyrosine phosphatases. J Immunol 156:4531–4534

Olsson-Alheim MY, Sundback J, Karre K et al (1999) The MHC class I molecule H-2Dp inhibits murine NK cells via the inhibitory receptor Ly49A. J Immunol 162:7010–7014

Orr MT, Sun JC, Hesslein DG et al (2009) Ly49H signaling through DAP10 is essential for optimal natural killer cell responses to mouse cytomegalovirus infection. J Exp Med 206:807–817

Orr MT, Murphy WJ, Lanier LL (2010) 'Unlicensed' natural killer cells dominate the response to cytomegalovirus infection. Nat Immunol 11:321–327

Palmieri G, Tullio V, Zingoni A et al (1999) CD94/NKG2-A inhibitory complex blocks CD16-triggered Syk and extracellular regulated kinase activation, leading to cytotoxic function of human NK cells. J Immunol 162:7181–7188

Peterson ME, Long EO (2008) Inhibitory receptor signaling via tyrosine phosphorylation of the adaptor Crk. Immunity 29:578–588

Proteau MF, Rousselle E, Makrigiannis AP (2004) Mapping of the BALB/c Ly49 cluster defines a minimal natural killer cell receptor gene repertoire. Genomics 84:669–677

Raulet DH, Held W, Correa I et al (1997) Specificity, tolerance and developmental regulation of natural killer cells defined by expression of class I-specific Ly49 receptors. Immunol Rev 155:41–52

Robbins SH, Tessmer MS, Mikayama T et al (2004) Expansion and contraction of the NK cell compartment in response to murine cytomegalovirus infection. J Immunol 173:259–266

Romagne F, Andre P, Spee P et al (2009) Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. Blood 114:2667–2677

Rouhi A, Lai CB, Cheng TP et al (2009) Evidence for high bi-allelic expression of activating Ly49 receptors. Nucleic Acids Res 37:5331–5342

Ruggeri L, Capanni M, Urbani E et al (2002) Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 295:2097–2100

Sasawatari S, Yoshizaki M, Taya C et al (2010) The Ly49Q receptor plays a crucial role in neutrophil polarization and migration by regulating raft trafficking. Immunity 32:200–213

Scarpellino L, Oeschger F, Guillaume P et al (2007) Interactions of Ly49 family receptors with MHC class I ligands in trans and cis. J Immunol 178:1277–1284

Shlomchik WD, Couzens MS, Tang CB et al (1999) Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science 285:412–415

Silver ET, Lavender KJ, Gong DE et al (2002) Allelic variation in the ectodomain of the inhibitory Ly-49G2 receptor alters its specificity for allogeneic and xenogeneic ligands. J Immunol 169:4752–4760

Smith HR, Karlhofer FM, Yokoyama WM (1994) Ly-49 multigene family expressed by IL-2-activated NK cells. J Immunol 153:1068–1079

Smith KM, Wu J, Bakker AB et al (1998) Ly-49D and Ly-49H associate with mouse DAP12 and form activating receptors. J Immunol 161:7–10

Smith HR, Heusel JW, Mehta IK et al (2002) Recognition of a virus-encoded ligand by a natural killer cell activation receptor. Proc Natl Acad Sci U S A 99:8826–8831

Stebbins CC, Watzl C, Billadeau DD et al (2003) Vav1 dephosphorylation by the tyrosine phosphatase SHP-1 as a mechanism for inhibition of cellular cytotoxicity. Mol Cell Biol 23:6291–6299

Sun JC, Lanier LL (2008) Cutting edge: viral infection breaks NK cell tolerance to "missing self ". J Immunol 181:7453–7457

Sun JC, Beilke JN, Lanier LL (2009) Adaptive immune features of natural killer cells. Nature 457:557–561

Tai LH, Goulet ML, Belanger S et al (2007) Recognition of H-2 K(b) by Ly49Q suggests a role for class Ia MHC regulation of plasmacytoid dendritic cell function. Mol Immunol 44:2638–2646

Tai LH, Goulet ML, Belanger S et al (2008) Positive regulation of plasmacytoid dendritic cell function via Ly49Q recognition of class I MHC. J Exp Med 205:3187–3199

Takei F, Brennan J, Mager DL (1997) The Ly-49 family: genes, proteins and recognition of class I MHC. Immunol Rev 155:67–77

Tonks NK, Neel BG (1996) From form to function: signaling by protein tyrosine phosphatases. Cell 87:365–368

Tortorella D, Gewurz BE, Furman MH et al (2000) Viral subversion of the immune system. Annu Rev Immunol 18:861–926

Toyama-Sorimachi N, Tsujimura Y, Maruya M et al (2004) Ly49Q, a member of the Ly49 family that is selectively expressed on myeloid lineage cells and involved in regulation of cytoskeletal architecture. Proc Natl Acad Sci U S A 101:1016–1021

Vahlne G, Lindholm K, Meier A et al (2010) In vivo tumor cell rejection induced by NK cell inhibitory receptor blockade: maintained tolerance to normal cells even in the presence of IL-2. Eur J Immunol 40:813–823

Valiante NM, Phillips JH, Lanier LL et al (1996) Killer cell inhibitory receptor recognition of human leukocyte antigen (HLA) class I blocks formation of a pp 36/PLC-gamma signaling complex in human natural killer (NK) cells. J Exp Med 184:2243–2250

Van Beneden K, Stevenaert F, De Creus A et al (2001) Expression of Ly49E and CD94/NKG2 on fetal and adult NK cells. J Immunol 166:4302–4311

Van Beneden K, De Creus A, Stevenaert F et al (2002) Expression of inhibitory receptors Ly49E and CD94/NKG2 on fetal thymic and adult epidermal TCR V gamma 3 lymphocytes. J Immunol 168:3295–3302

Van Den Broeck T, Stevenaert F, Taveirne S et al (2008) Ly49E-dependent inhibition of natural killer cells by urokinase plasminogen activator. Blood 112:5046–5051

Vivier E, Anfossi N (2004) Inhibitory NK-cell receptors on T cells: witness of the past, actors of the future. Nat Rev Immunol 4:190–198

Vyas YM, Mehta KM, Morgan M et al (2001) Spatial organization of signal transduction molecules in the NK cell immune synapses during MHC class I-regulated noncytolytic and cytolytic interactions. J Immunol 167:4358–4367

Vyas YM, Maniar H, Dupont B (2002) Cutting edge: differential segregation of the SRC homology 2-containing protein tyrosine phosphatase-1 within the early NK cell immune synapse distinguishes noncytolytic from cytolytic interactions. J Immunol 168:3150–3154

Vyas YM, Maniar H, Lyddane CE et al (2004) Ligand binding to inhibitory killer cell Ig-like receptors induce colocalization with Src homology domain 2-containing protein tyrosine phosphatase 1 and interruption of ongoing activation signals. J Immunol 173:1571–1578

Wang JW, Howson JM, Ghansah T et al (2002) Influence of SHIP on the NK repertoire and allogeneic bone marrow transplantation. Science 295:2094–2097

Wilhelm BT, Gagnier L, Mager DL (2002) Sequence analysis of the ly49 cluster in C57BL/6 mice: a rapidly evolving multigene family in the immune system. Genomics 80:646–661

Wong S, Freeman JD, Kelleher C et al (1991) Ly-49 multigene family. New members of a superfamily of type II membrane proteins with lectin-like domains. J Immunol 147:1417–1423

Wu MF, Raulet DH (1997) Class I-deficient hemopoietic cells and nonhemopoietic cells dominantly induce unresponsiveness of natural killer cells to class I-deficient bone marrow cell grafts. J Immunol 158:1628–1633

Yang J, Liang X, Niu T et al (1998) Crystal structure of the catalytic domain of protein-tyrosine phosphatase SHP-1. J Biol Chem 273:28199–28207

Yokoyama WM, Kim S (2006) Licensing of natural killer cells by self-major histocompatibility complex class I. Immunol Rev 214:143–154

Yokoyama WM, Plougastel BF (2003) Immune functions encoded by the natural killer gene complex. Nat Rev Immunol 3:304–316

Yu YY, George T, Dorfman JR et al (1996) The role of Ly49A and 5E6(Ly49C) molecules in hybrid resistance mediated by murine natural killer cells against normal T cell blasts. Immunity 4:67–76

Yu MC, Su LL, Zou L et al (2008) An essential function for beta-arrestin 2 in the inhibitory signaling of natural killer cells. Nat Immunol 9:898–907

Immunoregulatory Roles for Fc Receptor-Like Molecules

Götz R.A. Ehrhardt and Max D. Cooper

Contents

Abstract Fc receptor-like (FCRL) molecules comprise a family of imunoregulatory transmembrane proteins that are preferentially, but not exclusively expressed on B lineage cells. A strong regulatory potential on B cell activation has been characterized for the different FCRL proteins, but their biological roles are just beginning to be elucidated. We review recent advances in the understanding of FCRL1-6 expression and function, and indicate their potential roles in the pathogenesis of immunodeficiencies, lymphoid malignancies and autoimmune diseases.

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1 Introduction

Adaptive immune responses serve as a key defense mechanism for the control of infections in vertebrates. While immune responses must be of sufficient strength to contain invading pathogens, antigen-specific responses require regulatory mechanisms to ensure termination or downmodulation to avoid excessive damage to the host tissue. For both branches of the adaptive immune system regulatory molecules have been identified, which control the signaling cascades initiated by engagement of the T cell and B cell antigen receptors. Elucidation of the functional properties of immunoregulating elements of the immune system has important implications for understanding natural immune responses, as well as those elicited by vaccinations. This review concerns a family of six partially characterized immunoregulators named Fc receptor-like (FCRL) molecules. The FCRLs have garnered increasing interest due to their differential patterns of lymphocyte expression and potential involvement in the pathogenesis of autoimmune disorders, immunodeficiency, and lymphoid malignancies in humans.

2 Identification of FCRL Molecules Reveals a New Level of B Cell Regulation

The first report of *FCRL* genes by the laboratory of Ricardo Dalla-Favera described a novel gene at a chromosomal translocation breakpoint in a multiple myeloma (MM) cell line (Hatzivassiliou et al. 2001). The resulting fusion protein consisted of the signal peptide and a small portion of the extracellular domain of the receptor, now known as *FCRL4*, fused to the constant region domain of Igα. Sequence analysis of a full-length cDNA for this novel gene suggested an immunoglobulin-domain type receptor. Accordingly, the gene was named immunoglobulin superfamily receptor translocation-associated 1 (*IRTA1*) (Hatzivassiliou et al. 2001). A related neighboring gene identified in this study was named *IRTA2*. Independently, Davis and co-workers identified these genes and three additional members of this receptor family using a bioinformatics approach that was based on sequence similarities to the classical Fc receptors FcγRI, FcγRII, and FcγRIII. The sequence similarities to known Fc receptors coupled with their neighboring genomic localization and conserved organization led to the name Fc receptor homologs (FcRH) 1–5 (Davis et al. 2001). Members of this receptor family were also identified by other research groups and given names that reflect the methodological approaches leading to their discovery: IgSF-FcR-Gp42 (IFGP) as a result of sequence similarity to the immunoglobulin domains of FcγRI (Guselnikov et al. 2002), SH2 domain-containing phosphatase anchor protein 1 (SPAP1) indicating the ability upon tyrosine phosphorylation to recruit the tyrosine phosphatase SHP-1 (Xu et al. 2001), and BXMAS1 reflecting the upregulation of the transcript in response to

Table 1 Nomenclature of FCRL molecules

Name	Species	Alias
FCRL1	*Homo sapiens*	FcRH1, IRTA5, IFGP1, BXMAS1
FCRL2	*Homo sapiens*	FcRH2, IRTA4, IFGP4, BXMAS2, SPAP1
FCRL3	*Homo sapiens*	FcRH3, IRTA3, IFGP3, BXMAS3, SPAP2
FCRL4	*Homo sapiens*	IRTA1, FcRH4, IFGP2
FCRL5	*Homo sapiens*	IRTA2, FcRH5, IFGP5, BXMAS
FCRL6	*Homo sapiens*	FcRH6, IFGP6
FCRLA	*Homo sapiens*	FCRL, FREB, FcRX
FCRLB	*Homo sapiens*	FcRL2, FREB2, FcRY
Fcrl1	*Mus musculus*	FcRH1, IFGP1, BXMAS1
Fcrl5	*Mus musculus*	FcRH3, mBXMH2
Fcrl6	*Mus musculus*	FcRH6
Fcrls	*Mus musculus*	FcRH2, IFGP2, MSR2
Fcrla	*Mus musculus*	Fcrl1, FREB, FcRX
Fcrlb	*Mus musculus*	Fcrl2, FREB2, FcRY

Adapted from (Maltais et al. 2006). Table lists the current nomenclature, species, and formerly used names

ligation of the BCR (Nakayama et al. 2001). A unified nomenclature introduced in 2006 renamed these genes as *FCRL* genes (Maltais et al. 2006) (Table 1).

The *FCRL* genes belong to an ancient multigene family that is phylogenetically conserved in vertebrate species, at least from amphibians onward (reviewed in Davis 2007). In humans this gene family encodes six transmembrane receptors, FCRL1–6, and two intracellular proteins, FCRLA and FCRLB (Chikaev et al. 2005; Mechetina et al. 2002). The genes for the six transmembrane proteins of the FCRL family are highly conserved among humans and non-human primates, whereas mice have orthologous genes for only two of the five B cell-specific Fcrl family members (Fig. 1) and these do not display a high degree of sequence conservation. Therefore, the FCRL proteins add to the list of differences between the human and rodent adaptive immune systems (Mestas and Hughes 2004). The immunoregulatory potential and expression patterns of the FCRLs suggest that, with some notable exceptions, they govern primarily the activity of mature B lymphocytes. In this review, we focus on the human FCRL1–5 transmembrane receptors that are preferentially expressed by B lineage cells and FCRL6, which is expressed by T cells and NK cells.

3 FCRL Structural Properties

FCRL genes are located in close proximity to FcγRI and FcεRI in the q21–q23 region of chromosome 1 in humans. The murine *Fcrl* region is divided into two loci located on chromosomes 1 and 3. The similarities to the known Fc receptor genes extend to genomic organization. Members of both *FcR* and *FCRL* families have a

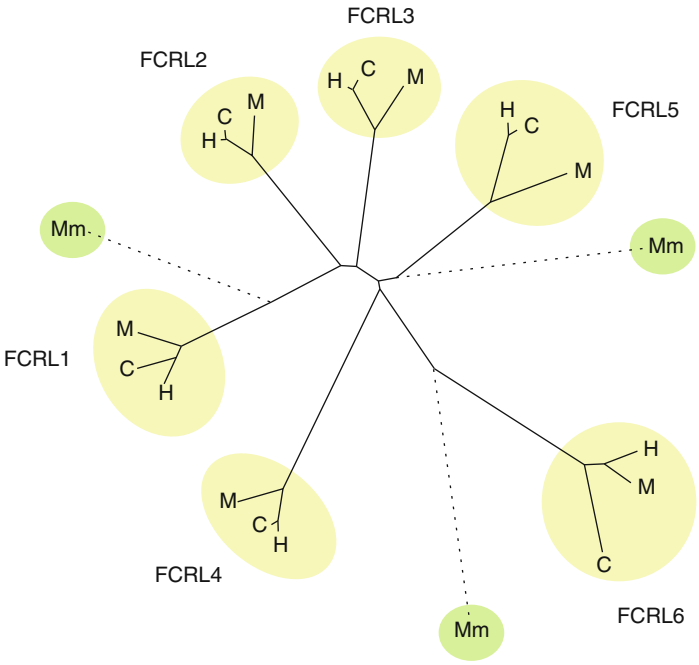

Fig. 1 Phylogenetic tree of FCRL family members in *Homo sapiens* (H), *Pan troglodytes* (C), *Macaca mulatta* (M), and *Mus musculus* (Mm). Protein alignments were performed using the ClustalW2 algorithm of the EMBL-EBI webserver. The graphic treefile was generated using the *drawtree* application of the HMMER package version 3.0 of the webserver of the Institut Pasteur, Paris

characteristic signal peptide coding sequence that is split into two exons, and each extracellular Ig domain is encoded by a single exon.

The six FCRL molecules have three to nine extracellular Ig domains and individual FCRs may be expressed in different isoforms (Fig. 2). For example, FCRL5 has splice isoforms indicative of secreted and GPI-anchored isoforms in addition to the transmembrane isoform (Hatzivassiliou et al. 2001). Importantly, the intracellular domains of the FCRL1–6 proteins in humans contain consensus sequences for the immunoreceptor tyrosine-based activation motifs (ITAM; D/E-x-x-Y-x-x-L/I-$x_{6–8}$-Y-x-x-L/I) and/or immunoreceptor tyrosine-based inhibitory motifs (ITIM; I/V/L/S-x-Y-x-x-L) (Ravetch and Lanier 2000; Reth 1992; Vely and Vivier 1997). The intracellular domain of FCRL1 has two ITAM sequences and the intracellular domain of FCRL4 contains three ITIM sequences. The cytoplasmic tails of FCRL2, 3, and 5 contain both ITAM and ITIM motifs, which suggest functional flexibility due to the potential for performing activating and inhibitory functions in a context-dependent manner. This dual potential may extend to FCRL4, because its membrane proximal ITIM sequence has the characteristics of an immunoreceptor tyrosine-based switch motif (ITSM) (Shlapatska et al. 2001). Additionally, the membrane proximal ITIM sequence of FCRL4 could form a non-

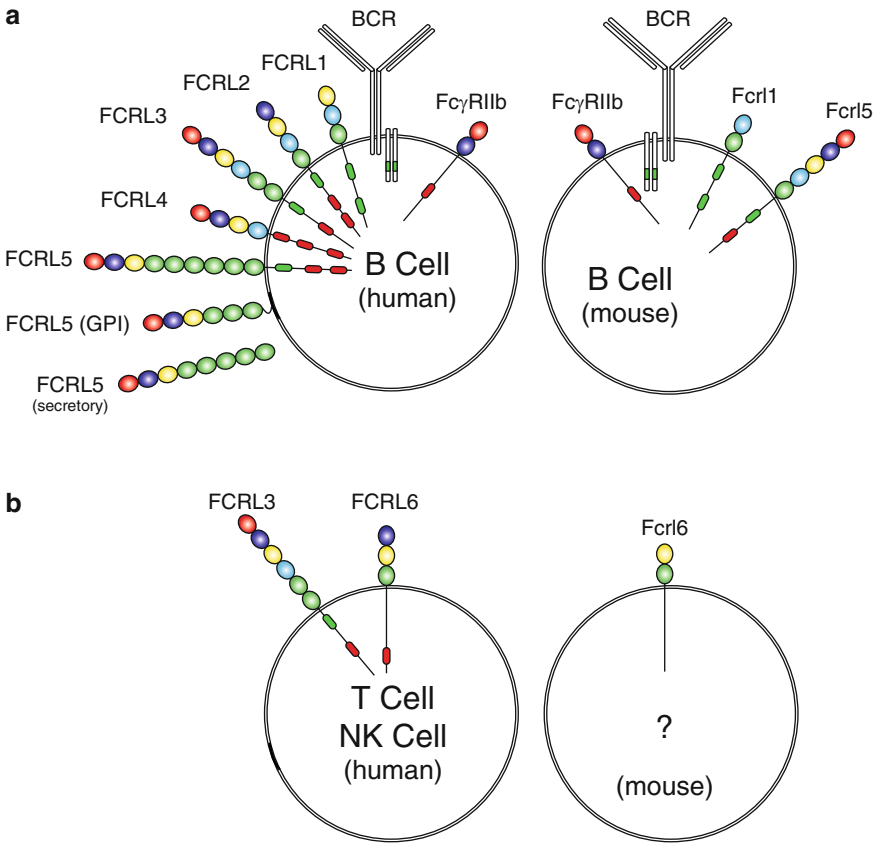

Fig. 2 FCRL family members expressed on (**a**) human and murine B lymphocytes and (**b**) non-B lineage cells. Extracellular Ig domains are indicated by *colored ellipses* with colors indicating similarities between the Ig domains. Intracellular ITAM and ITIM sequences are indicated by *green squares* and *red squares*, respectively

canonical ITAM in combination with the neighboring ITIM. The intracellular domain of human FCRL6 has a single ITIM sequence that is not conserved in the murine ortholog. Sequence similarities between the extracellular Ig domains are greater for the membrane proximal Ig domains (indicated by the green ellipses in Fig. 2), which share 53–83% identity versus 22–59% identity for the more membrane distal Ig domains. Despite the sequence similarities of FCRL molecules to the classical Fc receptors, it is important to note that immunoglobulin-binding potential has not been experimentally confirmed. Although cells overexpressing FCRL5 initially were reported to bind a mixture of IgG antibodies, none of the FCRL molecules were found to bind purified immunoglobulins when assayed by flow cytometry or Bio-Layer Interferometry (Polson et al. 2006; and our unpublished observations). The identification of ligands for these orphan receptors and the cells that express the ligands thus remains a high priority.

4 FCRL Expression Patterns

The cellular expression patterns for FCRL family members have been investigated by analysis of transcript levels by northern blots, RT-PCR, and in situ hybridizations, and by protein expression assessment by flow cytometry and immunohistochemical methods using both monoclonal and polyclonal antibodies. These studies indicate that the FCRL1-5 molecules are expressed predominantly by B lineage cells of the immune system, whereas FCRL6 is expressed by subpopulations of T and NK lineage cells. Intriguingly, the FCRL1-5 family members are variably expressed by B lineage cells according to their stages of maturation and tissue localization.

FCRL1 transcript and protein analyses indicate that this receptor is expressed by pro-B and pre-B cells as well as by all mature B cell populations. Although in situ hybridization analysis of tonsillar tissue initially suggested exclusive exp ression by mantle zone B cells (Miller et al. 2002), quantitative RT-PCR analysis has indicated relatively high FCRL1 transcript levels in naïve and memory B cells and lower transcript levels in pre-germinal center (pre-GC), germinal center (GC), and plasma cell (PC) populations (Leu et al. 2005). In accordance with its transcript expression pattern, FCRL1 is detectable on all peripheral blood B cells but not on most of the GC B cells (Leu et al. 2005; Polson et al. 2006).

In contrast with FCRL1, in situ hybridization with FCRL2-specific probes identifies intraepithelial and interfollicular lymphocytes in tonsillar tissue sections and some of the centrocytes in the light zone of GCs (Miller et al. 2002). This tissue distribution pattern of FCRL2 mRNA expression is concordant with the immunofluorescence analysis of FCRL2 protein expression by tonsillar and peripheral blood B cells. The composite data indicate that FCRL2 is preferentially expressed by memory B cells (Huttmann et al. 2006; Polson et al. 2006) and at low levels or not at all by GC B cells.

