Chapter 7

General Discussion

Contents

7.0 Discussion and Summary.

Most immunologists would probably agree that a lot of the remaining unsolved problems in immunology relate, in one way or another, to the question of tolerance and the regulation of immune responses. Although much progress had been made in understanding the initiation of an immune response, our insight into how this is subsequently turned off or regulated is still emerging. Traditionally, tolerance has been defined as a state of unresponsiveness to a particular antigen or insult. However in recent years this paradigm has been shifting to the appreciation that tolerance is an active process, and many mechanisms are in place to induce, and also maintain tolerance to self or innocuous antigens.

7.1 DCs take centre stage in the maintenance of immune regulation.

DCs arise from the $CD34^+$ cell compartment within the bone marrow. Myeloid progenitor cells can differentiate to immature DCs circulating in the blood or can migrate into the other tissues e.g. kidney to become interstitial DCs. Collectively these cells are broadly referred to as mDCs[1,2]. In addition, $CD14^+$ monocytes have been shown to be an important source of DC precursors during periods of physiological stress or injury[3,4]. Several studies have demonstrated that mDCs, under certain conditions, can promote tolerance and regulatory T cell induction[5,6]. The problem lies in identifying when and how to differentiate between immunogenic and tolerogenic populations. Extensive evidence from both mouse and human DC studies is that the lineage or origin of a DC is unlikely to determine whether an immunogenic or regulatory T cell response occurs. Furthermore, DCs are highly plastic and have been evolutionarily tailored to be responsive to their surroundings. It is more likely therefore, that the activation state and the site or tissue of action plays a more important role in determining immunity versus tolerance[7,8]. This becomes particularly important when one considers organ transplantation. DCs are found in virtually all organs and are therefore an important "passenger" cell population. Kidneys are the most frequently transplanted organ and resident DCs are a very important constituent involved in initiating the direct pathway of allo-recognition in transplantation, in addition to playing a central role in the innate immune response following injurious stimuli to the organ. Our knowledge into the local regulation of this DC population is remarkably finite, but promising data has suggested that in-vivo mobilised renal DCs have the potential to prolong allograft survival[9] thereby warranting further investigation into understanding renal DCs biology and how the local renal network could generate and maintain such a potent regulatory population.

A major caveat in DC biology is that we currently do not have sufficient tools to discriminate between regulatory and immunogenic DCs in vivo. The only apparent success to date has been with the discrimination between mDCs and plasmacytoid DCs (pDCs). This is due to the clear expression of BDCA-2[10] and the IL-3R[11,12] by pDCs and their strong IFNα signature upon activation. In this respect understanding the full cytokine repertoire of mDCs may prove to be very beneficial in establishing such a regime for discriminating between immunogenic and tolerogenic myeloid DC populations in vivo. In previous years one may have suggested the comparison between immature DCs with mature DCs as a model of tolerogenic and immunogenic DCs respectively. However more recent data has accrued showing that the maturation status alone, as currently defined at least, is inadequate for differentiating between an immunogenic versus a regulatory DC[5,6,13]. In light of the avid interest of many groups pursuing in-vitro generated tolerogenic DCs for therapeutic use in autoimmunity[14,15] and transplantation[16,17], these cells can provide a useful tool to investigate unexplored diverging functional characteristics between immunogenic and tolerogenic DCs. Furthermore, current clinical approaches for the treatment of inflammation mostly focus on the inhibition of pro-inflammatory responses. Cell-mediated therapy has significant potential to provide nontoxic and highly specific treatments for autoimmunity and transplantation. Deeper understanding into the mechanisms of how immune regulation is maintained may provide new targets in the treatment of chronic inflammation.

To address these issues we focussed on investigating the production and regulation of soluble factors secreted by DCs and tolerogenic DCs. In this thesis (**chapter 2, 3**) we explored the regulation and expression of IL-12 family members in DCs and describe the production of a potent regulatory cytokine, IL-35 by tolerogenic DCs. We demonstrated that uptake of apoptotic DCs by DCs profoundly affected the transcription regulation of the IL-12/IL-35 axis. We addressed an important question; which is that tolerogenic DCs are efficient at ingesting apoptotic material and that their regulatory cytokine prolife is maintained even when challenged with necrotic material. We identified a unique role for IFN family members and IL-27 in the regulation of the balance between fP and fH production (**chapter 4**), which provides new targets for investigation in DC biology. In (**chapter 5**), we described the expression of a regulatory protein, Ebi3 within non-haematopoietic cells of the kidney. Finally, the effects of culturing DCs in the presence of renal fibroblasts are demonstrated (**chapter 6**) which provides new insights into how the local stromal network within the kidney may dictate the development of DCs with a distinct regulatory phenotype.

7.2 The IL-12 family in immune-regulation.

The IL-12 family has been the subject of intense investigation in recent years and together its members are increasingly viewed as being key players in the regulation of immunity and immunological tolerance. In many aspects the IL-12 family perfectly illustrates the grander balance on-going within the entire immune system, with IL-12 and IL-23 representing the immunogenic arm, and IL-27 and IL-35 representing immune regulation. Whereas IL-12 and IL-23 can induce and maintain the generation of inflammatory Th1 and Th17 cells, IL-27 and IL-35 can induce Tr1 and iTr35 subsets of regulatory T cells[18,19]. Of the two regulatory cytokines, unquestionably more data has been generated with regard to IL-27 compared to IL-35. Several studies have demonstrated a direct role for IL-27 in the inhibition of Th17 commitment[20,21]. Additionally IL-27 can suppress immune responses indirectly. For example, IL-27 promotes the generation of CD39 expressing regulatory DCs which in turn can down-regulate innate immune NALP3 inflammasome activation[22]. This will lead to diminished availability of caspase cleaved cytokines, IL-1β and IL-18, and thereby potentially alleviate the induction of Th1 and Th17 populations. This epitomises the far reaching capabilities of the IL-12 family members in regulating immune responses, and demonstrates the importance of the knowledge that can be gained from fully understanding the regulation, expression and function of all its members, particularly the most recently identified, anti-inflammatory member, IL-35.

Unlike IL-12, IL-23 and IL-27, production of IL-35 has not been demonstrated in APCs. However in light of the expression of all chains by DCs, one could speculate that these cells may produce this potent anti-inflammatory cytokine. Our work focused on tolerogenic DCs generated in the presence of dexamethasone. Over the last decade a consensus has developed that tolerogenic DCs should lack IL-12[17]. We and others have shown that DCs generated in the presence of dexamethasone fulfil this requirement[23,24], but for the first time we have identified that these cells maintained, and had elevated transcriptional levels of *IL27B* and *IL12A*, together making