FCRL3 transcripts are found in memory, naïve, and GC B cell populations (Davis et al. 2001). In situ hybridization analysis reveals selective mRNA expression of FCRL3 by GC cells within the centrocyte-rich light zone (Miller et al. 2002). FCRL3 molecules are found in relatively low levels on peripheral blood and tonsillar B cells and in higher levels on splenic B cells (Nagata et al. 2009; Polson et al. 2006). Notably, FCRL3 represents the exception to the rule that expression of the *FCRL1–5* genes is limited to B lineage cells, in which FCRL3 expression has also been demonstrated on NK cells in the circulation (Polson et al. 2006). Furthermore, FCRL3 expression has been demonstrated for a subpopulation of the $CD25^{+}$/$Foxp3^{+}$ regulatory T cells (Nagata et al. 2009; Swainson et al. 2010).

FCRL4 mRNA and protein expression levels indicate a more selective pattern of cellular expression. FCRL4 transcripts have been demonstrated in memory B cell populations by quantitative RT-PCR and in situ hybridization (Ehrhardt et al. 2003; Hatzivassiliou et al. 2001). FCRL4 protein expression is limited to a subpopulation of memory B cells that are found primarily in mucosa-associated lymphoid tissues

(Ehrhardt et al. 2005; Falini et al. 2003; Polson et al. 2006). The FCRL4-positive B cells are rarely found in blood, spleen, and bone marrow samples from healthy individuals. Furthermore, this subpopulation of memory B cells is morphologically and functionally distinct from its more abundant and broadly distributed FCRL4-negative memory B cell counterparts. Comparative transcriptome analysis of $FCRL4^+$ versus $FCRL4^-$ memory B cells has revealed distinctive gene expression profiles for these two memory B cell populations. The $FCRL4^+$ memory B cells express relatively high mRNA levels for the chemokine receptors, CCR1 and CCR5, CD95, and the TNF superfamily member, RANKL; among B lineage cells, they are uniquely positive for the CD11c integrin (Ehrhardt et al. 2008). Adding to the list of differentially expressed transcripts, the src-family kinases HCK and FGR are upregulated in the $FCRL4^+$ population, whereas the src-family kinase LCK is upregulated in $FCRL4^-$ memory B cells. Notably, most of the $FCRL4^+$ memory B cells lack the commonly used CD27 memory B cell marker (Ehrhardt et al. 2005; Johrens et al. 2005; Moir et al. 2008); variable levels of FCRL4 and CD27 co-expression have been noted and this may reflect variability between tissue samples from different individuals (Falini et al. 2003; Lazzi et al. 2006). Importantly, functional analyses indicate very different responses of the two memory B cell subpopulations to T-dependent and T-independent stimulation. *Ex vivo* analysis indicates that $FCRL4^-$ memory B cells respond to antigen receptor ligation as a means of simulating T cell-independent stimulation, as well as to treatment with IL2, IL10, and CD40L, as a means of simulating T cell-dependent stimulation. The $FCRL4^+$ memory B cells instead respond robustly to T cell-dependent stimulation, but they fail to respond well to T cell-independent stimulation (Ehrhardt et al. 2005).

FCRL5 transcripts are expressed by cells in the tonsillar interfollicular and intraepithelial regions, which are rich in memory B cells, and also within the centrocyte-rich light zones of GCs (Hatzivassiliou et al. 2001). Immunofluorescence analysis of blood, spleen, and tonsillar tissues indicates a relatively low level of FCRL5 expression by GC B cells and higher expression levels in the naïve and memory B cell compartments (Polson et al. 2006). It is presently unclear why the substantial protein expression levels on naïve B cells indicated by immunofluorescence are not matched by FCRL5 mRNA expression by mantle zone cells in in situ hybridization studies. FCRL5 protein levels are highest in $CD38^{++}/CD138^+$ PCs in the bone marrow, tonsil, and spleen, suggesting an FCRL5 immunoregulatory role for PCs (Polson et al. 2006).

FCRL6 is not expressed by B lineage cells, in striking contrast with the first five members of the FCRL family. Using FCRL6-specific monoclonal antibodies, FCRL6 expression instead has been demonstrated for mature $CD56^{dim}$ NK cells, a subpopulation of $CD56^+/CD3^+$ NKT cells, and $CD8^+$ T cells of both effector and effector memory subtypes (Wilson et al. 2007). FCRL6 expression has also been shown for $CD8^+$ γδ T cells and relatively rare cytotoxic $CD4^+$ T cells (Schreeder et al. 2008).

The differential patterns for FCRL expression displayed by human B lymphocytes suggest that these receptors exert their functional roles primarily during the

later stages of differentiation and on memory B cells in particular. The remarkably restricted pattern of FCRL4 expression also highlights the heterogeneity of the human memory B cell pool and suggests that distinctive control mechanisms may govern activation of the $FCRL4^+$ versus $FCRL4^-$ memory B cells.

5 FCRL Functional Properties

Co-receptors that have regulatory control over the activation status of cells belonging to the immune system typically are characterized by the presence of ITAMs or ITIMs. The FCRL family members have intracellular domain sequences that fit the consensus ITAM and ITIM sequences, which indicated the immunoregulatory potential of these receptors. Studies on FCRL1-bearing B cell lines and primary B cells suggest a co-activator function on antigen receptor signaling for this receptor (Leu et al. 2005). Co-ligation of FCRL1 with the BCR on Daudi B cells results in increased calcium mobilization over that initiated by BCR ligation alone. Stimulation of primary tonsillar B cells with anti-FCRL1 antibodies induces a proliferative response and its co-ligation enhances the response to BCR ligation (Leu et al. 2005). The two ITAM sequences in the intracellular domain of FCRL1 undergo tyrosine phosphorylation following antibody-mediated FCRL1 ligation (Leu et al. 2005). Leu et al. have also observed association of the p85 subunit of PI3kinase and the adaptor molecule Grb2 to the tyrosine phosphorylated intracellular domain of FCRL1 (unpublished observations), thereby revealing a potential mechanism for the activating function of FCRL1. Furthermore, the FCRL1 transmembrane domain is unique among the FCRL family members in containing a negatively charged amino acid that could foster FCRL1 association with a positively charged transmembrane polypeptide to promote a co-activator role.

FCRL3 has been shown to inhibit BCR signaling when overexpressed and co-ligated with the BCR on cells of a GC-derived cell line (Kochi et al. 2009). This inhibitory activity was accompanied by binding of SHP-1 to the intracellular domain of FCRL3. SHP-1 binding to synthetic phosphopeptide mimics showed that binding of the phosphatase occurred chiefly on the ITIM sequence surrounding tyrosine 692 of the intracellular domain of FCRL3, a finding that confirmed earlier observations (Xu et al. 2001). Phosphopeptides corresponding to the ITAM sequences in FCRL3 that are dually phosphorylated have been shown to associate with the Syk and ZAP70 tyrosine kinases, thereby lending support for an activating potential for this receptor (Kochi et al. 2009).

A variant B cell line has been used as a model system to evaluate the immunoregulatory activity of FCRL2, 4, and 5, all of which have consensus ITIM sequences in their intracellular domains. The mouse memory B cell line A20-IIA1.6 lacks expression of endogenous Fc receptors and was used originally to demonstrate the inhibitory potential of the PD-1 receptor (Okazaki et al. 2001). For our studies on the inhibitory potential of the FCRL ITIMs, a chimeric receptor consisting of the extracellular and transmembrane domains of FcγRIIb fused to the

intracellular domain of the FCRL of interest was stably expressed in the A20-IIA1.6 cells. Activation of the B cell receptor (BCR) on these cells with $F(ab')_2$ fragments of BCR-specific antibodies leads to the activation of several downstream signal transduction cascades. BCR ligation with intact anti-BCR antibodies instead co-ligates the BCR with the chimeric FcγRIIb/FCRL fusion protein to allow evaluation of the effects of the intracellular domains of FCRL molecules on BCR signaling.

We observed a strong negative effect of FCRL4 co-ligation on BCR signaling using the model system described above. Unlike the relatively modest dampening of BCR-mediated signaling observed after co-ligation of the BCR with FcγRIIb (Muta et al. 1994), which is mediated primarily by recruitment of the SHIP inositol phosphatase, FCRL4 co-ligation with the BCR results in tyrosine phosphorylation of the tyrosine residues in the two membrane distal ITIM sequences of the intracellular domain of FCRL4, ablates almost completely the BCR-induced calcium mobilization and strongly inhibits Akt and MAPK activation (Ehrhardt et al. 2003). This potent inhibitory effect reflects the recruitment of the intracellular tyrosine phosphatases SHP-1 and/or SHP-2. As mentioned earlier, the two membrane proximal ITIM sequences of the intracellular domain of FCRL4 could potentially form a non-canonical ITAM recognition sequence. Although co-ligation of the chimeric FcγRIIb/FCRL4 receptor did not lead to tyrosine phosphorylation of the membrane proximal ITIM sequence, a phosphopeptide mimic of this potential ITAM in which both tyrosine residues were phosphorylated was able to precipitate PLCγ. This finding suggests the possibility that in addition to its inhibitory function, FCRL4 could assume an activating role in a context-dependent manner.

FCRL5 co-ligation also inhibits BCR signaling in the model system described above. This inhibitory effect is mediated via SHP-1 recruitment to the phosphorylated tyrosine residues at positions 924 and 954 in the FCRL5 ITIMs. The recruitment of the SHP-1 tyrosine phosphatase leads to dephosphorylation of the Igα/β signaling chains of the BCR complex, thus suggesting a potential mechanism for FCRL5-mediated inhibition of antigen receptor signaling. Importantly, the inhibition of BCR signaling in this in vitro model system could be reproduced by co-ligation of endogenous FCRL5 on primary memory B cells (Haga et al. 2007). The inhibition was sensitive to the *ex vivo* pre-treatment of the primary B cells with pharmacologic SHP-1 inhibitors. Unpublished work from our laboratory (T. Jackson et al.) indicates an equally potent inhibitory potential for FCRL2 on BCR signal transduction.

Although studies addressing the functional properties of the FCRL family members have relied mainly on in vitro experiments involving artificial co-ligation with the BCR, they convincingly demonstrate the regulatory potential of these receptors. The data so far implicate the FCRL proteins primarily as elements for negative regulatory control of B cell activation (see Fig. 3), although the ITAM-like sequences present in most of these receptors may contribute flexibility in fine-tuning the immune responses, especially for B lineage cells in the memory B cell and PC stages during which most of the FCRLs are expressed.

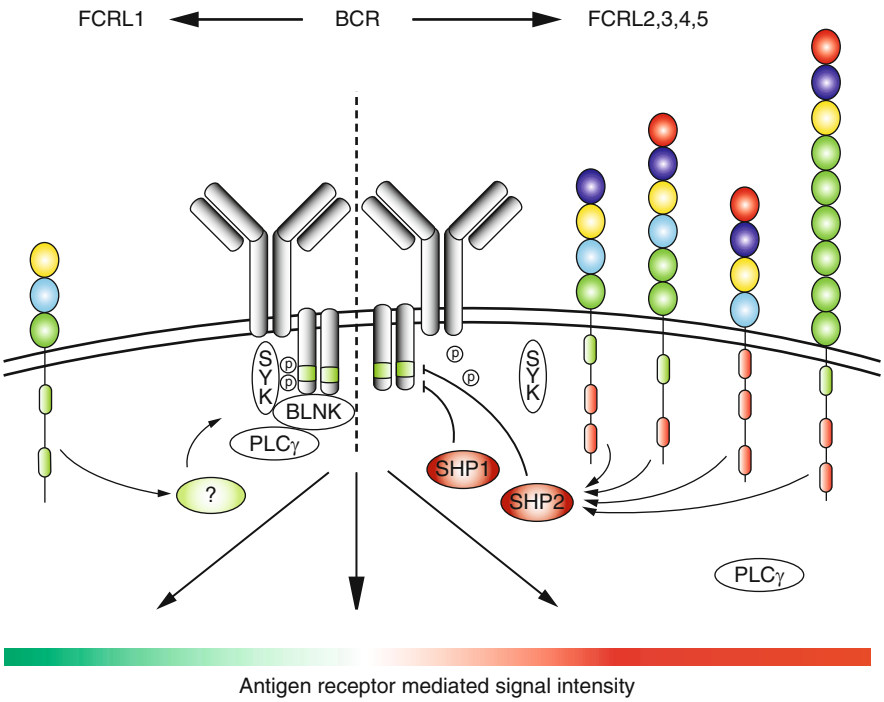

Fig. 3 Signaling potential of FCRL family members. Co-ligation of FCRL2, 3, 4, and 5 leads to inhibition of BCR signaling via recruitment of tyrosine phosphatases, whereas co-engagement of FCRL1 leads to increased BCR signaling via an as yet undefined mechanism. Effects on BCR-mediated signaling are indicated by the *bar* at the bottom of the graphic, with *green color* indicating activating potential and *red color* indicating inhibitory potential

6 FCRL Involvement in Human Diseases

The potent immunomodulatory potential of FCRL molecules suggested that these proteins may have important roles in the regulation of immune responses. The corollary implication is that the dysregulation of these receptors could contribute to the pathogenesis of autoimmune disorders, hemopoietic malignancies, and severity of infections. Indeed, a number of recent reports suggest involvement of FCRLs and the cells that express them in several such disorders. Attention was attracted to FCRL3 by a report that noted the correlation of a polymorphism in the promoter region of the *FCRL3* gene with increased susceptibility to rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and autoimmune thyroid disease (Kochi et al. 2005). This SNP is located in a weak NFκB-binding site in the FCRL3 promoter and is associated with more efficient NFκB binding that enhances transcription. This finding was initially supported (Ikari et al. 2006) and extended to Grave's disease (Simmonds et al. 2006), while some subsequent studies have not

indicated significant correlation between this SNP and disease susceptibility (Hu et al. 2006; Sanchez et al. 2006; Takata et al. 2008). The explanation for the inconsistency may lie in the differences between study populations. In this regard, a recent analysis indicated significant association of the investigated FCRL3 SNP with RA in Asian study populations, but not for cohorts of European descent (Lee et al. 2010).

Two recent studies have shown FCRL3 expression by a subpopulation of naturally occurring regulatory T cells (nT_{reg}) whose members are characterized by memory phenotype, high expression levels of programmed cell death 1 (PD-1), and impaired responsiveness to exogenous IL-2 (Nagata et al. 2009; Swainson et al. 2010). One of these studies implicates an elevation of FCRL3 in the pathogenesis of autoimmune diseases. The levels of FCRL3 expression by the nT_{reg} subpopulation were shown to vary with the occurrence of a SNP noted in the promoter region of the *FCRL3* gene; a C/C constellation at position-196 correlated with a higher level of FCRL3 expression, a C/T constellation corresponded with an intermediate FCRL3 expression level, and a T/T sequence was associated with a lower FCRL3 expression level (Swainson et al. 2010). Importantly, the $FCRL3^+$ T_{reg} cells displayed reduced responsiveness to antigenic stimulation in the presence of IL-2 and were impaired in their ability to suppress T cell proliferation.

Patients with B cell lymphocytic leukemia (B-CLL), a malignancy characterized by a rather homogenous phenotypical appearance, can be separated into those individuals likely to have an indolent course versus those likely to have more aggressive disease. The categorization is based on the somatic mutational status of the antigen receptor and the expression levels of CD38 and ZAP70 (Damle et al. 1999; Hamblin et al. 1999). An indolent B-CLL course is predicted by mutated antigen receptors and a lack of ZAP70 and CD38 expression, whereas an aggressive course of the disease is indicated by an unmutated antigen receptor and expression of CD38 and ZAP70 by the malignant cells. Several recent reports indicate increased FCRL2 expression levels on B-CLL cells in patients predicted to have the less aggressive phenotype (Huttmann et al. 2006; Li et al. 2008; Nuckel et al. 2009). It will be interesting to determine whether there is a causative interrelationship between expression of the inhibitory FCRL2 protein and the indolent disease course for this subgroup of B-CLL patients.

FCRL4 occupies a unique position among the FCRL family members by virtue of its highly restricted pattern of expression by a tissue-based subpopulation of memory B cells. Gene array data and immunofluorescence analyses also show that, among the B lineage cells, FCRL4-bearing memory cells are uniquely positive for CD11c expression (Ehrhardt et al. 2008). Although commonly found on various dendritic cell populations, CD11c is used as a hairy cell leukemia (HCL) marker (Goodman et al. 2003). The presence of CD11c on primary $FCRL4^+$ memory B cells may suggest that the elusive normal counterpart of the malignant HCL cells belongs to this memory B cell population. Recent findings also implicate FCRL4-bearing B cells in the immunopathology of combined variable immunodeficiency (CVID). Analysis of peripheral blood mononuclear cells in these individuals

indicated an expansion of B cells characterized by FCRL4 expression, low levels of CD21 expression, and poor responsiveness to antigen receptor ligation. Unlike the FCRL4-bearing memory B cells normally found in tonsils, the FCRL4$^+$ B cells found in the circulation of CVID patients were shown to have unmutated antigen receptor genes and therefore were postulated to represent an innate-like B cell population (Rakhmanov et al. 2009).

Circulatory FCRL4$^+$ memory B cells have recently been implicated in the immunopathology of two chronic infectious diseases, HIV and malaria. Although normally restricted to tissues of the mucosal immune system, FCRL4$^+$ memory B cells with characteristically low levels of CD21 expression and high levels of CD20 expression were abundant in blood samples of HIV-1 viremic individuals, but not in HIV-1 aviremic individuals or healthy controls (Moir et al. 2008). These atypically distributed memory B cells were found to have an *exhausted* phenotype in which they did not respond to stimulation with the cytokines IL2/IL10 and CD40L as a means for simulated T cell help. Importantly, the repertoire of the circulating FCRL4$^+$ memory B cells in HIV-infected patients was shown to be enriched for antibodies specific for the HIV-1 p120 envelope protein. CD21^{low}/CD20^{high}/FCRL4$^+$ memory B cells have also been found in the circulation of individuals exposed to the malaria pathogen *Plasmodium fulciparum* (Weiss et al. 2009). Although no direct involvement has been demonstrated for FCRL4 in the exhausted B cell phenotype in HIV-1 viremic individuals, the above findings are reminiscent of those observed for the other arm of the adaptive immune system in chronic viral infections, wherein exhausted antigen-specific T cells are characterized by expression of the inhibitory PD-1 receptor. Moreover, interference of PD-1 binding to its ligand PD-1L leads to re-invigoration of these exhausted T cells (Day et al. 2006). It will be interesting to determine whether interference of FCRL4 binding to its unknown ligands may lead to a similar re-invigoration of antigen-specific B cells and enhanced immune responses to these persistent pathogens.

Finally, elevated levels of the secreted isoform of FCRL5 have been found in patients with several B cell malignancies using an ELISA-based monitoring system. The levels of soluble FCRL5 correlated with the tumor burden in patients with HCL, B-CLL, MM, and mantle cell leukemia (Ise et al. 2005, 2007). The mechanism for the abundance of soluble FCRL5 in these disorders awaits clarification, but these findings suggest that FCRL5 may serve as a diagnostic marker and potential therapeutic target for these malignancies.

7 Concluding Remarks

Although we are just beginning to understand the FCRL orphan receptors and their functions, pathophysiological roles for several FCRL family members have been implied in autoimmune disorders, malignancies, immunodeficiencies, and infectious diseases, underscoring their potential importance in these health problems. The high degree of conservation of *FCRL* genes in humans and non-human

primates, combined with the absence of many of the *FCRL* genes in rodents, indicates the need for future research in non-human primate models. This exploration may be especially important for gaining an understanding of the dysregulation of $FCRL4^+$ memory B cells in HIV and malaria, two infectious diseases that together affect more than 300 million people worldwide. The cellular expression profile of the FCRL family features a memory B cell centric pattern that extends to the PC compartment in the case of FCRL5. Memory B cells and PCs are critical to the establishment and maintenance of humoral immunity, and their FCRL expression subjects these cells to immunoregulatory mechanisms that are still not well understood. Given the fact that most preventive vaccines function by eliciting neutralizing antibodies, better understanding of the FCRL family of immunoregulators could lead to novel insights in eliciting protective immune responses to pathogens previously impervious to vaccine strategies.

References

Chikaev NA, Bykova EA, Najakshin AM, Mechetina LV, Volkova OY, Peklo MM, Shevelev AY, Vlasik TN, Roesch A, Vogt T, Taranin AV (2005) Cloning and characterization of the human FCRL2 gene. Genomics 85:264–272

Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94:1840–1847

Davis RS (2007) Fc receptor-like molecules. Annu Rev Immunol 25:525–560

Davis RS, Wang YH, Kubagawa H, Cooper MD (2001) Identification of a family of Fc receptor homologs with preferential B cell expression. Proc Natl Acad Sci USA 98:9772–9777

Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, Mncube Z, Duraiswamy J, Zhu B, Eichbaum Q, Altfeld M, Wherry EJ, Coovadia HM, Goulder PJ, Klenerman P, Ahmed R, Freeman GJ, Walker BD (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443:350–354

Ehrhardt GR, Davis RS, Hsu JT, Leu CM, Ehrhardt A, Cooper MD (2003) The inhibitory potential of Fc receptor homolog 4 on memory B cells. Proc Natl Acad Sci USA 100:13489–13494

Ehrhardt GR, Hsu JT, Gartland L, Leu CM, Zhang S, Davis RS, Cooper MD (2005) Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. J Exp Med 202:783–791

Ehrhardt GR, Hijikata A, Kitamura H, Ohara O, Wang JY, Cooper MD (2008) Discriminating gene expression profiles of memory B cell subpopulations. J Exp Med 205:1807–1817

Falini B, Tiacci E, Pucciarini A, Bigerna B, Kurth J, Hatzivassiliou G, Droetto S, Galletti BV, Gambacorta M, Orazi A, Pasqualucci L, Miller I, Kuppers R, Dalla-Favera R, Cattoretti G (2003) Expression of the IRTA1 receptor identifies intraepithelial and subepithelial marginal zone B cells of the mucosa-associated lymphoid tissue (MALT). Blood 102:3684–3692

Goodman GR, Bethel KJ, Saven A (2003) Hairy cell leukemia: an update. Curr Opin Hematol 10:258–266

Guselnikov SV, Ershova SA, Mechetina LV, Najakshin AM, Volkova OY, Alabyev BY, Taranin AV (2002) A family of highly diverse human and mouse genes structurally links leukocyte FcR, gp42 and PECAM-1. Immunogenetics 54:87–95

Haga CL, Ehrhardt GR, Boohaker RJ, Davis RS, Cooper MD (2007) Fc receptor-like 5 inhibits B cell activation via SHP-1 tyrosine phosphatase recruitment. Proc Natl Acad Sci USA 104:9770–9775

Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK (1999) Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94:1848–1854

Hatzivassiliou G, Miller I, Takizawa J, Palanisamy N, Rao PH, Iida S, Tagawa S, Taniwaki M, Russo J, Neri A, Cattoretti G, Clynes R, Mendelsohn C, Chaganti RS, Dalla-Favera R (2001) IRTA1 and IRTA2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy. Immunity 14:277–289

Hu X, Chang M, Saiki RK, Cargill MA, Begovich AB, Ardlie KG, Criswell LA, Seldin MF, Amos CI, Gregersen PK, Kastner DL, Remmers EF (2006) The functional -169T–>C single-nucleotide polymorphism in FCRL3 is not associated with rheumatoid arthritis in white North Americans. Arthritis Rheum 54:1022–1025

Huttmann A, Klein-Hitpass L, Thomale J, Deenen R, Carpinteiro A, Nuckel H, Ebeling P, Fuhrer A, Edelmann J, Sellmann L, Duhrsen U, Durig J (2006) Gene expression signatures separate B-cell chronic lymphocytic leukaemia prognostic subgroups defined by ZAP-70 and CD38 expression status. Leukemia 20:1774–1782

Ikari K, Momohara S, Nakamura T, Hara M, Yamanaka H, Tomatsu T, Kamatani N (2006) Supportive evidence for a genetic association of the FCRL3 promoter polymorphism with rheumatoid arthritis. Ann Rheum Dis 65:671–673

Ise T, Maeda H, Santora K, Xiang L, Kreitman RJ, Pastan I, Nagata S (2005) Immunoglobulin superfamily receptor translocation associated 2 protein on lymphoma cell lines and hairy cell leukemia cells detected by novel monoclonal antibodies. Clin Cancer Res 11:87–96

Ise T, Nagata S, Kreitman RJ, Wilson WH, Wayne AS, Stetler-Stevenson M, Bishop MR, Scheinberg DA, Rassenti L, Kipps TJ, Kyle RA, Jelinek DF, Pastan I (2007) Elevation of soluble CD307 (IRTA2/FcRH5) protein in the blood and expression on malignant cells of patients with multiple myeloma, chronic lymphocytic leukemia, and mantle cell lymphoma. Leukemia 21:169–174

Johrens K, Shimizu Y, Anagnostopoulos I, Schiffmann S, Tiacci E, Falini B, Stein H (2005) T-bet-positive and IRTA1-positive monocytoid B cells differ from marginal zone B cells and epithelial-associated B cells in their antigen profile and topographical distribution. Haematologica 90:1070–1077

Kochi Y, Yamada R, Suzuki A, Harley JB, Shirasawa S, Sawada T, Bae SC, Tokuhiro S, Chang X, Sekine A, Takahashi A, Tsunoda T, Ohnishi Y, Kaufman KM, Kang CP, Kang C, Otsubo S, Yumura W, Mimori A, Koike T, Nakamura Y, Sasazuki T, Yamamoto K (2005) A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat Genet 37:478–485

Kochi Y, Myouzen K, Yamada R, Suzuki A, Kurosaki T, Nakamura Y, Yamamoto K (2009) FCRL3, an autoimmune susceptibility gene, has inhibitory potential on B-cell receptor-mediated signaling. J Immunol 183:5502–5510

Lazzi S, Bellan C, Tiacci E, Palummo N, Vatti R, Oggioni M, Amato T, Schuerfeld K, Tonini T, Tosi P, Falini B, Leoncini L (2006) IRTA1+ monocytoid B cells in reactive lymphadenitis show a unique topographic distribution and immunophenotype and a peculiar usage and mutational pattern of IgVH genes. J Pathol 209:56–66

Lee YH, Woo JH, Choi SJ, Ji JD, Song GG (2010) Fc receptor-like 3–169 C/T polymorphism and RA susceptibility: a meta-analysis. Rheumatol Int 30:947–953

Leu CM, Davis RS, Gartland LA, Fine WD, Cooper MD (2005) FcRH1: an activation coreceptor on human B cells. Blood 105:1121–1126

Li FJ, Ding S, Pan J, Shakhmatov MA, Kashentseva E, Wu J, Li Y, Soong SJ, Chiorazzi N, Davis RS (2008) FCRL2 expression predicts IGHV mutation status and clinical progression in chronic lymphocytic leukemia. Blood 112:179–187

Maltais LJ, Lovering RC, Taranin AV, Colonna M, Ravetch JV, Dalla-Favera R, Burrows PD, Cooper MD, Davis RS (2006) New nomenclature for Fc receptor-like molecules. Nat Immunol 7:431–432

Mechetina LV, Najakshin AM, Volkova OY, Guselnikov SV, Faizulin RZ, Alabyev BY, Chikaev NA, Vinogradova MS, Taranin AV (2002) FCRL, a novel member of the leukocyte Fc receptor family possesses unique structural features. Eur J Immunol 32:87–96

Mestas J, Hughes CC (2004) Of mice and not men: differences between mouse and human immunology. J Immunol 172:2731–2738

Miller I, Hatzivassiliou G, Cattoretti G, Mendelsohn C, Dalla-Favera R (2002) IRTAs: a new family of immunoglobulinlike receptors differentially expressed in B cells. Blood 99:2662–2669

Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, Roby G, Kottilil S, Arthos J, Proschan MA, Chun TW, Fauci AS (2008) Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med 205:1797–1805

Muta T, Kurosaki T, Misulovin Z, Sanchez M, Nussenzweig MC, Ravetch JV (1994) A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. Nature 368:70–73

Nagata S, Ise T, Pastan I (2009) Fc receptor-like 3 protein expressed on IL-2 nonresponsive subset of human regulatory T cells. J Immunol 182:7518–7526

Nakayama Y, Weissman SM, Bothwell AL (2001) BXMAS1 identifies a cluster of homologous genes differentially expressed in B cells. Biochem Biophys Res Commun 285:830–837

Nuckel H, Collins CH, Frey UH, Sellmann L, Durig J, Siffert W, Duhrsen U (2009) FCRL2 mRNA expression is inversely associated with clinical progression in chronic lymphocytic leukemia. Eur J Haematol 83:541–549

Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T (2001) PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci USA 98:13866–13871

Polson AG, Zheng B, Elkins K, Chang W, Du C, Dowd P, Yen L, Tan C, Hongo JA, Koeppen H, Ebens A (2006) Expression pattern of the human FcRH/IRTA receptors in normal tissue and in B-chronic lymphocytic leukemia. Int Immunol 18:1363–1373

Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G, van der Burg M, van Dongen JJ, Wiech E, Visentini M, Quinti I, Prasse A, Voelxen N, Salzer U, Goldacker S, Fisch P, Eibel H, Schwarz K, Peter HH, Warnatz K (2009) Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. Proc Natl Acad Sci USA 106:13451–13456

Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. Science 290:84–89

Reth M (1992) Antigen receptors on B lymphocytes. Annu Rev Immunol 10:97–121

Sanchez E, Callejas JL, Sabio JM, de Haro M, Camps M, de Ramon E, Garcia-Hernandez FJ, Koeleman B, Martin J, Gonzalez-Escribano MF (2006) Polymorphisms of the FCRL3 gene in a Spanish population of systemic lupus erythematosus patients. Rheumatology (Oxford) 45:1044–1046

Schreeder DM, Pan J, Li FJ, Vivier E, Davis RS (2008) FCRL6 distinguishes mature cytotoxic lymphocytes and is upregulated in patients with B-cell chronic lymphocytic leukemia. Eur J Immunol 38:3159–3166

Shlapatska LM, Mikhalap SV, Berdova AG, Zelensky OM, Yun TJ, Nichols KE, Clark EA, Sidorenko SP (2001) CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. J Immunol 166:5480–5487

Simmonds MJ, Heward JM, Carr-Smith J, Foxall H, Franklyn JA, Gough SC (2006) Contribution of single nucleotide polymorphisms within FCRL3 and MAP3K7IP2 to the pathogenesis of Graves' disease. J Clin Endocrinol Metab 91:1056–1061

Swainson LA, Mold JE, Bajpai UD, McCune JM (2010) Expression of the autoimmune susceptibility gene FcRL3 on human regulatory T cells is associated with dysfunction and high levels of programmed cell death-1. J Immunol 184:3639–3647

Takata Y, Inoue H, Sato A, Tsugawa K, Miyatake K, Hamada D, Shinomiya F, Nakano S, Yasui N, Tanahashi T, Itakura M (2008) Replication of reported genetic associations of PADI4, FCRL3, SLC22A4 and RUNX1 genes with rheumatoid arthritis: results of an independent Japanese population and evidence from meta-analysis of East Asian studies. J Hum Genet 53:163–173

Vely F, Vivier E (1997) Conservation of structural features reveals the existence of a large family of inhibitory cell surface receptors and noninhibitory/activatory counterparts. J Immunol 159:2075–2077

Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B, Kayentao K, Ongoiba A, Doumbo OK, Pierce SK (2009) Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. J Immunol 183:2176–2182

Wilson TJ, Presti RM, Tassi I, Overton ET, Cella M, Colonna M (2007) FcRL6, a new ITIM-bearing receptor on cytolytic cells, is broadly expressed by lymphocytes following HIV-1 infection. Blood 109:3786–3793

Xu MJ, Zhao R, Zhao ZJ (2001) Molecular cloning and characterization of SPAP1, an inhibitory receptor. Biochem Biophys Res Commun 280:768–775

FcγRs in Health and Disease

Falk Nimmerjahn and Jeffrey V. Ravetch

Contents

Abstract Genetic defects affecting the humoral immune response and especially the production of antibodies of the immunoglobulin G (IgG) isotype result in a heightened susceptibility to infections. Studies over the last years have demonstrated the crucial role of Fc-receptors for IgG (FcγRs) widely expressed on innate immune effector cells in mediating the protective function of IgG. During the last years, additional ligands interacting with FcγRs as well as additional receptors binding to IgG glycosylation variants have been identified. In this review, we discuss how the interaction of these different ligands with classical and novel Fcγ-receptors influences the immune response and which strategies microorganisms have developed to prevent them.

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1 Introduction

Apart from physical barriers, cells of the innate immune system, including mast cells, neutrophils, monocytes, and macrophages represent one of the first lines of defense against pathogenic microorganisms. Armed with a multitude of receptors, such as the Toll-like receptor family, recognizing danger and pathogen associated molecular patterns (DAMPs and PAMPs), they are able to recognize microorganisms directly and thereby prevent an overwhelming infection (Iwasaki and Medzhitov 2010). In the absence of adaptive immunity, however, many infections cannot be cleared, emphasizing the importance of the interplay between the two arms of the immune system. Antibodies, the hallmark of the adaptive immune response, are essential in providing protection against recurrent infections (Ballow 2002). While low affinity antibodies of the IgM isotype are characteristic for the early immune response, antibodies of the IgG subclass dominate the later response and are typically of higher affinity and exquisite specificity for the respective target antigen. Genetic defects affecting the production of high affinity IgG antibodies often result in a heightened susceptibility to microbial infections emphasizing the protective role of this isotype (Ballow 2002). Studies performed in mouse model systems over the last decade have led to the conclusion that the underlying mechanism of this protective activity of IgG, which consists of four different subclasses in mice (IgG1, IgG2a/c, IgG2b, IgG3) and humans (IgG1–IgG4), is the recruitment and activation of innate immune effector cells via receptors specific for the IgG Fc (fragment crystallizable) domain (Fcγ-receptors, FcγR) (Hogarth 2002; Nimmerjahn and Ravetch 2008b; Takai 2002). Depending on the effector cell, crosslinking of these receptors results in cell degranulation, release of different cytokines/chemokines, phagocytosis, or antibody dependent cellular cytotoxicity (ADCC). Thus, Fc-receptors provide the link between the high specificity of the adaptive immune system and the powerful effector functions of cells of the innate immune system and target these proinflammatory activities to sites of infection or healthy tissues during autoimmune disease (Hogarth 2002; Nimmerjahn and Ravetch 2008b; Takai 2002). In contrast to the early IgM dominated immune response, which largely depends on the activation of the classical complement pathway, marking microorganisms for phagocytosis via complement receptors or initiation of bacterial lysis through the generation of lytic membrane attack complexes, activation of the complement pathway is not essential for IgG mediated effector functions, although it can enhance the activity of certain IgG subclasses (Azeredo da Silveira et al. 2002; Carroll 2004; Clynes and Ravetch 1995; Nimmerjahn and Ravetch 2005; Ravetch and Clynes 1998; Sylvestre et al. 1996; Uchida et al. 2004). While this proinflammatory activity of IgG is desirable in the case of an infection, it leads to severe tissue and organ damage if directed toward healthy tissues during autoimmune disease (Hogarth 2002; Nimmerjahn and Ravetch 2008b; Takai 2002). Several factors, including the individual IgG subclass and the antibody glycosylation pattern, can influence the quality and strength of this interaction (Nimmerjahn and Ravetch 2006). Besides IgG, other molecules such as members of the evolutionary conserved pentraxin family can bind to FcγRs

(Marnell et al. 2005; Woof and Burton 2004). More recently, it was shown that certain antibody glycosylation variants can bind to other cell surface receptors belonging to the family of C-type lectins unrelated to the family of classical FcγRs (Anthony et al. 2008a, b; Kaneko et al. 2006b; Nimmerjahn and Ravetch 2008a). This review summarizes our current understanding of how the interaction of these different ligands with cellular FcγRs is regulated and how this might help develop novel therapeutic avenues for the optimization of antimicrobial and the amelioration of autoreactive antibody responses.

2 The Family of Canonical Fcγ-Receptors

FcγRs are a conserved family of glycoproteins that belong to the IgG superfamily and are comprised of a ligand binding a-subunit consisting of two or three C2-type extracellular domains (Fig. 1). In mice, monkeys, humans and other mammalian species the family of FcγRs is comprised of several activating (FcγRIA, IIA, IIC, IIIA in humans; FcγRI, III, IV in mice) and one inhibitory (FcγRIIB) member (Hogarth 2002; Nimmerjahn and Ravetch 2008b; Takai 2002; Willcocks et al. 2009). Whereas the inhibitory FcγR has an immunoreceptor tyrosine based inhibitory motif (ITIM) in its cytosolic domain, the majority of activating FcγRs have to

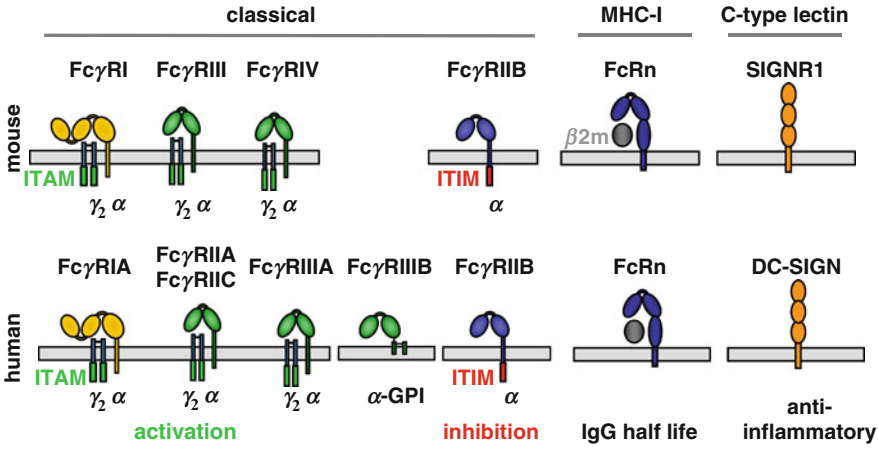

Fig. 1 The extended family of mouse and human Fcγ-receptors. In mice and humans the family of classical FcγRs consists of several activating and one inhibitory member. In addition, humans have a GPI-linked FcγR (FcγRIIIB) exclusively expressed on neutrophils. The neonatal FcRn is responsible for IgG half life in mice and humans and belongs to the family of major histocompatibility class I (MHCI) molecules. More recently, mouse SIGNR1 and its human orthologue DC-SIGN joined the family of IgG binding proteins with a selective specificity for IgG glycoforms rich in terminal sialic acid residues (see text for further details)

associate with signaling adaptor proteins containing immunoreceptor tyrosine based activation motifs (ITAM) such as the FcR common γ-chain (γ-chain). In humans and monkeys, there are some exceptions to this rule as FcγRIIA and FcγRIIC have an ITAM in their cytosolic domain and do not require signaling adaptor molecules for cell surface expression and functionality (Fig. 1) (Willcocks et al. 2009). Besides these differences in signal transduction, the other distinguishing feature among the family members is the affinity and specificity for the different IgG subclasses. In analogy to the high affinity Fc-receptor for IgE (FcεR), there is one high affinity receptor for IgG (FcγRI or CD64), which has nanomolar affinity for select IgG subclasses (IgG2a/c in mice; IgG1/3 in humans) and is saturated in the presence of serum IgG on cells such as monocytes in the blood. All the other receptors have a low to medium affinity in the micromolar range and can only bind to IgG if present in the form of immune complexes (ICs) (Nimmerjahn and Ravetch 2008b). Structural analysis of human IgG together with FcγRIIIA showed that IgG–FcgR binding occurs in a one to one ratio and that the sugar side chains of IgG and FcγRs are critically involved in this binding (Ferrara et al. 2006; Mimura et al. 2001; Radaev et al. 2001; Sondermann et al. 2000). FcγRs are expressed on the majority of innate immune effector cells such as basophils, eosinophils, mast cells, monocytes, macrophages, NK cells and neutrophils. Among these cell lineages, monocytes and macrophages express the broadest repertoire of activating FcγRs (I, III, and IV), whereas neutrophils express FcγRIII and IV, and NK cells selectively express FcγRIII. In addition, dendritic cells (DCs) essential for the initiation of adaptive immune responses, and B cells do express FcγRs. Early studies have also identified FcγR expression on some T cell populations although the role of these receptors for T cell activity or development is unclear and requires further analysis (Anderson and Grey 1974; Leclerc et al. 1977; Stout and Herzenberg 1975). A hallmark of this receptor family is the coexpression of activating and inhibitory receptors on the majority of innate immune effector cells and DCs. Thus, the simultaneous triggering of activating and inhibitory signaling pathways sets a threshold for cell activation and regulates cellular effector functions. Indeed, deletion of the inhibitory FcγRIIB resulted in heightened IgG dependent proinflammatory reactions, DC maturation and antigen presentation *in vivo* (Boruchov et al. 2005; Dhodapkar et al. 2005; Kalergis and Ravetch 2002; Nimmerjahn and Ravetch 2008b; Takai et al. 1996). An exception to this rule is NK cells which solely express FcγRIIIA (CD16) in humans and FcγRIII in mice. Recently, a small subpopulation of NK cells has been shown to express FcγRIIB, although the functional role of this NK cell subset remains to be established (Dutertre et al. 2008). B cells do not express activating FcγRs, but express the inhibitory Fc-receptor, which regulates positive signals initiated via the B cell receptor. The importance of FcγRIIB on B cells was demonstrated by the development of autoantibodies and a severe autoimmune disease similar to human systemic lupus erythematosus (SLE) in mice deficient for this receptor (Baerenwaldt and Nimmerjahn 2008; Bolland and Ravetch 1999, 2000; Daeron and Lesourne 2006; Ravetch and Lanier 2000; Takai 2002; Takai et al. 1996).

Based on genomic localization and sequence similarity in the extracellular portion, orthologous receptors can be identified between mice and humans (Hirano et al. 2007; Nimmerjahn and Ravetch 2006). Thus, the high affinity receptors FcγRIA/FcγRI and the low affinity receptors FcγRIIB cluster in the same group (Fig. 1). Similarly, human FcγRIIIA/mouse FcγRIV and human FcγRIIA/mouse FcγRIII share a high level of sequence homology in their extracellular domains (Nimmerjahn and Ravetch 2006). Despite this similarity caution should be taken in a one to one transfer of data obtained in mouse models to the human system as the ligands (the IgG subclasses) and the cellular expression pattern of some of the receptors differ between mice and humans. Thus, human FcγRI binds two IgG subclasses (IgG1and IgG3) with high affinity whereas mouse FcγRI only binds IgG2a/c with nanomolar affinity. Similarly, mouse FcγRIV (not expressed on NK cells) has the capacity to recognize IgE whereas human FcγRIIIA (expressed on NK cells) does not (Hirano et al. 2007; Mancardi et al. 2008). Moreover, humans express several allelic variants of the low affinity receptors FcγRIIA and FcγRIIIA which greatly differ in their affinity for the different IgG subclasses (Bruhns et al. 2009; Ravetch and Nimmerjahn 2008). Thus, human IgG2 binds only to an FcγRIIA variant carrying a histidine at position 131 (FcγRIIA-131H) whereas the allele carrying an arginine allele (FcγRIIA-131R) has a very low affinity for this IgG subclass. Similarly, FcγRIIIA with a valine residue at position 158 (FcγRIIIA-158V) has a much higher affinity for IgG1 and IgG3 than its allelic counterpart with a phenylalanine residue (FcγRIIIA-158F) at this position. Studies with human patient cohorts suggest that these differences in affinity impact the outcome of therapeutic success with antitumor antibodies, for example (Cartron et al. 2002; Weng et al. 2004; Weng and Levy 2003). To mimic these allelic differences in the future, novel mouse models carrying these allelic variants of the human FcγRs will be essential. Nonetheless, classical mouse models have been and will continue to be detrimental for deciphering the role of the family of FcγRs for antibody activity in infection and autoimmunity which will be discussed in the following paragraphs.

3 Regulation of IgG Activity *In Vivo*

Given the crucial role of FcγRs for IgG mediated effector functions, several studies have addressed the issue about which activating FcγRs were involved in mediating the activity of the individual IgG subclasses. *In vitro* studies indicated that IgG1 can only bind to FcγRIII, IgG2a/c can bind to all activating FcγRs and IgG2b binds to FcγRIII and FcγRIV. IgG3, in contrast, did not bind with significant affinity to any of the known FcγRs in C57BL/6 mice, although binding to an FcγRI allele in NOD mice was described (Gavin et al. 1998). Consistent with these in vitro data, IgG1 activity was abrogated in mouse strains deficient in FcγRIII (Hazenbos et al. 1996; Meyer et al. 1998; Nimmerjahn and Ravetch 2005, 2006). In contrast, the situation for IgG2a/c and IgG2b is more complicated: in some model systems the activity of

these subclasses was abrogated in FcγRIII knockout animals whereas in others it was not. In models of IgG2b dependent platelet depletion, B cell depletion, and nephrotoxic nephritis, for example, blockade of FcγRIV function prevented platelet and B cell depletion and kidney inflammation (Hamaguchi et al. 2006; Kaneko et al. 2006a; Nimmerjahn et al. 2005; Nimmerjahn and Ravetch 2005). In the case of IgG2b dependent autoimmune hemolytic anemia (AIHA), acute glomerular inflammation and IC induced lung inflammation, both FcγRIII and FcγRIV were essential for the activity of this IgG subclass (Baudino et al. 2008; Giorgini et al. 2008; Syed et al. 2009). For IgG2a/c a similar picture emerges as for IgG2b with additional contributions of the high affinity Fc-receptor depending on the system model used (Baudino et al. 2008; Bevaart et al. 2006; Ioan-Facsinay et al. 2002; Otten et al. 2008). Together with the aforementioned correlation of allelic variants of FcγRIIA and FcγRIIIA with the activity of therapeutic antibodies in human cancer patients this indicates that the low affinity FcγRs are critical for IgG dependent effector functions in mice and humans.

While these studies confirmed the crucial role of FcγRs for IgG activity they did not explain another observation that was made in several *in vivo* model systems of autoimmunity and infection, suggesting that the different IgG subclasses have a different activity *in vivo*. Using IgG subclass switch variants of platelet or red blood cell (RBC) specific antibodies it was demonstrated that IgG2a/c and IgG2b were superior to the other IgG subclasses in mediating phagocytosis of opsonized platelets or RBCs compared to IgG1 and IgG3, respectively (Fossati-Jimack et al. 2000; Nimmerjahn et al. 2005; Nimmerjahn and Ravetch 2005). Similarly, IgG2a/c mediated depletion of B cells, syngeneic melanoma cells, and T cell lymphomas was far more efficient compared to antibodies with an IgG1 Fc-fragment, for example, consistent with earlier results obtained in vitro (Kaminski et al. 1986; Kipps et al. 1985; Lambert et al. 2004; Nimmerjahn and Ravetch 2005; Uchida et al. 2004). Further confirming this observation glomerular inflammation induced by IgG subclass switch variants was most severe for the IgG2a/c subclass followed by IgG2b and a much weaker activity of IgG1 (Giorgini et al. 2008). Similarly, antimicrobial antibodies of the IgG2a/c subclass yielded enhanced protection from the development of poliomyelitis following infection with lactate dehydrogenase elevating virus or enhanced phagocytosis of opsonized *Cryptococcus neoformans* (Markine-Goriaynoff and Coutelier 2002; Schlageter and Kozel 1990). A possible explanation for this hierarchy of activities was afforded by the affinities of the different IgG subclasses toward their triggering activating and inhibitory FcγR pairs. Thus, IgG1 has a higher affinity for the inhibitory FcγRIIB than toward the activating FcγRIII, resulting in a high threshold for activation. In contrast, IgG2a/c and IgG2b have a much higher affinity for the activating FcγRIV than for the inhibitory FcγR, thus being less influenced by cotriggering. Consistent with this in vitro data, deletion of the inhibitory FcγRIIB most strongly enhances the activity of IgG1 in models of platelet depletion and tumor cell destruction (Nimmerjahn and Ravetch 2005). Of note, this affinity based model of IgG subclass activity can be influenced by several factors, including the varying FcγR repertoire of individual innate immune effector cells and DCs and cytokines that can alter the ratio of

activating to inhibitory FcγR expression. Thus, TH1-type cytokines, LPS, and the anaphylatoxin C5a have been shown to increase activating FcγR expression (Nimmerjahn and Ravetch 2008b; Schmidt and Gessner 2005). In contrast, TH2-signature cytokines such as IL4 had the opposite effect, with the exception of B cells where IL4 was suggested to downmodulate expression of FcγRIIB (Nimmerjahn and Ravetch 2006). Apart from the expression level of the individual FcγRs, recent studies have highlighted the role of the sugar side-chain of IgG which is attached to the asparagine 297 (N297) residue in the IgG heavy chain in modulating the affinity to activating FcγRs which will be discussed in the next chapter.

4 Influence of Antibody Glycosylation and Novel Fcγ-Receptors

All immunoglobulin isotypes are glycoproteins with a varying amount of sugar side chains attached to the protein backbone. In contrast to IgM, IgA, and IgE which contain multiple exposed sugar side chains, the IgG associated sugar domain is constrained by the groove formed by the two individual IgG heavy chains (Nimmerjahn and Ravetch 2008b). Deletion of this sugar domain results in an altered conformation and severely impaired binding to cellular FcγRs and diminished activation of the complement pathway (Shields et al. 2001). Compared to the homogeneous composition of the sugar domain of the other Ig isotypes the IgG associated sugar moiety is heterogeneous. Thus, in a healthy individual more than 30 different IgG glycosylation variants can be detected in the serum of mice and humans (Arnold et al. 2007). This heterogeneity stems from variable additions of terminal and branching sugar residues such as sialic acid, galactose, *N*-acetylglucosamine and fucose. Interestingly, this glycosylation pattern of serum IgG changes during active autoimmune disease in mice and humans where glycoforms rich in terminal sialic acid and galactose residues were diminished (Bond et al. 1990; Kaneko et al. 2006b; Mizuochi et al. 1990; Nimmerjahn and Ravetch 2008a). A similar change in serum IgG glycosylation pattern was observed with older age, whereas during pregnancy IgG glycoforms rich in terminal sialic acid and galactose residues were increased and correlated with remission of disease (Arnold et al. 2007; Rook et al. 1991; van de Geijn et al. 2009). Although the function of this altered glycosylation pattern is not fully understood, recent evidence points toward an important role of differential IgG glycosylation in modulation of IgG activity (Jefferis 2009). The absence of the branching fucose residue, for example, results in a selective increase of affinity of IgG subclasses to human FcγRIIIA and its mouse orthologue FcγRIV (Nimmerjahn and Ravetch 2005; Shields et al. 2002; Shinkawa et al. 2003). This increased affinity results in enhanced *in vivo* activity as demonstrated by the ability to prevent tumor growth in various model systems (Nimmerjahn and Ravetch 2007a). In contrast, the presence of high levels of terminal sialic acid residues diminished the affinity of human and mouse IgG for the family of classical FcγRs, consistent with a lower proinflammatory activity (Anthony et al. 2008a; Kaneko et al. 2006b; Scallon et al. 2007). Importantly,

however, sialic acid rich IgG glycovariants not only display reduced proinflammatory activity, as a result of the diminished binding to classical FcγRs, but they actually gained an active anti-inflammatory activity of IgG, which was able to suppress the proinflammatory activity of other autoantibodies (Anthony et al. 2008a, b; Kaneko et al. 2006b; Nimmerjahn and Ravetch 2007b). This finding might explain the long known anti-inflammatory activity of infusion of high doses of pooled serum IgG from several thousands of donors (IVIg therapy), which has been in use for nearly three decades as an efficient symptomatic treatment of different human autoimmune diseases, including thrombocytopenia, rheumatoid arthritis and chronic inflammatory demyelinating polyneuropathy (Nimmerjahn and Ravetch 2008a). A recent study identified SIGNR-1 (specific ICAM3 grabbing nonintegrin related 1) and its human orthologue DC-SIGN (dendritic cell specific ICAM3 grabbing nonintegrin) as cellular receptors that can specifically bind to IgG glycovariants containing high levels of terminal sialic acid residues. Consistent with this in vitro binding, IVIg lost its anti-inflammatory activity in SIGNR-1 knockout mice in a model of rheumatoid arthritis (Anthony et al. 2008b). During the steady state SIGNR-1 expression is most dominant on splenic marginal zone macrophages characterized by expression of another cell surface receptor called MARCO (macrophage receptor with collagenous structure) (Fig. 2). Consistent with an important function of this SIGNR-1 positive splenic macrophage population, splenectomized mice, mice with a disturbed splenic structure or osteopetrotic op/op mice lacking this macrophage subpopulation were no longer protected by IVIg (Anthony et al. 2008b; Bruhns et al. 2003). Thus, in analogy to the family of Toll-like receptors which can recognize microbial as well as self ligands, these studies highlight the dual function of receptors such as SIGNR1 and DC-SIGN in recognition of microbial and self ligands. Further studies showed that IVIg infusion changes the threshold for innate immune effector cell activation through ICs through an upregulation of the inhibitory FcγRIIB and a downregulation of activating FcγRs in different mouse model systems and in patients with chronic inflammatory demyelinating polyneuropathy (Fig. 2) (Bruhns et al. 2003; Kaneko et al. 2006a, b; Samuelsson et al. 2001; Tackenberg et al. 2009). It is tempting to speculate that pathogens, such as HIV and *Mycobacterium tuberculosis*, which bind to DC-SIGN use this anti-inflammatory pathway to escape an initial immune response. Besides the important function of sialic acid residues, it has been proposed that the additional lack of galactose residues, which exposes the mannose rich core sugar structure, would result in the capacity to activate the lectin pathway of complement activation via mannan binding lectin (MBL) and thereby enhance the proinflammatory activity of this so called IgG-G0 glycovariant (lacking terminal sialic acid and galactose residues) (Malhotra et al. 1995). However, recent *in vivo* studies using MBL1/2 knockout animals could not confirm these previous in vitro observations and rather suggest that IgG glycoforms with or without galactose are still fully dependent on cellular FcγRs and do not gain proinflammatory activity through the MBL pathway of complement activation (Nimmerjahn et al. 2007).

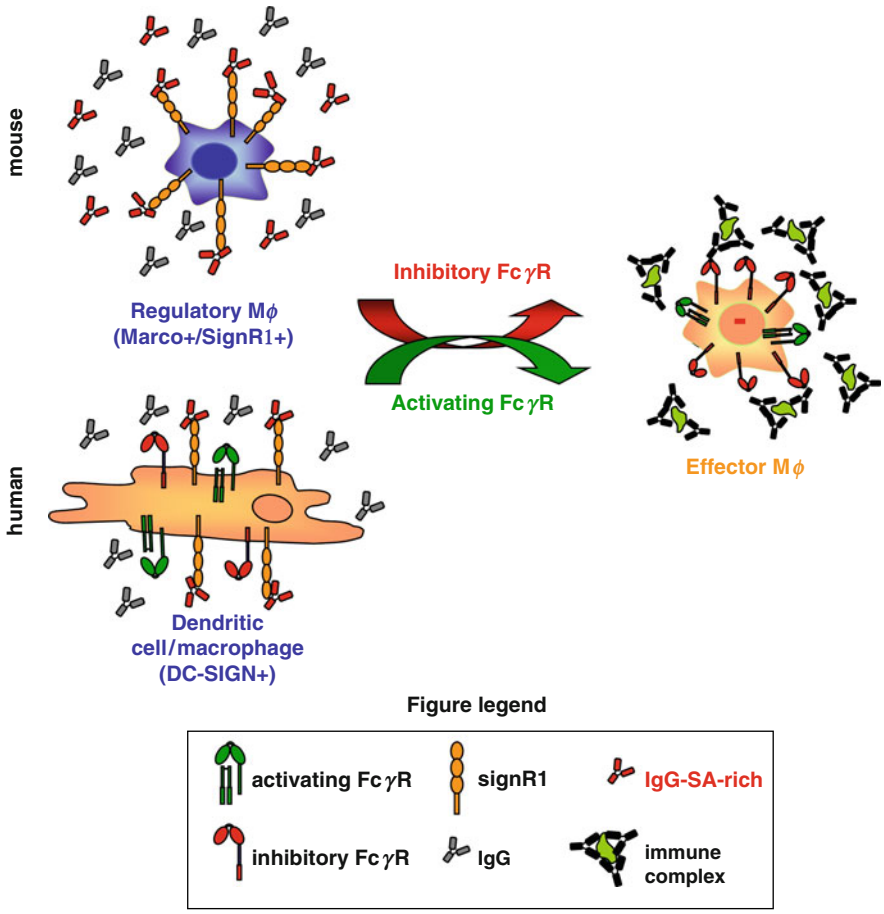

Fig. 2 Model for the anti-inflammatory activity of IgG. Immunoglobulin G glycovariants rich in terminal sialic acid residues (SA-rich IgG) loose affinity for classical FcγRs but gain the capacity to bind to C-type lectin receptors such as mouse SIGNR1 and human DC-SIGN. Binding of SA-rich IgG to splenic resident MARCO positive macrophages heightens the threshold for innate immune effector cell activation by upregulation of inhibitory FcγRIIB expression and lowering expression of activating FcγRs. In humans, DC-SIGN positive dendritic cells or macrophages might be involved in the IgG dependent anti-inflammatory pathway

5 Role of FcγRs for Infection

A role for IgG antibodies in the prevention of infection is demonstrated by the heightened susceptibility of patients with hypogammaglobulinemia or hyper-IgM syndrome to recurrent infections with encapsulated bacteria, viruses and with some protozoan infections (Ballow 2002; Wood 2009). Major efforts have been undertaken to identify broadly neutralizing antibodies capable of protecting the host from

infection with a wide range of microorganisms (especially highly pathogenic viruses such as influenza and HIV) from the same or different species (Karlsson Hedestam et al. 2008; Walker and Burton 2008). One general issue with respect to many human pathogens is that they do not infect mice. Therefore, one has to rely on in vitro model systems and readouts to evaluate the capacity of pathogen specific antibodies to prevent infection of host cells. Such an approach may be severely biased by the choice of target cells used in such an assay. For example, HIV infects both T cells and myeloid cells, of which only the latter cell lineage expresses FcγRs. In screening for neutralizing antibodies by using T cells as a readout one selectively screens for antibodies preventing the interaction of viral ligands with cellular receptors such as CD4 expressed on T cells, which would be solely dependent on the specificity (the F(ab)2 fragment) of the antibody. By doing so one might miss a potential role for the antibody Fc-fragment and FcγRs in antibody mediated protection from a productive infection or viral replication (Fig. 3). Along these lines, it was recently demonstrated that different cell lines used for in vitro neutralization assays with antibodies specific for the anthrax toxin express different

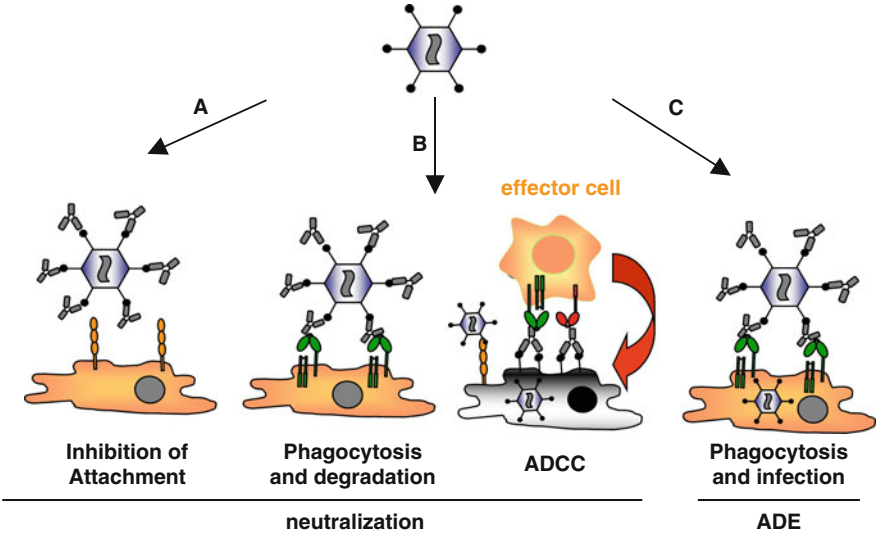

Fig. 3 Pathways of IgG dependent pathogen neutralization or enhancement of infection. Pathogen specific IgG can either block infection (neutralization) of host cells (A, B) or lead to antibody dependent enhancement (ADE) of pathogen infectivity (C). (A) Binding of IgG to the surface of bacterial or viral receptors essential for the attachment to host cells blocks pathogen entry and prevents an infection. This mechanism of neutralization is solely dependent on the specificity of the antibody and independent of the Fc-fragment. (B) Opsonized pathogens or pathogen specific IgG binding to infected cells results in pathogen clearance via phagocytosis and destruction of the microorganism in acidic endosomal/lysosomal vesicles or to killing of infected host cells by effector cells via antibody dependent cell-mediated cytotoxicity (ADCC). (C) For some opsonized microorganisms FcγR dependent uptake results in enhanced infection of target cells instead of degradation in lysosomal vesicles (see text for further details)

levels of FcγRs and that toxin neutralization was more efficient in the cell line expressing higher levels of FcγRs (Verma et al. 2009). Moreover, blocking FcγRs impaired IgG dependent anthrax toxin neutralization in this in vitro assay. Thus, in vitro neutralization assays should screen for both, FcγR dependent and independent mechanisms of neutralization to ensure the highest level of predictability for *in vivo* functionality. The importance of ADCC or other FcγR dependent pathways for inhibition of viral replication and spread (also referred to as antibody dependent cell-mediated viral inhibition; ADCVI) *in vivo* have only recently become appreciated. Thus, γ-chain deficient mice lacking the activity of all activating FcγRs were more susceptible to influenza virus infection due to reduced phagocytosis and ADCC dependent killing of infected cells (Fig. 3) (Huber et al. 2001). Along the same lines, the capacity of immune serum to trigger ADCC in vitro has been associated with protection from HIV infection in some (but not all) studies and nonneutralizing antibodies (as defined by classical in vitro assays) were able to inhibit HIV replication in human macrophages in vitro (Florese et al. 2006; Gomez-Roman et al. 2005; Holl et al. 2006a). More recently it was demonstrated that the protective activity of the broadly neutralizing HIV specific antibody b12 directed against the CD4 binding site of gp120 mediates its activity at least in part via engaging activating FcγRs but not the complement pathway *in vivo* (Hessell et al. 2007). Several studies have addressed the role of individual activating FcγRs for this antiviral activity and have suggested that especially FcγRIA and FcγRIIA were involved in conferring protection (David et al. 2006; Forthal and Moog 2009; Holl et al. 2004, 2006b; Perez-Bercoff et al. 2003). Apart from a simple ADCC and phagocytosis followed by destruction of antibody bound virus in endosomal/lysosomal vessicles, other mechanisms such as FcγR dependent induction of transcription factors are discussed to be involved in inhibition of virus infection and replication (Bergamaschi et al. 2009). Although more studies are necessary to elucidate the molecular pathways involved in FcγR dependent protection from virus infection, the available data suggest a critical involvement of IgG-Fc dependent pathways in parallel to the IgG-F(ab)2 dependent interference with virus binding to cellular receptors (Fig. 3).

Consistent with the results obtained for HIV and influenza, antibody dependent phagocytosis of *Bordetella pertussis* was enhanced via FcγRs but not via complement receptor 3 (CR3) in mouse models *in vivo* and with human effector cells in vitro (Hellwig et al. 2001; Rodriguez et al. 2001). With respect to parasitic infections, mice deficient in activating FcγRs were less efficient in clearing microfilariae of *Brugia malayi* and showed a decreased antibody mediated phagocytosis of *Plasmodium bergei* and enhanced susceptibility to infection in this murine malaria model (Gray and Lawrence 2002; Yoneto et al. 2001). Confirming the role of the inhibitory FcγRIIB in setting a threshold for cell activation, mice deficient in the inhibitory FcγR were more efficient in the phagocytosis of bacteria and showed enhanced resistance to infection with *Plasmodium chabaudi chabaudi*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Clatworthy and Smith 2004; Clatworthy et al. 2007; Gjertsson et al. 2002). Another line of evidence for an involvement of FcγRs in antibacterial responses in humans comes from IgG

subclass deficiencies. An IgG2 deficiency, for example, predisposes to infections with microorganisms such as *Haemophilus influenzae* (causing upper respiratory tract infections and pneumonia), *S. pneumoniae* (causing pneumonia, peritonitis and meningitis) and *Nisei meningitides* (causing meningitis and septicemia) (Ballow 2002; Pathan et al. 2003). IgG2 is a poor activator of the complement pathway and selectively binds the FcγRIIA-131H allele (Ravetch and Nimmerjahn 2008). Supporting a role for this activating FcγR in phagocytosis of opsonized bacteria of these species, it was demonstrated that humans with the low affinity FcγRIIA-131R allele had a higher susceptibility to invasive pneumococcal disease and higher risk for fulminant meningococcal septic shock (Bredius et al. 1994; Fijen et al. 2000; Rodriguez et al. 1999; Willcocks et al. 2009; Yee et al. 2000; Yuan et al. 2003). Moreover, neutrophils of FcγRIIA-131H donors were more efficient in phagocytosing opsonized bacteria in vitro (Fijen et al. 2000; Pathan et al. 2003; Rodriguez et al. 1999). Consistent with this finding murine IgG1 antibodies against cell wall components of *S. pneumoniae*, which cannot activate the complement pathway, could protect mice from lethal infection (Briles et al. 1984a, b). Taken together, there is evidence that FcγRs are involved in antibody dependent control at least of certain bacterial and viral infections in mice and humans. For genetic association studies in humans, larger patient cohorts will be essential to provide convincing evidence as some of the current studies show contradicting results (Smith et al. 2003). Of note, FcγRs are not only involved in protection from microbial infections but can also be responsible for promoting susceptibility of the host, a phenomenon that has been termed antibody mediated enhancement (ADE) of infection (Fig. 3). Thus, *Leishmania major* infection of susceptible mouse strains is severely impaired in γ-chain knockout animals (Kima et al. 2000; Padigel and Farrell 2005). Similar results have been obtained for many viruses including HIV and members of the dengue virus family (Brouwer et al. 2004; Littaua et al. 1990; Loke et al. 2002; Takeda et al. 1988) . Despite this FcγR dependent effect of ADE, this does not preclude the use of neutralizing antibodies to block virus infection. Thus, dengue virus specific IgG was efficient in protecting animals from infection if used in an aglycosyl form which can no longer interact with cellular FcγRs (Balsitis et al. 2010).

6 Microbial Immune Escape Mechanisms Targeting the IgG–FcγR Interaction

Further evidence that the interaction of antimicrobial antibodies with FcγRs is of importance is provided by the fact that several viruses and bacteria have developed strategies to interfere with this interaction. Prime examples are protein A (*S. aureus*) and protein G (*Streptococcus spec.*) which immobilize IgG to inactivate its effector functions (Langone 1982). Herpes simplex virus (HSV) as well as cytomegalovirus express viral FcγRs which compete with cellular FcγRs for IgG

binding and thereby prevent direct virus recognition and detection of infected cells through innate immune effector cells (Atalay et al. 2002; Dubin et al. 1991; Frank and Friedman 1989; Sprague et al. 2008). As demonstrated for gp68 of human cytomegalovirus (HCMV), viral FcγRs bind to IgG independent of the N297 attached sugar moiety, which is crucial for binding to cellular FcγRs. In contrast to classical FcγRs which bind to IgG in the CH2/hinge domain in a one to one ratio, HCMV gp68 binds IgG more closely to the CH3 domain with nanomolar affinity and in a two to one stoichiometry (Sprague et al. 2008). More recently, several secreted glycosidases and proteases derived from *Streptococcus pyogenes* with a high specificity for the IgG attached sugar moiety or the IgG Fc-fragment have been identified (Collin and Olsen 2001; Johansson et al. 2008). Thus, Endoglycosidase S (EndoS) efficiently cleaves the N297-attached sugar moiety after the first *N*-acetylglucosamine residue, which results in a decreased binding to cellular FcγRs. In a variety of models of IgG dependent autoimmune disease, injection of purified EndoS resulted in decreased autoantibody activity and reduced tissue damage, suggesting that EndoS might help *S. pyogenes* to inactivate the antimicrobial activity of IgG (Albert et al. 2008; Allhorn et al. 2008; Nandakumar et al. 2007a). Interestingly, EndoS treatment showed an IgG subclass specific activity and was not able to impair the activity of the most potent subclass IgG2a/c (Albert et al. 2008). In contrast, the protease Ide S efficiently cleaves the IgG Fc-fragment of all human IgG subclasses and of mouse IgG2a/c, resulting in inactivation of (auto) antibody activity in vitro and *in vivo* (Johansson et al. 2008; Nandakumar et al. 2007b). Taken together, pathogenic microorganisms have evolved several mechanisms to escape the potent effector pathways initiated through the IgG Fc-fragment. These studies may provide the basis to develop not only novel antimicrobial therapies, but also be helpful to limit the destructive potential of autoantibodies during autoimmune disease (Allhorn and Collin 2009; Nandakumar and Holmdahl 2008).

7 Other Ligands for Fcγ-Receptors

Apart from IgG, there is evidence that other proteins unrelated to IgG can bind to FcγRs and use the potent effector functions initiated via these receptors. The most prominent examples are members of the pentraxin superfamily which are evolutionary conserved proteins with a multimeric cyclic structure (Agrawal et al. 2009). Two members of this family, C-reactive protein (CRP) and serum amyloid P (SAP), have been shown to bind to human and mouse FcγRs (Bharadwaj et al. 1999, 2001; Lu et al. 2008; Marjon et al. 2009; Thomas-Rudolph et al. 2007). Whereas these proteins are present only in minute amounts in the serum in the steady state they become greatly upregulated during inflammatory responses such as microbial infections. Similar to antimicrobial antibodies, CRP and SAP can directly bind to a variety of pathogens including bacteria, fungi and viruses marking them for phagocytosis by neutrophils and macrophages of the host (Agrawal et al. 2009; Marnell et al. 2005). Besides the activation of the classical complement pathway

through these pentraxins a role of FcγRs in the CRP dependent phagocytosis of microorganism was suggested by in vitro studies with FcγR expressing cell lines and more recently by a cocrystal structure of SAP together with FcγRIIA, demonstrating that CRP and SAP can bind to the low affinity FcγRIIA and FcγRIIB and to the high affinity FcγRI (Bharadwaj et al. 1999, 2001; Lu et al. 2008). Although the relevance of this binding for the phagocytosis of CRP opsonised microorganisms *in vivo* remains to be established it was recently demonstrated that FcγR dependent uptake of CRP opsonised *S. pneumoniae* enhances the immune response against this microorganism (Thomas-Rudolph et al. 2007). In addition to this antimicrobial function CRP has also been shown to have an anti-inflammatory activity which seems to be dependent on cellular FcγRs. Thus, mice deficient in FcγRs are not protected from lethal LPS challenge, nephrotoxic nephritis and ITP by injection of human CRP. For the latter two autoimmune models it has been shown that FcγRI was crucial for this CRP dependent suppression of autoimmune disease although the exact mechanism of this activity requires further studies (Marjon et al. 2009; Rodriguez et al. 2007).

8 Conclusions

Work over the last years has highlighted the central importance of FcγRs in mediating the proinflammatory activity of IgG. By setting a threshold for innate immune effector cell activation activating and inhibitory FcγRs modulate the strength of the immune response and prevent an unwanted activation of the immune system which might result in destruction of healthy tissues. The identification of novel IgG glycosylation variants involved in the long known but poorly understood anti-inflammatory activity of IgG was an important step toward a more complete picture of the underlying mechanism of this activity. It is tempting to speculate that molecules such as SIGNR-1 which have the capacity to recognize pathogens directly during an infection might have another function during the steady state and help to maintain the immune system in a resting state. The immune escape mechanisms which have been developed by different microorganisms are as complex as these novel pathways responsible for IgG effector functions. It is a safe assumption that many more microbial molecules that target the IgG–FcγR interaction will be identified in future studies, which might not only be useful for fighting microbial infections but also for the development of novel strategies to interfere with the deleterious activities of IgG during autoimmune disease.

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References

Agrawal A, Singh PP, Bottazzi B, Garlanda C, Mantovani A (2009) Pattern recognition by pentraxins. Adv Exp Med Biol 653:98–116

Albert H, Collin M, Dudziak D, Ravetch JV, Nimmerjahn F (2008) *In vivo* enzymatic modulation of IgG glycosylation inhibits autoimmune disease in an IgG subclass-dependent manner. Proc Natl Acad Sci USA 105:15005–15009

Allhorn M, Collin M (2009) Sugar-free antibodies–the bacterial solution to autoimmunity? Ann NY Acad Sci 1173:664–669

Allhorn M, Olin AI, Nimmerjahn F, Collin M (2008) Human IgG/Fc gamma R interactions are modulated by streptococcal IgG glycan hydrolysis. PLoS One 3:e1413

Anderson CL, Grey HM (1974) Receptors for aggregated IgG on mouse lymphocytes: their presence on thymocytes, thymus-derived, and bone marrow-derived lymphocytes. J Exp Med 139:1175–1188

Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV (2008a) Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 320:373–376

Anthony RM, Wermeling F, Karlsson MC, Ravetch JV (2008b) Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci USA 105:19571–19578

Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA (2007) The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 25:21–50

Atalay R, Zimmermann A, Wagner M, Borst E, Benz C, Messerle M, Hengel H (2002) Identification and expression of human cytomegalovirus transcription units coding for two distinct Fcgamma receptor homologs. J Virol 76:8596–8608

Azeredo da Silveira S, Kikuchi S, Fossati-Jimack L, Moll T, Saito T, Verbeek JS, Botto M, Walport MJ, Carroll M, Izui S (2002) Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high affinity anti-erythrocyte autoantibody. J Exp Med 195:665–672

Baerenwaldt A, Nimmerjahn F (2008) Immune regulation – Fc gamma RIIB – regulating the balance between protective and autoreactive immune responses. Immun Cell Biol 86:482–484

Ballow M (2002) Primary immunodeficiency disorders: antibody deficiency. J Allergy Clin Immunol 109:581–591

Balsitis SJ, Williams KL, Lachica R, Flores D, Kyle JL, Mehlhop E, Johnson S, Diamond MS, Beatty PR, Harris E (2010) Lethal antibody enhancement of dengue disease in mice is prevented by Fc modification. PLoS Pathog 6:e1000790

Baudino L, Nimmerjahn F, Azeredo da Silveira S, Martinez-Soria E, Saito T, Carroll M, Ravetch JV, Verbeek JS, Izui S (2008) Differential contribution of three activating IgG Fc receptors (FcgammaRI, FcgammaRIII, and FcgammaRIV) to IgG2a- and IgG2b-induced autoimmune hemolytic anemia in mice. J Immunol 180:1948–1953

Bergamaschi A, David A, Le Rouzic E, Nisole S, Barre-Sinoussi F, Pancino G (2009) The CDK inhibitor p21Cip1/WAF1 is induced by FcgammaR activation and restricts the replication of human immunodeficiency virus type 1 and related primate lentiviruses in human macrophages. J Virol 83:12253–12265

Bevaart L, Jansen MJ, van Vugt MJ, Verbeek JS, van de Winkel JG, Leusen JH (2006) The high-affinity IgG receptor, FcgammaRI, plays a central role in antibody therapy of experimental melanoma. Cancer Res 66:1261–1264

Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW (1999) The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. J Exp Med 190:585–590

Bharadwaj D, Mold C, Markham E, Du Clos TW (2001) Serum amyloid P component binds to Fc gamma receptors and opsonizes particles for phagocytosis. J Immunol 166:6735–6741

Bolland S, Ravetch JV (1999) Inhibitory pathways triggered by ITIM-containing receptors. Adv Immunol 72:149–177

Bolland S, Ravetch JV (2000) Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity 13:277–285

Bond A, Cooke A, Hay FC (1990) Glycosylation of IgG, immune complexes and IgG subclasses in the MRL-lpr/lpr mouse model of rheumatoid arthritis. Eur J Immunol 20:2229–2233

Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW (2005) Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. J Clin Invest 115:2914–2923

Bredius RG, Derkx BH, Fijen CA, de Wit TP, de Haas M, Weening RS, van de Winkel JG, Out TA (1994) Fc gamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. J Infect Dis 170:848–853

Briles DE, Forman C, Hudak S, Claflin JL (1984a) The effects of idiotype on the ability of IgG1 anti-phosphorylcholine antibodies to protect mice from fatal infection with Streptococcus pneumoniae. Eur J Immunol 14:1027–1030

Briles DE, Forman C, Hudak S, Claflin JL (1984b) The effects of subclass on the ability of anti-phosphocholine antibodies to protect mice from fatal infection with Streptococcus pneumoniae. J Mol Cell Immunol 1:305–309

Brouwer KC, Lal RB, Mirel LB, Yang C, van Eijk AM, Ayisi J, Otieno J, Nahlen BL, Steketee R, Lal AA, Shi YP (2004) Polymorphism of Fc receptor IIa for IgG in infants is associated with susceptibility to perinatal HIV-1 infection. AIDS 18:1187–1194

Bruhns P, Samuelsson A, Pollard JW, Ravetch JV (2003) Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity 18:573–581

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daeron M (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113:3716–3725

Carroll MC (2004) The complement system in regulation of adaptive immunity. Nat Immunol 5:981–986

Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H (2002) Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood 99:754–758

Clatworthy MR, Smith KG (2004) FcgammaRIIb balances efficient pathogen clearance and the cytokine-mediated consequences of sepsis. J Exp Med 199:717–723

Clatworthy MR, Willcocks L, Urban B, Langhorne J, Williams TN, Peshu N, Watkins NA, Floto RA, Smith KG (2007) Systemic lupus erythematosus-associated defects in the inhibitory receptor FcgammaRIIb reduce susceptibility to malaria. Proc Natl Acad Sci USA 104:7169–7174

Clynes R, Ravetch JV (1995) Cytotoxic antibodies trigger inflammation through Fc receptors. Immunity 3:21–26

Collin M, Olsen A (2001) Effect of SpeB and EndoS from Streptococcus pyogenes on human immunoglobulins. Infect Immun 69:7187–7189

Daeron M, Lesourne R (2006) Negative signaling in Fc receptor complexes. Adv Immunol 89: 39–86

David A, Saez-Cirion A, Versmisse P, Malbec O, Iannascoli B, Herschke F, Lucas M, Barre-Sinoussi F, Mouscadet JF, Daeron M, Pancino G (2006) The engagement of activating FcgammaRs inhibits primate lentivirus replication in human macrophages. J Immunol 177:6291–6300

Dhodapkar KM, Kaufman JL, Ehlers M, Banerjee DK, Bonvini E, Koenig S, Steinman RM, Ravetch JV, Dhodapkar MV (2005) Selective blockade of inhibitory Fcgamma receptor enables human dendritic cell maturation with IL-12p70 production and immunity to antibody-coated tumor cells. Proc Natl Acad Sci USA 102:2910–2915

Dubin G, Socolof E, Frank I, Friedman HM (1991) Herpes simplex virus type 1 Fc receptor protects infected cells from antibody-dependent cellular cytotoxicity. J Virol 65:7046–7050

Dutertre CA, Bonnin-Gelize E, Pulford K, Bourel D, Fridman WH, Teillaud JL (2008) A novel subset of NK cells expressing high levels of inhibitory FcgammaRIIB modulating antibody-dependent function. J Leukoc Biol 84:1511–1520

Ferrara C, Stuart F, Sondermann P, Brunker P, Umana P (2006) The carbohydrate at FcgammaR-IIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. J Biol Chem 281:5032–5036

Fijen CA, Bredius RG, Kuijper EJ, Out TA, De Haas M, De Wit AP, Daha MR, De Winkel JG (2000) The role of Fcgamma receptor polymorphisms and C3 in the immune defence against Neisseria meningitidis in complement-deficient individuals. Clin Exp Immunol 120:338–345

Florese RH, Van Rompay KK, Aldrich K, Forthal DN, Landucci G, Mahalanabis M, Haigwood N, Venzon D, Kalyanaraman VS, Marthas ML, Robert-Guroff M (2006) Evaluation of passively transferred, nonneutralizing antibody-dependent cellular cytotoxicity-mediating IgG in protection of neonatal rhesus macaques against oral SIVmac251 challenge. J Immunol 177:4028–4036

Forthal DN, Moog C (2009) Fc receptor-mediated antiviral antibodies. Curr Opin HIV AIDS 4:388–393

Fossati-Jimack L, Ioan-Facsinay A, Reininger L, Chicheportiche Y, Watanabe N, Saito T, Hofhuis FM, Gessner JE, Schiller C, Schmidt RE, Honjo T, Verbeek JS, Izui S (2000) Markedly different pathogenicity of four immunoglobulin G isotype-switch variants of an antierythrocyte autoantibody is based on their capacity to interact *in vivo* with the low-affinity Fcgamma receptor III. J Exp Med 191:1293–1302

Frank I, Friedman HM (1989) A novel function of the herpes simplex virus type 1 Fc receptor: participation in bipolar bridging of antiviral immunoglobulin G. J Virol 63:4479–4488

Gavin AL, Tan PS, Hogarth PM (1998) Gain-of-function mutations in FcgammaRI of NOD mice: implications for the evolution of the Ig superfamily. EMBO J 17:3850–3857

Giorgini A, Brown HJ, Lock HR, Nimmerjahn F, Ravetch JV, Verbeek JS, Sacks SH, Robson MG (2008) Fc gamma RIII and Fc gamma RIV are indispensable for acute glomerular inflammation induced by switch variant monoclonal antibodies. J Immunol 181:8745–8752

Gjertsson I, Kleinau S, Tarkowski A (2002) The impact of Fcgamma receptors on Staphylococcus aureus infection. Microb Pathog 33:145–152

Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, Robert-Guroff M (2005) Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol 174:2185–2189

Gray CA, Lawrence RA (2002) A role for antibody and Fc receptor in the clearance of Brugia malayi microfilariae. Eur J Immunol 32:1114–1120

Hamaguchi Y, Xiu Y, Komura K, Nimmerjahn F, Tedder TF (2006) Antibody isotype-specific engagement of Fcgamma receptors regulates B lymphocyte depletion during CD20 immunotherapy. J Exp Med 203:743–753, Epub 2006 Mar 6

Hazenbos WL, Gessner JE, Hofhuis FM, Kuipers H, Meyer D, Heijnen IA, Schmidt RE, Sandor M, Capel PJ, Daeron M, van de Winkel JG, Verbeek JS (1996) Impaired IgG-dependent anaphylaxis and Arthus reaction in Fc gamma RIII (CD16) deficient mice. Immunity 5:181–188

Hellwig SM, van Oirschot HF, Hazenbos WL, van Spriel AB, Mooi FR, van De Winkel JG (2001) Targeting to Fcgamma receptors, but not CR3 (CD11b/CD18), increases clearance of *Bordetella pertussis*. J Infect Dis 183:871–879

Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, Lanigan CM, Landucci G, Forthal DN, Parren PW, Marx PA, Burton DR (2007) Fc receptor but not complement binding is important in antibody protection against HIV. Nature 449:101–104

Hirano M, Davis RS, Fine WD, Nakamura S, Shimizu K, Yagi H, Kato K, Stephan RP, Cooper MD (2007) IgEb immune complexes activate macrophages through FcgammaRIV binding. Nat Immunol 8:762–771

Hogarth PM (2002) Fc receptors are major mediators of antibody based inflammation in autoimmunity. Curr Opin Immunol 14:798–802

Holl V, Hemmerter S, Burrer R, Schmidt S, Bohbot A, Aubertin AM, Moog C (2004) Involvement of Fc gamma RI (CD64) in the mechanism of HIV-1 inhibition by polyclonal IgG purified from infected patients in cultured monocyte-derived macrophages. J Immunol 173:6274–6283

Holl V, Peressin M, Decoville T, Schmidt S, Zolla-Pazner S, Aubertin AM, Moog C (2006a) Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. J Virol 80:6177–6181

Holl V, Peressin M, Schmidt S, Decoville T, Zolla-Pazner S, Aubertin AM, Moog C (2006b) Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. Blood 107:4466–4474

Huber VC, Lynch JM, Bucher DJ, Le J, Metzger DW (2001) Fc receptor-mediated phagocytosis makes a significant contribution to clearance of influenza virus infections. J Immunol 166:7381–7388

Ioan-Facsinay A, de Kimpe SJ, Hellwig SM, van Lent PL, Hofhuis FM, van Ojik HH, Sedlik C, da Silveira SA, Gerber J, de Jong YF, Roozendaal R, Aarden LA, van den Berg WB, Saito T, Mosser D, Amigorena S, Izui S, van Ommen GJ, van Vugt M, van de Winkel JG, Verbeek JS (2002) FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity 16:391–402

Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. Science 327:291–295

Jefferis R (2009) Glycosylation as a strategy to improve antibody-based therapeutics. Nat Rev Drug Discov 8:226–234

Johansson BP, Shannon O, Bjorck L (2008) IdeS: a bacterial proteolytic enzyme with therapeutic potential. PLoS One 3:e1692

Kalergis AM, Ravetch JV (2002) Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. J Exp Med 195:1653–1659

Kaminski MS, Kitamura K, Maloney DG, Campbell MJ, Levy R (1986) Importance of antibody isotype in monoclonal anti-idiotype therapy of a murine B cell lymphoma. A study of hybridoma class switch variants. J Immunol 136:1123–1130

Kaneko Y, Nimmerjahn F, Madaio MP, Ravetch JV (2006a) Pathology and protection in nephrotoxic nephritis is determined by selective engagement of specific Fc receptors. J Exp Med 203:789–797, Epub 2006 Mar 6

Kaneko Y, Nimmerjahn F, Ravetch JV (2006b) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313:670–673

Karlsson Hedestam GB, Fouchier RA, Phogat S, Burton DR, Sodroski J, Wyatt RT (2008) The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. Nat Rev Microbiol 6:143–155

Kima PE, Constant SL, Hannum L, Colmenares M, Lee KS, Haberman AM, Shlomchik MJ, McMahon-Pratt D (2000) Internalization of Leishmania mexicana complex amastigotes via the Fc receptor is required to sustain infection in murine cutaneous leishmaniasis. J Exp Med 191:1063–1068

Kipps TJ, Parham P, Punt J, Herzenberg LA (1985) Importance of immunoglobulin isotype in human antibody-dependent, cell-mediated cytotoxicity directed by murine monoclonal antibodies. J Exp Med 161:1–17

Lambert SL, Okada CY, Levy R (2004) TCR vaccines against a murine T cell lymphoma: a primary role for antibodies of the IgG2c class in tumor protection. J Immunol 172:929–936

Langone JJ (1982) Protein A of Staphylococcus aureus and related immunoglobulin receptors produced by streptococci and pneumococci. Adv Immunol 32:157–252

Leclerc JC, Plater C, Fridman WH (1977) The role of the Fc receptor (FcR) of thymus-derived lymphocytes. I. Presence of FcR on cytotoxic lymphocytes and absence of direct role in cytotoxicity. Eur J Immunol 7:543–548

Littaua R, Kurane I, Ennis FA (1990) Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. J Immunol 144:3183–3186
Loke H, Bethell D, Phuong CX, Day N, White N, Farrar J, Hill A (2002) Susceptibility to dengue hemorrhagic fever in vietnam: evidence of an association with variation in the vitamin d receptor and Fc gamma receptor IIa genes. Am J Trop Med Hyg 67:102–106
Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD (2008) Structural recognition and functional activation of FcgammaR by innate pentraxins. Nature 456:989–992
Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med 1:237–243
Mancardi DA, Iannascoli B, Hoos S, England P, Daeron M, Bruhns P (2008) FcgammaRIV is a mouse IgE receptor that resembles macrophage FcepsilonRI in humans and promotes IgE-induced lung inflammation. J Clin Invest 118:3738–3750
Marjon KD, Marnell LL, Mold C, Du Clos TW (2009) Macrophages activated by C-reactive protein through Fc gamma RI transfer suppression of immune thrombocytopenia. J Immunol 182:1397–1403
Markine-Goriaynoff D, Coutelier JP (2002) Increased efficacy of the immunoglobulin G2a subclass in antibody-mediated protection against lactate dehydrogenase-elevating virus-induced polioencephalomyelitis revealed with switch mutants. J Virol 76:432–435
Marnell L, Mold C, Du Clos TW (2005) C-reactive protein: ligands, receptors and role in inflammation. Clin Immunol 117:104–111
Meyer D, Schiller C, Westermann J, Izui S, Hazenbos WL, Verbeek JS, Schmidt RE, Gessner JE (1998) FcgammaRIII (CD16)-deficient mice show IgG isotype-dependent protection to experimental autoimmune hemolytic anemia. Blood 92:3997–4002
Mimura Y, Sondermann P, Ghirlando R, Lund J, Young SP, Goodall M, Jefferis R (2001) Role of oligosaccharide residues of IgG1-Fc in Fc gamma RIIb binding. J Biol Chem 276:45539–45547
Mizuochi T, Hamako J, Nose M, Titani K (1990) Structural changes in the oligosaccharide chains of IgG in autoimmune MRL/Mp-lpr/lpr mice. J Immunol 145:1794–1798
Nandakumar KS, Holmdahl R (2008) Therapeutic cleavage of IgG: new avenues for treating inflammation. Trends Immunol 29:173–178
Nandakumar KS, Collin M, Olsen A, Nimmerjahn F, Blom AM, Ravetch JV, Holmdahl R (2007a) Endoglycosidase treatment abrogates IgG arthritogenicity: Importance of IgG glycosylation in arthritis. Eur J Immunol 37:2973–2982
Nandakumar KS, Johansson BP, Bjorck L, Holmdahl R (2007b) Blocking of experimental arthritis by cleavage of IgG antibodies *in vivo*. Arthritis Rheum 56:3253–3260
Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 310:1510–1512
Nimmerjahn F, Ravetch JV (2006) Fcgamma receptors: old friends and new family members. Immunity 24:19–28
Nimmerjahn F, Ravetch JV (2007a) Antibodies, Fc receptors and cancer. Curr Opin Immunol 19:239–245
Nimmerjahn F, Ravetch JV (2007b) The antiinflammatory activity of IgG: the intravenous IgG paradox. J Exp Med 204:11–15
Nimmerjahn F, Ravetch JV (2008a) Anti-inflammatory actions of intravenous immunoglobulin. Annu Rev Immunol 26:513–533
Nimmerjahn F, Ravetch JV (2008b) Fc gamma receptors as regulators of immune responses. Nat Rev Immunol 8:34–47
Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV (2005) FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity 23:41–51
Nimmerjahn F, Anthony RM, Ravetch JV (2007) Agalactosylated IgG antibodies depend on cellular Fc receptors for *in vivo* activity. Proc Natl Acad Sci USA 104:8433–8437
Otten MA, van der Bij GJ, Verbeek SJ, Nimmerjahn F, Ravetch JV, Beelen RH, van de Winkel JG, van Egmond M (2008) Experimental antibody therapy of liver metastases reveals functional redundancy between Fc gammaRI and Fc gammaRIV. J Immunol 181:6829–6836

Padigel UM, Farrell JP (2005) Control of infection with Leishmania major in susceptible BALB/c mice lacking the common gamma-chain for FcR is associated with reduced production of IL-10 and TGF-beta by parasitized cells. J Immunol 174:6340–6345

Pathan N, Faust SN, Levin M (2003) Pathophysiology of meningococcal meningitis and septicaemia. Arch Dis Child 88:601–607

Perez-Bercoff D, David A, Sudry H, Barre-Sinoussi F, Pancino G (2003) Fcgamma receptor-mediated suppression of human immunodeficiency virus type 1 replication in primary human macrophages. J Virol 77:4081–4094

Radaev S, Motyka S, Fridman WH, Sautes-Fridman C, Sun PD (2001) The structure of a human type III Fcgamma receptor in complex with Fc. J Biol Chem 276:16469–16477

Ravetch JV, Clynes RA (1998) Divergent roles for Fc receptors and complement *in vivo*. Annu Rev Immunol 16:421–432

Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. Science 290:84–89

Ravetch JV, Nimmerjahn F (2008) Fc receptors. In: Paul WE (ed) Fundamental immunology, 5th edn. Lippincott-Raven, Philadelphia, pp 684–705

Rodriguez ME, van der Pol WL, Sanders LA, van de Winkel JG (1999) Crucial role of FcgammaRIIa (CD32) in assessment of functional anti-Streptococcus pneumoniae antibody activity in human sera. J Infect Dis 179:423–433

Rodriguez ME, Hellwig SM, Hozbor DF, Leusen J, van der Pol WL, van de Winkel JG (2001) Fc receptor-mediated immunity against Bordetella pertussis. J Immunol 167:6545–6551

Rodriguez W, Mold C, Kataranovski M, Hutt JA, Marnell LL, Verbeek JS, Du Clos TW (2007) C-reactive protein-mediated suppression of nephrotoxic nephritis: role of macrophages, complement, and Fcgamma receptors. J Immunol 178:530–538

Rook GA, Steele J, Brealey R, Whyte A, Isenberg D, Sumar N, Nelson JL, Bodman KB, Young A, Roitt IM et al (1991) Changes in IgG glycoform levels are associated with remission of arthritis during pregnancy. J Autoimmun 4:779–794

Samuelsson A, Towers TL, Ravetch JV (2001) Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. Science 291:484–486

Scallon BJ, Tam SH, McCarthy SG, Cai AN, Raju TS (2007) Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality. Mol Immunol 44:1524–1534

Schlageter AM, Kozel TR (1990) Opsonization of *Cryptococcus neoformans* by a family of isotype-switch variant antibodies specific for the capsular polysaccharide. Infect Immun 58:1914–1918

Schmidt RE, Gessner JE (2005) Fc receptors and their interaction with complement in autoimmunity. Immunol Lett 100:56–67

Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li B, Fox JA, Presta LG (2001) High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem 276:6591–6604

Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277:26733–26740, Epub 2002 May 1

Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, Hanai N, Shitara K (2003) The absence of fucose but not the presence of galactose or bisecting *N*-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–3473, Epub 2002 Nov 8

Smith I, Vedeler C, Halstensen A (2003) FcgammaRIIa and FcgammaRIIIb polymorphisms were not associated with meningococcal disease in Western Norway. Epidemiol Infect 130:193–199

Sondermann P, Huber R, Oosthuizen V, Jacob U (2000) The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc gammaRIII complex. Nature 406:267–273

Sprague ER, Reinhard H, Cheung EJ, Farley AH, Trujillo RD, Hengel H, Bjorkman PJ (2008) The human cytomegalovirus Fc receptor gp68 binds the Fc CH2-CH3 interface of immunoglobulin G. J Virol 82:3490–3499

Stout RD, Herzenberg LA (1975) The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. J Exp Med 142:611–621

Syed SN, Konrad S, Wiege K, Nieswandt B, Nimmerjahn F, Schmidt RE, Gessner JE (2009) Both FcgammaRIV and FcgammaRIII are essential receptors mediating type II and type III autoimmune responses via FcRgamma-LAT-dependent generation of C5a. Eur J Immunol 39:3343–3356

Sylvestre D, Clynes R, Ma M, Warren H, Carroll MC, Ravetch JV (1996) Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice. J Exp Med 184:2385–2392

Tackenberg B, Jelcic I, Baerenwaldt A, Oertel WH, Sommer N, Nimmerjahn F, Lunemann JD (2009) Impaired inhibitory Fc gamma receptor IIB expression on B cells in chronic inflammatory demyelinating polyneuropathy. Proc Natl Acad Sci USA 106:4788–4792

Takai T (2002) Roles of Fc receptors in autoimmunity. Nat Rev Immunol 2:580–592

Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV (1996) Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. Nature 379:346–349

Takeda A, Tuazon CU, Ennis FA (1988) Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. Science 242:580–583

Thomas-Rudolph D, Du Clos TW, Snapper CM, Mold C (2007) C-reactive protein enhances immunity to Streptococcus pneumoniae by targeting uptake to Fc gamma R on dendritic cells. J Immunol 178:7283–7291

Uchida J, Hamaguchi Y, Oliver JA, Ravetch JV, Poe JC, Haas KM, Tedder TF (2004) The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med 199:1659–1669

van de Geijn FE, Wuhrer M, Selman MH, Willemsen SP, de Man YA, Deelder AM, Hazes JM, Dolhain RJ (2009) Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study. Arthritis Res Ther 11:R193

Verma A, Ngundi MM, Meade BD, De Pascalis R, Elkins KL, Burns DL (2009) Analysis of the Fc gamma receptor-dependent component of neutralization measured by anthrax toxin neutralization assays. Clin Vaccine Immunol 16:1405–1412

Walker BD, Burton DR (2008) Toward an AIDS vaccine. Science 320:760–764

Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 21:3940–3947, Epub 2003 Sep 15

Weng WK, Czerwinski D, Timmerman J, Hsu FJ, Levy R (2004) Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. J Clin Oncol 22:4717–4724, Epub 2004 Oct 13

Willcocks LC, Smith KG, Clatworthy MR (2009) Low-affinity Fcgamma receptors, autoimmunity and infection. Expert Rev Mol Med 11:e24

Wood P (2009) Primary antibody deficiency syndromes. Ann Clin Biochem 46:99–108

Woof JM, Burton DR (2004) Human antibody-Fc receptor interactions illuminated by crystal structures. Nat Rev Immunol 4:89–99

Yee AM, Phan HM, Zuniga R, Salmon JE, Musher DM (2000) Association between FcgammaRIIa-R131 allotype and bacteremic pneumococcal pneumonia. Clin Infect Dis 30:25–28

Yoneto T, Waki S, Takai T, Tagawa Y, Iwakura Y, Mizuguchi J, Nariuchi H, Yoshimoto T (2001) A critical role of Fc receptor-mediated antibody-dependent phagocytosis in the host resistance to blood-stage Plasmodium berghei XAT infection. J Immunol 166:6236–6241

Yuan FF, Wong M, Pererva N, Keating J, Davis AR, Bryant JA, Sullivan JS (2003) FcgammaRIIA polymorphisms in *Streptococcus pneumoniae* infection. Immunol Cell Biol 81:192–195

TGF-β Function in Immune Suppression

Akihiko Yoshimura and Go Muto

Contents

Abstract Transforming growth factor-β (TGF-β) has been shown to play an essential role in establishing immunological tolerance, yet recent studies have revealed the pro-inflammatory roles of TGF-β in inflammatory responses. TGF-β induces Foxp3-positive regulatory T cells (iTregs), while in the presence of IL-6, it induces pathogenic IL-17 producing Th17 cells. TGF-β inhibits the proliferation of T cells as well as cytokine production via Foxp3-dependent and independent mechanisms. On the one hand, little is known about molecular mechanisms

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involved in immune suppression via TGF-β; however, recent studies suggest that Smad2 as well as Smad3 play essential roles in Foxp3 induction and cytokine suppression, whereas Th17 differentiation is promoted via the Smad-independent pathway. Mutual suppression of signaling between TGF-β and inflammatory cytokines has been shown to be necessary for the balance of immunity and tolerance.

1 Introduction

Dysregulated immune suppression often results in immune disorders, such as autoimmunity and inflammatory diseases. Development of these diseases is due to both enhanced immune reactions and decreased immune suppression. Such balance is achieved by the interaction between effectors cells for positive immune reactions and suppressor cells for negative immune reactions. Helper T (Th) cells are known to function as central regulators of immune responses. After activation by antigenic stimulation, naïve helper T cells differentiate into either effector T cells responsible for positive reactions or regulatory T cells (Tregs) responsible for the negative reactions. The balance between effector T cells and Tregs seems to play an important role in the establishment of immunity or tolerance (Sakaguchi 2004).

Active immune suppression is also mediated by cytokines. The pleiotropic cytokines, transforming growth factor-β (TGF-β), and interleukin-10 (IL-10) play critical roles in suppressing the immune response (Li et al. 2006a; Wan and Flavell 2007; Moore et al. 2001; Li and Flavell 2008a). Recently, a direct connection between Treg and TGF-β has been discovered (Chen et al. 2003; Li and Flavell 2008b). TGF-β has been shown to induce Foxp3, a master regulator of Tregs in naïve T cells. However, TGF-β has also been identified as an inducer of effector T cells, such as Th17 cells (Korn et al. 2007a; Rubtsov and Rudensky 2007). It has been shown that Tregs and Th17 cells are interchangeable at least in, in vitro systems (Dong 2008). Thus, T cell development, tolerance, homeostasis, and differentiation are highly dependent on a regulatory network that is modulated by TGF-β. In this review, we will focus on the regulation of both helper T cells functions and differentiation via TGF-β and its signals.

2 TGF-β and Signal Transduction

TGF-β1, -β2, and -β3 are the three isoforms that have been identified in mammals. Among these three isoforms, TGF-β1 is predominantly expressed in the immune system and an important pleiotropic cytokine with potent immunoregulatory properties (Chang et al. 2002; Govinden and Bhoola 2003). Mice deficient in TGF-β1 develop a multiorgan autoimmune inflammatory disease and die a few weeks after birth (Shull et al. 1992; Kulkarni et al. 1993). T cells have been shown to play important roles in this severe inflammtion, since such neonatal death and inflammation were eliminated by depleting mature T cells (Diebold et al. 1995; Bommireddy

et al. 2004). Various transgenic mice whose T cells are unable to respond specifically to TGF-β have also been shown to develop autoimmunity, indicating that TGF-β signaling is essential for T cell homeostasis (Gorelik and Flavell 2000; Marie et al. 2006; Li et al. 2006b). Thus, in this review, TGF-β1 will be representative of all TGF-βs unless otherwise specified.

TGF-β is synthesized in an inactive form, the pre-pro-TGF-β precursor. The dimeric proprotein is called the latency-associated peptide (LAP). The LAP/TGF-β complex binds to the latent TGF-β-binding protein (LTBP), a 125- to 160-kDa protein, and the LTBP/LAP/TGF-β complex is then secreted from cells and bound to collagen and other tissue matrix proteins (Taylor 2009; Annes et al. 2003). It has also been shown that the LAP/TGF-β complex is highly expressed in Tregs. Additional stimuli, such as low pH, proteolysis, and binding to the cell surface processing proteins are required to liberate active TGF-β (Nakamura et al. 2004; Chen et al. 2008).

The major signaling pathways of the TGF-β receptors (TGF-βR) are relatively simple (Massague 1998). TGF-β first binds to the TGF-βR, which then primarily activates Smad transcription factors, including three structurally similar proteins: two receptor-associated Smads, Smad2 and Smad3; and one common Smad, Smad4 (Huse et al. 2001). Smad2 or Smad3 is directly phosphorylated and activated by TGF-βR and heterodimerizes with Smad4 or TIF1γ (Li and Flavell 2008b; He et al. 2006). The activated Smad-complex translocates into the nucleus, and, in a cooperative manner with other nuclear cofactors, regulates the transcription of target genes (Fig. 1). Apparently, however, there exist Smad-independent pathways (Derynck and Zhang 2003; Yu et al. 2002). Through mechanisms yet to be determined, TGF-β induces rapid activation of Ras-extracellular signal-regulated kinase (Erk), TGF-β-activated kinase-mitogen-activated protein kinase (MAPK) kinase 4-c-Jun N-terminal kinase (TAK-MKK4-JNK), TAK-MKK3/6-p38, Rho-Rac-cdc42 MAPK, and phosphatidylinositol 3-kinase (PI3K)-Akt pathways (Fig. 1). Therefore, TGF-β exerts its regulation of target cell function via a range of mechanisms.

3 How TGF-β Inhibits Immune Responses

Multiple types of immune cells can be regulated by TGF-β. The following mechanisms are proposed: (1) Suppression of effector helper T cell differentiation. (2) Conversion of naïve T cells into regulatory T cells. (3) Inhibition of the proliferation of T cells and B cells. (4) Inhibition of effector cytokine production, such as IL-2, IFN-γ, and IL-4. (5) Suppression of macrophages, dendritic cells (DCs) and natural killer (NK) cells. These are summarized in Fig. 2.

One of the most important effects of TGF-β on T cells is the suppression of IL-2 production (Brabletz et al. 1993), which leads to the anti-proliferative effect on activated T cells. Moreover, the addition of exogenous IL-2 partially relieved TGF-β-mediated suppression (Ruegemer et al. 1990). However, TGF-β still inhibits the effect of IL-2, indicating that TGF-β inhibits both the production and intracellular signaling of IL-2.

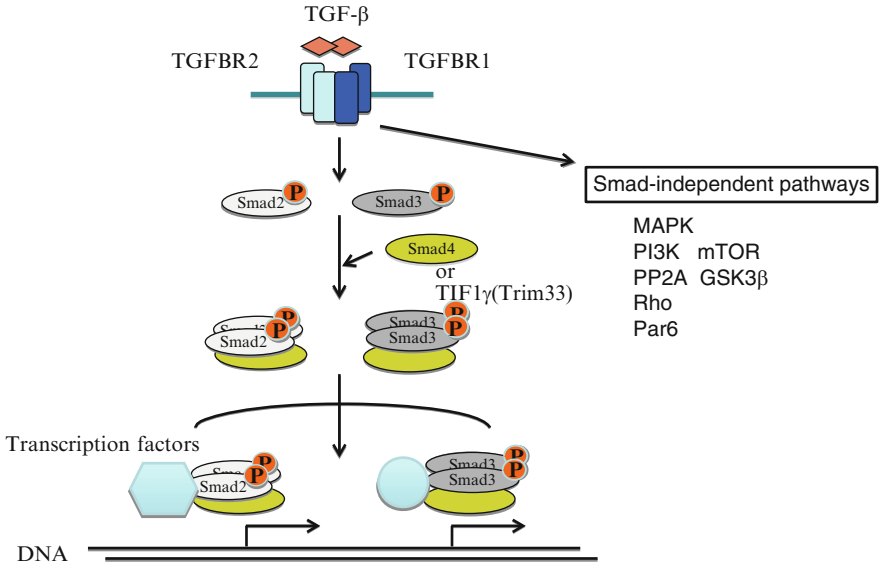

Fig. 1 Signal transduction of TGF-β Active TGF-β binds to a tetrameric complex composed of TGF-β receptor II (TGF-βRII) and TGF-β receptor I (TGF-βRI) and initiates signaling pathways that are dependent on the kinase activity of the receptors. Activated TGF-βRI phosphorylates the transcription factors Smad2 and Smad3, triggering their translocation into the nucleus in complex with the proteins Smad4. Smad complexes with additional transcription factors bind to the regulatory sequences in target genes and regulate gene expression. In addition, TGF-β activates Smad-independent pathways which trigger different cell type-specific responses

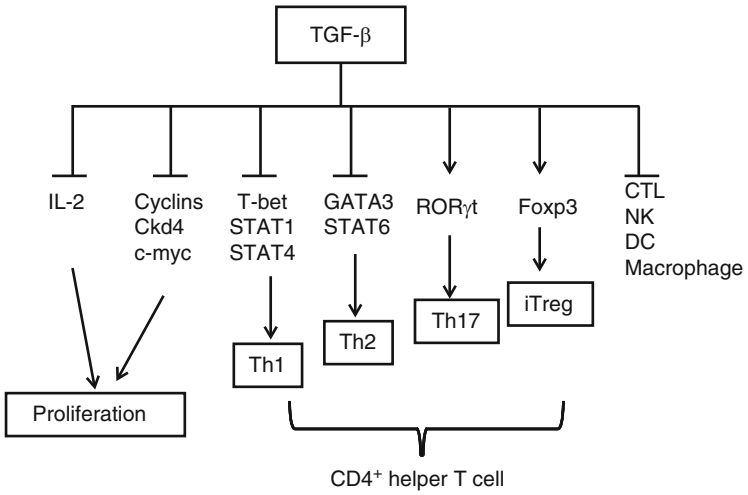

Fig. 2 Effect of TGF-β on immune cells. TGF-β inhibits proliferation of various immune cells, inhibits Th1 and Th2 differentiation, induces Th17 and iTregs, and inhibits maturation of other cells such as $CD8^{+}$CTL, NK cell, denderitic cell (DC), and macrophages

TGF-β also regulates cell proliferation through controlling the expression of cell cycle regulators, including cyclin-dependent kinase inhibitors (CKIs), such as p15, p21, and p27 (up-regulate), and cell cycle promoters, such as c-myc, cyclin D2, CDK2, and cyclin E (down-regulate) (Hannon and Beach 1994; Wolfraim et al. 2004; Polyak et al. 1994). TGF-β inhibits naïve T-cell proliferation more profoundly than that of activated T cells, which may be due to reduced TGF-β receptor II expression on activated T cells (Cottrez and Groux 2001).

In addition to T cells, TGF-β modulates the development and functions of various immune cells. DCs are potent antigen-presenting cells that activate naïve T cells and induce their proliferation and differentiation. TGF-β is necessary for the development of Langerhans cells (LCs), which are resident DCs present within epithelial cells in the epidermis (Jaksits et al. 1999; Zhang et al. 1999). TGF-β also regulates the maturation of differentiated DCs and DC-mediated T cell responses (Strobl and Knapp 1999; Strobl et al. 1996). Additionally, it regulates the antigen-presentation function of differentiated DCs in vitro (Geissmann et al. 1999). Autocrine TGF-β has been shown to be necessary for tolerogenic future of DCs by inducing indoleamine 2,3-dioxygenase (IDO), which is an enzyme that inhibits T cell proliferation (Belladonna et al. 2008). TGF-β inhibits macrophage activation, such as induction of inducible nitric-oxide synthase (iNOS) and matrix metalloproteinase (MMP)-12 via the Smad3 pathway (Werner et al. 2000) and also inhibits MyD88-dependent TLR signaling pathways (Naiki et al. 2005). Macrophages are also an important producer of TGF-β, which is activated by the phagocytosis of apoptotic cells. Usually, uptake of apoptotic cells elicits anti-inflammatory effect. Thus, induction of TGF-β is a mechanism involving the anti-inflammatory effect of apoptotic cells (Fadok et al. 1998).

TGF-β also suppresses NK cells, mast cells, granulocytes and also controls $CD8^+$ T-cell proliferation and effector functions (Li et al. 2006a; Laouar et al. 2005). Recent studies have shown that TGF-β is important for Treg-induced inhibition of the exocytosis of granules and the cytolytic function of $CD8^+$ T cells (Mempel et al. 2006).

Helper T cells play central roles in the immunosuppresive effect of TGF-β, because the neonatal lethality of TGF-β1-deficient mice was eliminated by the depletion of $CD4^+$ T cells (Rudner et al. 2003), and the crossing of TGF-β1-deficient mice onto a major histocompatibility complex (MHC) class II null background prevented this inflammation (Letterio et al. 1996). We will therefore focus on the effect of TGF-β on helper T cells in the following sections. See Fig. 3 for the role of cytokines in the helper T cell differentiation.

4 Induction and Regulation of Treg-Differentiation by TGF-β

TGF-β has been shown to induce Foxp3 during naive T cell activation (Chen et al. 2003). Foxp3 is a master transcriptional factor of Treg cells (Sakaguchi 2004). Foxp3 in $CD4^+$ T cells is responsible for the suppression activity of regulatory

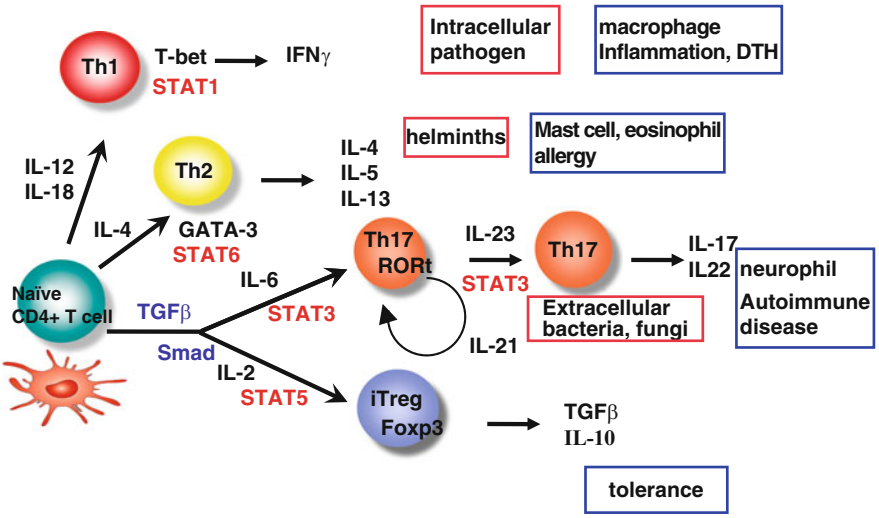

Fig. 3 Schematic overview of helper T cell differentiation. Upon antigen stimulation, $CD4^+$helper T (Th) cells follow distinct developmental pathways, attaining specialized properties and effector functions. Cells of the Th1 lineage, which evolved to enhance eradication of intracellular pathogens (e.g., intracellular bacteria, viruses and some protozoa), are characterized by their production of IFN-γ, a potent activator of cell-mediated immunity; cells of the Th2 lineage, which evolved to enhance elimination of parasitic infections (e.g., helminths), are characterized by production of IL-4, IL-5, and IL-13, which are potent activators of B-cell immunoglobulin (Ig)E production, eosinophil recruitment and mucosal expulsion mechanisms (mucous production and hypermotility). Immune pathogenesis that results from dysregulated Th1 responses to self or commensal floral antigens can promote tissue destruction and chronic inflammation, whereas dysregulated Th2 responses can cause allergy and asthma. Th17 cells secrete a distinctive set of immunoregulatory cytokines, including IL-17A, IL-17F, IL-22, and IL-21. These cytokines collectively play roles in inflammation and autoimmunity and in elimination of extracellular bacterial and fungal pathogens. Immature Th17 cells are differentiated by TGF-β +IL-6, and IL-23 is necessary for the pathogenic maturation of Th17 cells. TGF-β also induces differentiation of naïve T cells into Foxp3+ Tregs (iTregs) in the periphery. Transcription factors involved in helper T cell differentiation are also shown

T cells (Tregs). Thymus-derived Tregs are called naturally-occurring regulatory T cells (nTregs), while Foxp3-positive $CD4^+$T cells from Foxp3-naive T cells are called induced Tregs (iTregs). IL-2 is necessary for the proliferation of iTregs as well as nTregs (Fig. 3). Foxp3 inhibits secretion of proinflammatory cytokines, including IL-2, IFN-γ, IL-4 and IL-17, enhances expression of anti-inflammatory cytokines, such as IL-10, and TGF-β, and upregulates an inhibitor for co-stimulation, CTLA4 (Fontenot et al. 2003; Bettelli et al. 2005; Zhou et al. 2008). Thus, induction of iTregs could be an important immunosuppressive activity of TGF-β. TGF-β has also been implicated in the maintenance of Foxp3 in nTregs. TGF-β1 deficient mice showed normal nTreg development in the thymus but the peripheral Tregs were significantly reduced in number (Marie et al. 2005). Recently, however, TGF-β has been implicated in the development of nTregs during the

neonatal stage in the thymus (Yongzhong et al. 2008). The role of TGF-β in nTreg generation is still unclear.

When naïve T cells were stimulated with DCs in the presence of TGF-β, antigen-specific Foxp3+iTregs were generated (Yamazaki et al. 2006). These in vitro-generated iTregs can prevent experimental autoimmune diseases (Yamazaki et al. 2006). DCs in the presence of TGF-β or specific DC subsets (CD8; CD205+DCs) also promote nTreg expansion by selectively suppressing effector T cell expansion (Yamazaki et al. 2008).

5 Molecular Mechanism of Foxp3 Induction by TGF-β

Foxp3 expression is tightly regulated by various factors. The Foxp3 promoter/enhancer region contains three evolutionary conserved non-coding sequence (CNS) elements where several essential transcription factors bind. Rudensky's group described the function of three Foxp3 CNS elements (CNS1-3) in Treg cell fate determination in mice using a knockout strategy (Zheng et al. 2010). CNS1, which contains a TGF-β-NFAT response element, is superfluous in nTreg cell differentiation, but plays a prominent role in iTreg cell generation in gut-associated lymphoid tissues.

We, and others, have found Smad-binding elements in the CNS1 region of the Foxp3 promoter (Takaki et al. 2008; Tone et al. 2008). This region contains two consecutive Smad-binding elements and one NF-AT binding site. Previously, Smad3, but not Smad2, was implicated in the induction of Foxp3 (Tone et al. 2008) because Smad2 has a low DNA-binding activity compared to that of Smad3. However, using Smad2-deficient T cells, we demonstrated that both Smad2 and Smad3 are essential for TGF-β-mediated induction and maintenance of Foxp3 expression (Takimoto et al. 2010). Like *TGF-β1* knockout mice, T-cell specific Smad2- and Smad3-deficient mice possess normal nTreg cells in the thymus, but their number was decreased at the periphery (Takimoto et al. 2010).

TGF-β mediated Foxp3 expression is regulated by various factors. The IL-2/STAT5 signal is an essential factor for iTreg generation (Davidson et al. 2007; Yao et al. 2007; Burchill et al. 2007), whereas inflammatory cytokines IL-6 and IL-4 suppress iTreg (Takaki et al. 2008; Bettelli et al. 2006). STAT6 activated by IL-4 may bind to the Foxp3 promoter, thereby inducing chromatin remodeling (Takaki et al. 2008). Recently, retinoic acid, RA, has been discovered as a potent inducer and preserver of Foxp3 in iTregs (Mucida et al. 2007). The retinoic acid receptor directly interacts with the *foxp3* promoter (Takaki et al. 2008). A reporter assay using a series of deletion mutants revealed that an RA-responsive element (RARE) was present between +2114 to +2350 and interacts with the RA receptor complex, RARα/RXRα. This region was 300 bp upstream of a putative STAT6-binding site (Takaki et al. 2008). The mechanisms by which IL-6/STAT3 inhibits Foxp3 expression are still unknown.

STAT1 seems to have different effects on TGF-β-mediated Foxp3 gene expression in humans and mice. The STAT1-activating cytokines IL-27 and IFN-γ amplify TGF-β-induced FOXP3 expression in human T cells (Ouaked et al. 2009). This study shows STAT1 binding elements within the proximal region of the human *FOXP3* promoter. While IFNγ-activated STAT1 has been shown to inhibit Foxp3 induction in murine T cells (Caretto et al. 2010; Chang et al. 2009); the reason for this difference has not been clarified.

The Notch and TGF-β signaling pathways cooperatively regulate Foxp3 expression and regulatory T-cell maintenance (Samon et al. 2008). Pharmacologic inhibition of Notch signaling using γ-secretase inhibitor (GSI) treatment blocks TGF-β1-induced Foxp3 expression (Samon et al. 2008). In addition, the binding of Notch1, CSL, and Smad to conserved binding sites in the foxp3 promoter can be inhibited by treatment with GSI. Since Smads interact with various transcription factors, additional factors will undoubtedly be involved in the generation of iTregs.

6 TGF-β and Th17 Differentiation

Recently, a novel helper T cell subset that produces IL-17 (Th17) has been described and has been identified as a subset distinct from Th1 or Th2 cells (Infante-Duarte et al. 2000; Aggarwal et al. 2003). Th17 cells secrete a distinctive set of immunoregulatory cytokines, including IL-17A, IL-17F, IL-22, and IL-21 (Langrish et al. 2005; Harrington et al. 2005; Park et al. 2005a). These cytokines collectively play roles in inflammation and autoimmunity and in the elimination of extracellular bacterial and fungal pathogens. Murine autoimmue models, such as experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA), have been shown to be dependent on Th17 cells.

Th1 polarization is primarily driven by IL-12 and IFN-γ, while Th2 polarization is primarily driven by IL-4. These respective cytokines signal via STAT4, STAT1 and STAT6 to directly control the transcription factors T-bet and GATA3, which, in turn, determine Th1 and Th2 differentiation, respectively (Glimcher and Murphy 2000). Th1 cells produce IFN-γ, which facilitates their differentiation while inhibiting IL-4-mediated Th2 differentiation. Reciprocally, Th2 cells produce IL-4 and IL-10, which strongly inhibit IL-12/IFN-γ-driven Th1 differentiation.

The Th17 differentiation of naïve T cells is initiated by IL-6 and TGF-β (Mangan et al. 2006; Veldhoen et al. 2006a) (Fig. 3). In addition, IL-23, as well as IL-21, is thought to be a key cytokine for the maturation and/or maintenance of Th17 cells (Langrish et al. 2005; Nurieva et al. 2007; Korn et al. 2007b). IL-6, IL-21 and IL-23 all activate STAT3, which is thought to be essential for Th17 differentiation (Cho et al. 2006; Yang et al. 2007; Chen et al. 2006). It has also been reported that TGF-β and STAT3 play critical roles in the induction of the orphan nuclear receptor, RORγt, which directs Th17 cell differentiation by inducing the IL-23 receptor (Ivanov et al. 2006). The critical role of STAT3 in Th17 differentiation was also confirmed in human patients lacking functional STAT3 (Milner et al. 2008; Ma et al. 2008;

de Beaucoudrey et al. 2008). Th17 differentiation is strongly suppressed by STAT1, STAT4 and STAT6, but the molecualr mechanism has not been clarified.

7 Regulation of Effector Th-Differentiation by TGF-β

TGF-β inhibits Th1 and Th2 differentiation from naïve T cells in vitro (Li et al. 2006a). TGF-β blockade of Th1 cell differentiation is associated with reduced IL-12 receptor β2 (IL-12Rβ2) and T-bet expression (Gorelik et al. 2002a). T-bet is required for the induction of IL-12Rβ2 (Afkarian et al. 2002). Therefore, reduced IL-12Rβ2 levels upon TGF-β treatment is probably due to its inhibition of T-bet expression, which is dependent on the IFN-γ /Stat1 pathway (Afkarian et al. 2002). TGF-β also inhibits Th2 differentiation by suppressing Gata-3 expression and IL-4 mediated STAT6 activity (Gorelik et al. 2002b; Heath et al. 2000). It has been suggested that the role of TGF-β in Th17 differentiation is the suppression of Th1 and Th2 differentiation (i.e., suppression of the production of IFN-γ and IL-4), since these cyokines strongly inhibit Th17 differentiation. This is supported by a report showing that IL-6 alone was sufficient in inducing robust differentiation of Th17 cells in *Stat-6(-/-)T-bet(-/-)* mice, which are unable to generate Th1 and Th2 cells (Das et al. 2009). TGF-β, however, may play a specific role in Th17 differentiation, other than Th1 and Th2 suppression, because antibodies against IFN-γ and IL-4 could not completely replace TGF-β (Bettelli et al. 2006), and RORγt, the master regulator of Th17, was induced by TGF-β alone (Ichiyama et al. 2008).

Interestingly, TGF-β partially inhibits IFN-γ production and IL-12 mediated STAT4 phosphorylation in fully-differentiated Th1 cells, while IL-4 production and IL-4 mediated STAT6 activation in fully-differentiated Th2 cells were unaffected by TGF-β (Ludviksson et al. 2000). Recently, it has been shown that TGF-β induces robust IL-9 production in the presence of IL-4, which are now called Th9 cells (Veldhoen et al. 2008) (Fig. 3).

8 Treg Is a Major Source of Pro-TGF-β, and TGF-β Is One of the Effector Molecules of Tregs

Endogenous TGF-β during T/DC interaction participates in maintaining the balance between effector T cells and Tregs. For example, we have shown that SOCS3-deficient DCs, in which STAT3 was constitutively activated, selectively enhance expansion of nTregs (Matsumura et al. 2007). This effect was canceled in the presence of anti-TGF-β antibody. SOCS3-deficient Tregs produced higher levels of TGF-β than did WT DCs. TGF-β promoter analysis revealed that STAT3 binds to the region of the TGF-β promoter (Kinjyo et al. 2006). Adoptive transfer of SOCS3-deficient DCs suppresses EAE (Matsumura et al. 2007). Thus, TGF-β during T/DC interaction is important for the determination of immunity or tolerance.

Local TGF-β activation through Treg/DC interaction has been shown to be necessary for Th17 generation. T cell specific TGF-β1 knockout revealed that T cell-produced TGF-β1 promoted Th17 cell differentiation and was essential for the induction of the EAE model (Li et al. 2007). Local, but not systemic, administration of anti-TGF-β antibody inhibited EAE development (Veldhoen et al. 2006b).

Tregs express LAP on their membrane surface at high levels (Nakamura et al. 2004; Chen et al. 2008). The $CD25^+$ $CD4^+$ LAP^+ T cells are more potent in their regulatory activity than are $CD25^+$ $CD4^+$ LAP^- T cells, and the LAP^+ cells are considered to be a major source of active TGF-β. $CD25^-$ $CD4^+$ LAP^+ T cells also produce TGF-β. To be expressed on the cell surface as LAP, the TGF-β precursor must be cleaved by the endopeptidase furin in the Golgi. It has been shown that conditional deletion of furin in T cells allows for normal T-cell development but impairs the function of regulatory and effector T cells, which, in turn, produce less TGF-β. Furin-deficient T regulatory (Treg) cells are less protective in a T-cell transfer colitis model and fail to induce Foxp3 in T cells (Pesu et al. 2008). The LAP-activating receptors, such as CD36/TSP-1 and integrin $\alpha_V\beta_6$, are expressed on monocytes, endothelial cells, and DC, but not on T cells (Taylor 2009). Thus, LAP/TGF-β on Tregs will be activated via the interaction between Tregs and APCs. This is consistent with reports showing that conditional knockout of integrin $\alpha_V\beta_6$ or $\alpha_V\beta_8$ on DCs resulted in autoimmune diseases (Travis et al. 2007; Aluwihare et al. 2009). TGF-β produced by Tregs was required to inhibit Th1-cell differentiation and inflammatory-bowel disease in a transfer model (Marie et al. 2006). These data suggest that the major source of TGF-β in the immune system is regulatory T cells, which are activated by Treg/DC interaction (Fig. 4).

9 Smad-Dependent and Smad-Independent Regulation of Th Differentiation by TGF-β

The downstream mechanism for the regulation of T cells by TGF-β remains unclear. It has been reported that Smad2 or Smad3 regulates a distinctive sets of genes in fibroblasts and tumor cells (Massague 1998). *Smad2*-knockout (KO) mice are embryonic-lethal (Nomura and Li 1998), and *Smad3*-KO mice exhibit inflammatory diseases (Yang et al. 1999), suggesting that Smad2 is involved in mediating signals during development, while Smad3 is important for anti-inflammation. Moreover, the disruption of Smad4, specifically in T cells, results in colitis and an increased susceptibility to spontaneous colo-rectal tumorigenesis (Kim et al. 2006). These reports suggest that the Smad3/4 pathway is an important mediator of TGF-β signaling in immune regulation. However, the phenotypes of Smad3- or Smad4-deficient mice were much milder than those of T cell-specific *TGF-βRII* knockout mice (Li et al. 2006b), suggesting that Smad2 may also play a role in immune regulation.

T cell-specific *Smad2* conditional knockout mice revealed unexpected overlapping functions of Smad2 and Smad3 in TGF-β-induced Foxp3 induction as well as in

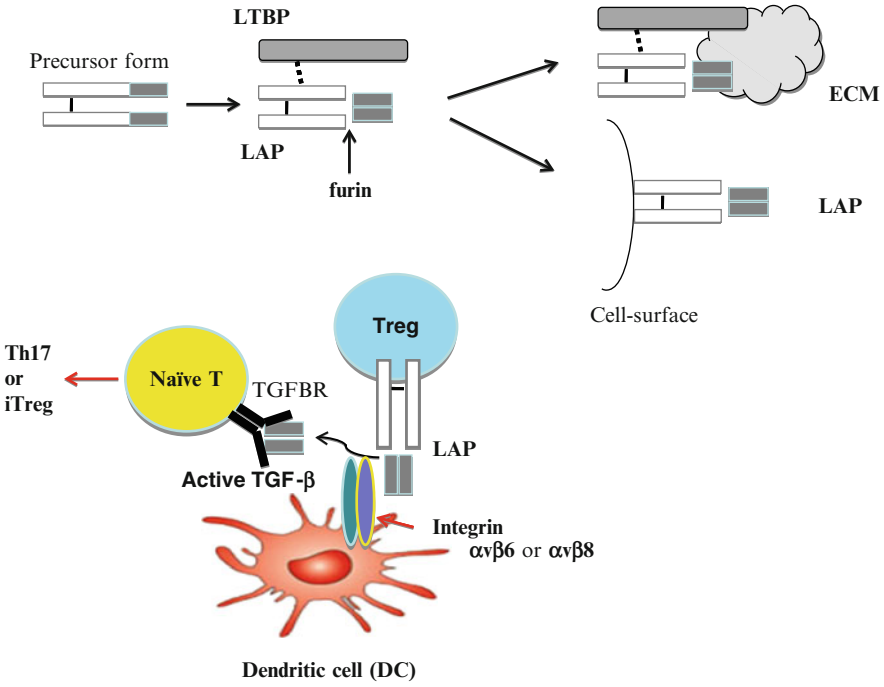

Fig. 4 Potential pathway of TGF-β activation in immunity. TGF-β is synthesized as dimeric precursor form. C-terminal region is cleaved by Furin in the Golgi, then expressed on the cell surface of Tregs or stored in the extracellular matrix (ECM).This latent form comprises a TGF-β dimer associated with the latency-associated protein (LAP) and latent TGF-β-binding protein (LTBP). Interaction of LAP with integrins expressed by DCs triggers degradation of LAP (mediated by unidentified proteases) and the release of the active form of TGF-β. This TGF-β promotes Th17 and iTreg generation from naive T cells

Th functions (Takimoto et al. 2010). *Smad2*/*Smad3*-double knockout mice, but not single knockout mice, developed fatal inflammatory diseases, with higher IFN-γ production and reduced Foxp3 expression in $CD4^+$ T cells at the periphery (Takimoto et al. 2010). TGF-β mediated induction of Foxp3, as well as suppression of IFN-γ and IL-2 was partialy impaired in Smad2-deficient T cells and Smad3-deficient T cells, and was completely eliminated in Smad2/3-double knockout T cells (Takimoto et al. 2010). Thus, Smad2 and Smad3 are redundantly essential for iTreg induction and Th suppression (Fig. 5).

Recent studies have demonstrated that TGF-β-induced Foxp3 antagonizes RORγt, which is also induced by TGF-β, to inhibit Th17 cell differentiation (Zhou et al. 2008; Ichiyama et al. 2008). It has not yet been clarified, however, how TGF-β induces both the transcription factor Foxp3 and RORγt, which have diametrically opposed physiological functions: one interacts with anti-inflammatory Tregs and the other induces inflammatory Th17 cells. It has been suggested that RORγt induction by TGF-β is independent of Smad4 (Yang et al. 2008).

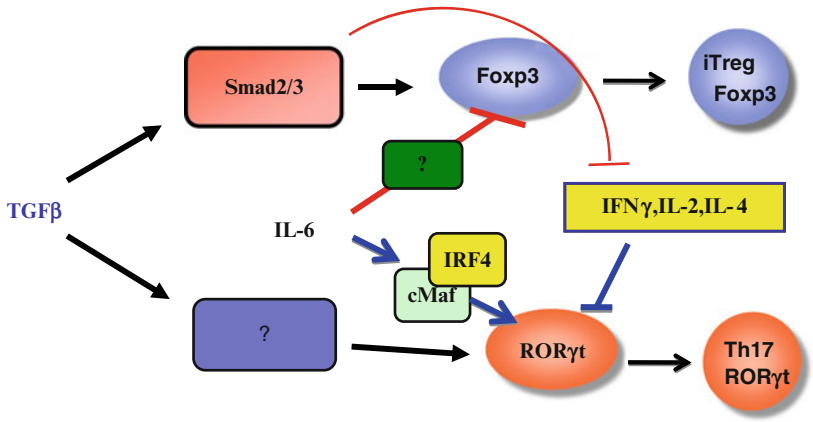

Fig. 5 Role of TGF-β signaling pathways in Th17 and iTreg differentiation. Both Foxp3 and RORγt is induced by TGF-β, however, Foxp3 induction is Smad-dependent, while RORγt induction is not. IL-6 inhibits Foxp3 expression by unknown mechanisms, and enhances RORγt expression via IRF4 and c-Maf. The Smad pathway also inhibits IFN-γ, IL-2, IL-4 production, thereby promoting Th17 differentiation

Takimoto et al. (2010) also demonstarted that both Smad2 and Smad3 were dispensable for the induction of RORγt (Takimoto et al. 2010) (Fig. 5).

Interestingly, however, Th17 development was indirectly regulated by Smad2/3 signaling. Th17 cell development was reduced in Smad-deficient $CD4^+$ T cells because of the higher production of Th17-inhibitory cytokines, such as IL-2 and IFNγ, from these T cells. Therefore, Smad signaling indirectly promotes the inducing of Th17 cell differentiation by suppressing Th17 inhibitory cytokine production (Fig. 5).

It is important to understand the role of IL-6/STAT3 in the generation of Th17 differentiation in the presence of TGF-β. IL-6 is apparently necessary for the suppression of Foxp3 and for maintaining high levels of RORγt (Zhou et al. 2008; Ivanov et al. 2006). STAT3 may suppress Foxp3 expression via a direct binding (Chaudhry et al. 2009). In addition, IRF4 (Huber et al. 2008) and c-Maf (Yang et al. 2005; Bauquet et al. 2009), which are upregulated by STAT3, have been shown to be necessary for RORγt expression. Since Foxp3 inhibits the transcriptional activity of RORγt, in the absence of IL-6/STAT3 signals, Foxp3 will overwhelm the activity of RORγt. Regulation of Th17 and iTregs through Smad-dependent and independent mechanisms are illustrated in Fig. 3.

10 Smad-Mediated Suppression of the Cytokine Production

TGF-β mediated suppression of IFNγ, IL-2 and IL-4 production was partially impaired in Smad2-KO T cells and Smad3-KO T cells (Takimoto et al. 2010; McKarns et al. 2004), and completely eliminated in Smad2/3-double KO T cells

(Takimoto et al. 2010). Therefore, suppression of cytokine production by TGF-β is Smad2/3-dependent.

TGF-β suppresses IL-2 production in T cells potentially through direct inhibition of IL-2 promoter activity. A cis-acting enhancer DNA element was identified as critical in suppressing IL-2 production via TGF-β (Brabletz et al. 1993). Tob, a member of an anti-proliferative gene family, was shown to bind to Smad2, thereby inhibiting IL-2 production (Tzachanis et al. 2001). The interaction between Tob and Smad3, however, was not observed. Runx1/3 also play essential roles in cytokine production from $CD4^+$ T cells, and may be potential interaction partners of Smad2 and/or Smad3 (Miyazono et al. 2004). It is notable that an essential NF-AT binding site is present, adjacent to the Smad binding elements in the Foxp3 promoter (Takaki et al. 2008; Tone et al. 2008). However, the interactions between these transcription factors for both Smad2 and Smad3 have not been identified.

TGF-β inhibits IFN-γ production by suppressing T-bet, which is a transcription factor critical for IFN-γ production and Th1 differentiation of $CD4^+$ T cells (Gorelik et al. 2002a). T-bet expression is induced by STAT1 and STAT4, thus Smads may inhibit IFNγ production by suppressing STAT1 and STAT4. TGF-β inhibits IL-4 production probably by suppressing IL-4-mediated STAT6 activation. The molecular mechanism by which Smads inhibit STAT have not been well understood. One paper has suggested that TGF-β1 suppresses IFN-γ-induced T-bet expression through the hemopoietic protein tyrosine phosphatase (PTP) Src homology region 2 domain-containing phosphatase-1 (Shp-1) (Park et al. 2005b). Shp-1 was shown to play a vital role in TGF-β1's suppressive effects, because the suppression activity of TGF-β was completely eliminated in Shp-1 deficient $CD4^+$ T cells. The way in which Smads are involved in the induction of Shp-1, however, still remains unclear.

11 Reciprocal Regulation of TGF-β Signaling and IFN-γ Signaling

There is extensive crosstalk between the TGF-β1/Smad signaling and the JAK-STAT pathway (Eickelberg et al. 2001; Ulloa et al. 1999). For example, IFN-γ suppresses TGF-β1 signaling through upregulation of the inhibitory Smad7. IFN-γ also inhibits TGF-β1 responses via STAT1-mediated sequestration of the nuclear coactivator p300/CREB-binding protein, preventing its association with Smads and blocking Smad transcriptional activity (Ghosh et al. 2001). In contrast, little is known about the suppression mechanisms of the JAK-STAT pathway via TGF-β1. TGF-β1 suppresses NO production from macrophages stimulated with LPS and IFN-γ, and TGF-β1 functions as a negative autocrine feedback regulator to prevent tissue injury caused by excessive NO (Ding et al. 1990; Nelson et al. 1991). Previous reports have suggested that TGF-β1 reduces IFN-γ-induced iNOS mRNA and protein levels (Ding et al. 1990; Mitani et al. 2005). We have also

found that TGF-β1 not only accelerated proteosomal degradation of iNOS but also inhibited iNOS mRNA transcription by suppressing STAT1 activation (Takaki et al. 2006). Additional analyses showed that TGF-βRI interacted with and phosphorylated IFNGR1, which is a novel mechanism of STAT1 repression by TGF-β1 (Takaki et al. 2006). Another study suggested that TGF-β inhibits IFN-γ mediated STAT1 activation via the induction of STAT1-PIAS1 (a protein inhibitor of activated STAT1) interaction (Reardon and McKay 2007).

SOCS1 is a potent inhibitor of signaling events stimulated by both IFN-γ, and in the absence of the SOCS1 protein, STAT1 is highly activated, and, subsequently, T cells are unconditionally hyperactivated (Yoshimura et al. 2007; Kubo et al. 2003). SOCS1-deficient mice die within three weeks after birth due to very severe inflammtion, just as TGF-β1-deficient mice do. We therefore hypothesized that TGF-β signaling was impaired in SOCS1-deficient T cells. SOCS1-deficient T cells were resistant to all effects of TGF-β. TGF-β could not suppress Th1-differentiation or IFN-γ production very efficiently in SOCS1-deficient $CD4^+$T cells (Tanaka et al. 2008). Moreover, TGF-β mediated induction of Foxp3 and RORγt was impaired in SOCS1-deficient T cells (Tanaka et al. 2008; Horino et al. 2008). Such TGF-β resistance was IFNγ-dependent, because TGF-β functioned normally in SOCS1/IFNγ-DKO T cells. In other words, SOCS1 is necessary for proper TGF-β signaling by protecting cells from the strong antagonistic effect of IFN-γ. Although the precise molecular mechanism for STAT1-mediated Smad suppression is still unknown, it is apparent that the reciprocal suppression of IFN-γ and TGF-β is significant in the detemination of immunity or tolerance.

12 Conclusions

The importance of active immune suppression is widely acknowledged. Studies on TGF-β and Tregs have shed light on immune suppression applications. Advances in these areas have been and are currently being translated into clinical benefits. Further investigations are warranted to clarify the mechanism through which TGF-β and Tregs control immune responses. In addition, as TGF-βs function in non-lymphoid systems, further studies on both the roles of TGF-β and Foxp3 in non-lymphoid systems and on the interaction between lymphoid and non-lymphoid systems are essential for achieving a more comprehensive view of our immune system.

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References

Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N et al (2002) T-bet is a STAT1-induced regulator of IL-12R expression in naive $CD4^+$ T cells. Nat Immunol 3:549–557

Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL (2003) Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem 278:1910–1914

Aluwihare P, Mu Z, Zhao Z, Yu D, Weinreb PH, Horan GS, Violette SM, Munger JS (2009) Mice that lack activity of alphavbeta6- and alphavbeta8-integrins reproduce the abnormalities of Tgfb1- and Tgfb3-null mice. J Cell Sci 122:227–232

Annes JP, Munger JS, Rifkin DB (2003) Making sense of latent TGF β activation. J Cell Sci 116:217–224

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(2):167–175

Belladonna ML, Volpi C, Bianchi R, Vacca C, Orabona C, Pallotta MT, Boon L, Gizzi S, Fioretti MC, Grohmann U, Puccetti P (2008) Cutting edge: Autocrine TGF-beta sustains default tolerogenesis by IDO-competent dendritic cells. J Immunol 181(8):5194–5198

Bettelli E, Dastrange M, Oukka M (2005) Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. Proc Natl Acad Sci USA 102:5138–5143

Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441:235–238

Bommireddy R, Engle SJ, Ormsby I, Boivin GP, Babcock GF, Doetschman T (2004) Elimination of both $CD4^+$ and $CD8^+$ T cells but not B cells eliminates inflammation and prolongs the survival of TGFbeta1-deficient mice. Cell Immunol 232:96–104

Brabletz T, Pfeuffer I, Schorr E, Siebelt F, Wirth T, Serfling E (1993) Transforming growth factor β and cyclosporin A inhibit the inducible activity of the interleukin-2 gene in T cells through a noncanonical octamer-binding site. Mol Cell Biol 13:1155–1162

Burchill MA, Yang J, Vogtenhuber C, Blazar BR, Farrar MA (2007) IL-2 receptor beta-dependent STAT5 activation is required for the develop- ment of Foxp3+ regulatory T cells. J Immunol 178:280–290

Caretto D, Katzman SD, Villarino AV, Gallo E, Abbas AK (2010) Cutting edge: the Th1 response inhibits the generation of peripheral regulatory T cells. J Immunol 184(1):30–34

Chang H, Brown CW, Matzuk MM (2002) Genetic analysis of the mammalian transforming growth factor-beta superfamily. Endocr Rev 23:787–823

Chang JH, Kim YJ, Han SH, Kang CY (2009) IFN-gamma-STAT1 signal regulates the differentiation of inducible Treg: potential role for ROS-mediated apoptosis. Eur J Immunol 39(5):1241–1251

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 (5955):986–991

Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM (2003) Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 198(12):1875–1886

Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu BM, Tato C, Yoshimura A, Hennighausen L, O'Shea JJ (2006) Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci USA 103:8137–8142

Chen ML, Yan BS, Bando Y, Kuchroo VK, Weiner HL (2008) Latency-associated peptide identifies a novel $CD4^+CD25^+$ regulatory T cell subset with TGFβ-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. J Immunol 180:7327–7337

Cho ML, Kang JW, Moon YM, Nam HJ, Jhun JY, Heo SB, Jin HT, Min SY, Ju JH, Park KS, Cho YG, Yoon CH, Park SH, Sung YC, Kim HY (2006) STAT3 and NF-kappaB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice. J Immunol 176:5652–5661

Cottrez F, Groux H (2001) Regulation of TGF-beta response during T cell activation is modulated by IL-10. J Immunol 167:773–778

Das J, Ren G, Zhang L, Roberts AI, Zhao X, Bothwell AL, Van Kaer L, Shi Y, Das G (2009) Transforming growth factor beta is dispensable for the molecular orchestration of Th17 cell differentiation. J Exp Med 206(13):2407–2416

Davidson TS, DiPaolo RJ, Andersson J, Shevach EM (2007) Cutting Edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells. J Immunol 178:4022–4026

de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P et al (2008) Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. J Exp Med 205:1543

Derynck R, Zhang YE (2003) Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature 425:577–584

Diebold RJ, Eis MJ, Yin M, Ormsby I, Boivin GP, Darrow BJ, Saffitz JE, Doetschman T (1995) Early-onset multifocal inflammation in the transforming growth factor beta 1-null mouse is lymphocyte mediated. Proc Natl Acad Sci USA 92:12215–12219

Ding A, Nathan CF, Graycar J, Derynck R, Stuehr DJ, Srimal S (1990) Macrophage deactivating factor and transforming growth factors-beta 1 -beta 2 and -beta 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. J Immunol 145:940–944

Dong C (2008) TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 8(5):337–348

Eickelberg O, Pansky A, Koehler E, Bihl M, Tamm M, Hildebrand P, Perruchoud AP, Kashgarian M, Roth M (2001) Molecular mechanisms of TGF-(beta) antagonism by interferon (gamma) and cyclosporine A in lung fibroblasts. Faseb J 15:797–806

Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE2, and PAF. J Clin Invest 101:890–898

Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. Nat Immunol 4:330–336

Geissmann F, Revy P, Regnault A, Lepelletier Y, Dy M, Brousse N, Amigorena S, Hermine O, Durandy A (1999) TGF-β1 prevents the noncognate maturation of human dendritic Langerhans cells. J Immunol 162:4567–4575

Ghosh AK, Yuan W, Mori Y, Chen S, Varga J (2001) Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. J Biol Chem 276:11041–11048

Glimcher LH, Murphy KM (2000) Lineage commitment in the immune system: the T helper lymphocyte grows up. Genes Dev 14:1693–1711

Gorelik L, Flavell RA (2000) Abrogation of TGFβ signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. Immunity 12:171–181

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

Gorelik L, Fields PE, Flavell RA (2002b) Cutting edge: TGF-β inhibits Th type 2 development through inhibition of GATA-3 expression. J Immunol 165:4773–4777

Govinden R, Bhoola KD (2003) Genealogy, expression, and cellular function of transforming growth factor-beta. Pharmacol Ther 98:257–265

Hannon GJ, Beach D (1994) p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. Nature 371:257–261

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT (2005) Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6:1123–1132

He W, Dorn DC, Erdjument-Bromage H, Tempst P, Moore MA, Massague J (2006) Hematopoiesis controlled by distinct TIF1gamma and Smad4 branches of the TGFbeta pathway. Cell 125:929–941

Heath VL, Murphy EE, Crain C, Tomlinson MG, O'Garra A (2000) TGF-β1 down-regulates Th2 development and results in decreased IL-4-induced STAT6 activation and GATA-3 expression. Eur J Immunol 30:2639–2649

Horino J, Fujimoto M, Terabe F, Serada S, Takahashi T, Soma Y, Tanaka K, Chinen T, Yoshimura A, Nomura S, Kawase I, Hayashi N, Kishimoto T, Naka T (2008) Suppressor of cytokine signaling-1 ameliorates dextran sulfate sodium-induced colitis in mice. Int Immunol 20(6):753–762

Huber M, Brüstle A, Reinhard K, Guralnik A, Walter G, Mahiny A, von Löw E, Lohoff M (2008) IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. Proc Natl Acad Sci USA 105(52):20846–20851

Huse M, Muir TW, Xu L, Chen YG, Kuriyan J, Massague J (2001) The TGF β receptor activation process: an inhibitor- to substrate-binding switch. Mol Cell 8:671–682

Ichiyama K, Yoshida H, Wakabayashi Y, Chinen T, Saeki K, Nakaya M, Takaesu G, Hori S, Yoshimura A, Kobayashi T (2008) Foxp3 inhibits RORgammat-mediated IL-17A mRNA transcription through direct interaction with RORgammat. J Biol Chem 283(25):17003–17008

Infante-Duarte C, Horton HF, Byrne MC, Kamradt T (2000) Microbial lipopeptides induce the production of IL-17 in Th cells. J Immunol 165:6107–6115

Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR (2006) The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126:1121–1133

Jaksits S, Kriehuber E, Charbonnier AS, Rappersberger K, Stingl G, Maurer D (1999) $CD34^+$ cell-derived $CD14^+$ precursor cells develop into Langerhans cells in a TGF-β1-dependent manner. J Immunol 163:4869–4877

Kim BG, Li C, Qiao W, Mamura M, Kasprzak B, Anver M, Wolfraim L, Hong S, Mushinski E, Potter M, Kim SJ, Fu XY, Deng C, Letterio JJ (2006) Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. Nature 441:1015–1019

Kinjyo I, Inoue H, Hamano S, Fukuyama S, Yoshimura T, Koga K, Takaki H, Himeno K, Takaesu G, Kobayashi T, Yoshimura A (2006) Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-beta 1. J Exp Med 203(4):1021–1031

Korn T, Oukka M, Kuchroo V, Bettelli E (2007a) Th17 cells: effector T cells with inflammatory properties. Semin Immunol 19(6):362–371

Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK (2007b) IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 448:484–487

Kubo M, Hanada T, Yoshimura A (2003) Suppressors of cytokine signaling and immunity. Nat Immunol 4:1169–1176

Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S (1993) Transforming growth factor β1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 90:770–774

Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 201:233–240

Laouar Y, Sutterwala FS, Gorelik L, Flavell RA (2005) Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. Nat Immunol 6:600–607

Letterio JJ, Geiser AG, Kulkarni AB, Dang H, Kong L, Nakabayashi T, Mackall CL, Gress RE, Roberts AB (1996) Autoimmunity associated with TGF-beta1-deficiency in mice is dependent on MHC class II antigen expressions II antigen expression. J Clin Invest 98:2109–2119

Li MO, Flavell RA (2008a) Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. Immunity 28(4):468–476

Li MO, Flavell RA (2008b) TGF-beta: a master of all T cell trades. Cell 134(3):392–404

Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA (2006a) Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 24:99–146

Li MO, Sanjabi S, Flavell RA (2006b) Transforming growth factor-b controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. Immunity 25:455–471

Li MO, Wan YY, Flavell RA (2007) T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity 26(5):579–591

Ludviksson BR, Seegers D, Resnick AS, Strober W (2000) The effect of TGF-beta1 on immune responses of naive versus memory $CD4^+$ Th1/Th2 T cells. Eur J Immunol 30:2101–2111

Ma CS, Chew GYJ, Simpson N et al (2008) Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. J Exp Med 205:1551

Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT (2006) Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 441:231–234

Marie JC, Letterio JJ, Gavin M, Rudensky AY (2005) TGF-beta1 maintains suppressor function and Foxp3 expression in $CD4^+CD25^+$ regulatory T cells. J Exp Med 201(7):1061–1067

Marie JC, Liggitt D, Rudensky AY (2006) Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. Immunity 25:441–454

Massague J (1998) TGF-beta signal transduction. Annu Rev Biochem 67:753–791

Matsumura Y, Kobayashi T, Ichiyama K, Yoshida R, Hashimoto M, Takimoto T, Tanaka K, Chinen T, Shichita T, Wyss-Coray T, Sato K, Yoshimura A (2007) Selective expansion of foxp3-positive regulatory T cells and immunosuppression by suppressors of cytokine signaling 3-deficient dendritic cells. J Immunol 179(4):2170–2179

McKarns SC, Schwartz RH, Kaminski NE (2004) Smad3 is essential for TGF-beta 1 to suppress IL-2 production and TCR-induced proliferation, but not IL-2-induced proliferation. J Immunol 172:4275–4284

Mempel TR, Pittet MJ, Khazaie K, Weninger W, Weissleder R, von Boehmer H, von Andrian UH (2006) Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. Immunity 25:129–141

Milner JD, Brenchley JM, Laurence A et al (2008) Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 452:773

Mitani T, Terashima M, Yoshimura H, Nariai Y, Tanigawa Y (2005) TGF-beta1 enhances degradation of IFN-gamma-induced iNOS protein via proteasomes in RAW 264.7 cells. Nitric Oxide 13:78–87

Miyazono K, Maeda S, Imamura T (2004) Coordinate regulation of cell growth and differentiation by TGF-β superfamily and Runx proteins. Oncogene 23:4232–4237

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

Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H (2007) Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 317:256–260

Naiki Y, Michelsen KS, Zhang W, Chen S, Doherty TM, Arditi M (2005) Transforming growth factor-β differentially inhibits MyD88-dependent, but not TRAM- and TRIF-dependent, lipopolysaccharide-induced TLR4 signaling. J Biol Chem 280:5491–5495

Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W (2004) TGF-beta 1 plays an important role in the mechanism of $CD4^+CD25^+$ regulatory T cell activity in both humans and mice. J Immunol 172(2):834–842

Nelson BJ, Ralph P, Green SJ, Nacy CA (1991) Differential susceptibility of activated macrophage cytotoxic effector reactions to the suppressive effects of transforming growth factor-beta 1. J Immunol 146:1849–1857

Nomura M, Li E (1998) Smad2 role in mesoderm formation, left-right patterning and craniofacial development. Nature 25:737–739

Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C (2007) Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 448:480–483

Ouaked N, Mantel PY, Bassin C, Burgler S, Siegmund K, Akdis CA, Schmidt-Weber CB (2009) Regulation of the foxp3 gene by the Th1 cytokines: the role of IL-27-induced STAT1. J Immunol 182(2):1041–1049

Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C (2005a) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 6:1133–1141

Park IK, Shultz LD, Letterio JJ, Gorham JD (2005b) TGF-beta1 inhibits T-bet induction by IFN-gamma in murine $CD4^+$ T cells through the protein tyrosine phosphatase Src homology region 2 domain-containing phosphatase-1. J Immunol 175(9):5666–5674

Pesu M, Watford WT, Wei L, Xu L, Fuss I, Strober W, Andersson J, Shevach EM, Quezado M, Bouladoux N, Roebroek A, Belkaid Y, Creemers J, O'Shea JJ (2008) T-cell-expressed proprotein convertase furin is essential for maintenance of peripheral immune tolerance. Nature 455:246–250

Polyak K, Kato JY, Solomon MJ, Sherr CJ, Massague J, Roberts JM, Koff A (1994) p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. Genes Dev 8:9–22

Reardon C, McKay DM (2007) TGF-beta suppresses IFN-gamma-STAT1-dependent gene transcription by enhancing STAT1-PIAS1 interactions in epithelia but not monocytes/macrophages. J Immunol 178(7):4284–4295

Rubtsov YP, Rudensky AY (2007) TGFbeta signalling in control of T-cell-mediated self-reactivity. Nat Rev Immunol 7(6):443–453

Rudner LA, Lin JT, Park IK, Cates JM, Dyer DA, Franz DM, Fresnch MA, Duncan EM, White HD, Gorham JD (2003) Necroinflammatory liver disease in BALB/c background, TGF-beta 1-deficient mice requires CD4+ T cells. J Immunol 170:4785–4792

Ruegemer JJ, Ho SN, Augustine JA, Schlager JW, Bell MP, McKean DJ, Abraham RT (1990) Regulatory effects of transforming growth factor-beta on IL-2- and IL-4-dependent T cell-cycle progression. J Immunol 144(5):1767–1776

Sakaguchi S (2004) Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 22:531–562

Samon JB, Champhekar A, Minter LM, Telfer JC, Miele L, Fauq A, Das P, Golde TE, Osborne BA (2008) Notch1 and TGFbeta1 cooperatively regulate Foxp3 expression and the maintenance of peripheral regulatory T cells. Blood 112(5):1813–1821

Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D et al (1992) Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. Nature 359:693–699

Strobl H, Knapp W (1999) TGF-β1 regulation of dendritic cells. Microbes Infect 1:1283–1289

Strobl H, Riedl E, Scheinecker C, Bello-Fernandez C, Pickl WF, Rappersberger K, Majdic O, Knapp W (1996) TGF-β1 promotes in vitro development of dendritic cells from $CD34^+$ hemopoietic progenitors. J Immunol 157:1499–1507

Takaki H, Minoda Y, Koga K, Takaesu G, Yoshimura A, Kobayashi T (2006) TGF-beta1 suppresses IFN-gamma-induced NO production in macrophages by suppressing STAT1 activation and accelerating iNOS protein degradation. Genes Cells 11(8):871–882

Takaki H, Ichiyama K, Koga K, Chinen T, Takaesu G, Sugiyama Y, Kato S, Yoshimura A, Kobayashi T (2008) STAT6 Inhibits TGF-β-1-mediated Foxp3 induction through direct binding to the Foxp3 promoter, which is reverted by retinoic acid receptor. J Biol Chem 283 (22):14955–14962

Takimoto T, Wakabayashi Y, Sekiya T, Inoue N, Morita R, Ichiyama K, Takahashi R, Asakawa M, Muto G, Mori T, Hasegawa E, Shizuya S, Hara T, Nomura M, Yoshimura A (2010) Smad2 and Smad3 are redundantly essential for the TGF-β-mediated regulation of regulatory T plasticity and Th1 development. J Immunol 185 (2):842–855

Tanaka K, Ichiyama K, Hashimoto M, Yoshida H, Takimoto T, Takaesu G, Torisu T, Hanada T, Yasukawa H, Fukuyama S, Inoue H, Nakanishi Y, Kobayashi T, Yoshimura A (2008) Loss of suppressor of cytokine signaling 1 in helper T cells leads to defective Th17 differentiation by enhancing antagonistic effects of IFN-gamma on STAT3 and Smads. J Immunol 180 (6):3746–3756

Taylor AW (2009) Review of the activation of TGF-β in immunity. J Leukocyte Biol 85:29–33

Tone Y, Furuuchi K, Kojima Y, Tykocinski ML, Greene MI, Tone M (2008) Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol 9(2):194–202

Travis MA, Reizis B, Melton AC, Masteller E, Tang Q, Proctor JM, Wang Y, Bernstein X, Huang X, Reichardt LF, Bluestone JA, Sheppard D (2007) αvβ8 on dendritic cells causes autoimmunity and colitis in mice. Nature 449:361–365

Tzachanis D, Freeman GJ, Hirano N, van Puijenbroek AA, Delfs MW, Berezovskaya A, Nadler LM, Boussiotis VA (2001) Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. Nat Immunol 2:1174–1182

Ulloa L, Doody J, Massague J (1999) Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. Nature 397:710–713

Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B (2006a) TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 24:179–189

Veldhoen M, Hocking RJ, Flavell RA, Stockinger B (2006b) Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. Nat Immunol 7(11):1151–1156

Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, Martin B, Wilhelm C, Stockinger B (2008) Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 9(12):1341–1346

Wan YY, Flavell RA (2007) 'Yin-Yang' functions of transforming growth factor-beta and T regulatory cells in immune regulation. Immunol Rev 220:199–213

Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, Chin MT, Topper JN, Perrella MA, Lee ME (2000) Transforming growth factor-β1 inhibition of macrophage activation is mediated via Smad3. J Biol Chem 275:36653–36658

Wolfraim LA, Walz TM, James Z, Fernandez T, Letterio JJ (2004) p21Cip1 and p27Kip1 act in synergy to alter the sensitivity of naive T cells to TGF-beta-mediated G1 arrest through modulation of IL-2 responsiveness. J Immunol 173:3093–3102

Yamazaki S, Patel M, Harper A, Bonito A, Fukuyama H, Pack M, Tarbell KV, Talmor M, Ravetch JV, Inaba K, Steinman RM (2006) Effective expansion of alloantigen-specific Foxp3+ CD25+ $CD4^+$ regulatory T cells by dendritic cells during the mixed leukocyte reaction. Proc Natl Acad Sci USA 103(8):2758–2763

Yamazaki S, Dudziak D, Heidkamp GF, Fiorese C, Bonito AJ, Inaba K, Nussenzweig MC, Steinman RM (2008) $CD8^+$ $CD205^+$ splenic dendritic cells are specialized to induce $Foxp3^+$ regulatory T cells. J Immunol 181(10):6923–6933

Yang XO, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C (1999) Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-β. EMBO J 18:1280–1291

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(5):2720–2729

Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, Dong C (2007) STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem 282:9358–9363

Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C (2008) Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity 29(1):44–56

Yao Z, Kanno Y, Kerenyi M, Stephens G, Durant L, Watford WT, Laurence A, Robinson GW, Shevach EM, Moriggl R, Hennighausen L, Wu C, O'Shea JJ (2007) Nonredundant roles for Stat5a/b in directly regulating Foxp3. Blood 109:4368–4375

Yongzhong L, Zhang P, Li J, Kulkarni AB, Perruche S, Chen W (2008) A critical function for TGF-b signaling in the development of natural $CD4^+CD25^+Foxp3^+$regulatory T cells. Nat Immunol 9:632–640

Yoshimura A, Naka T, Kubo M (2007) SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol 7:454–465

Yu L, Hebert MC, Zhang YE (2002) TGF-β receptor-activated p38 MAP kinase mediates Smad-independent TGF-β responses. EMBO J 21:3749–3759

Zhang Y, Zhang YY, Ogata M, Chen P, Harada A, Hashimoto S, Matsushima K (1999) Transforming growth factor-β1 polarizes murine hematopoietic progenitor cells to generate Langerhans cell-like dendritic cells through a monocyte/macrophage differentiation pathway. Blood 93:1208–1220

Zheng Y, Josefowicz S, Chaudhry A, Peng XP, Forbush K, Rudensky AY (2010) Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. Nature 463(7282):808–812

Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR (2008) TGF-beta-induced Foxp3 inhibits T_H17 cell differentiation by antagonizing RORγt function. Nature 453:236–240

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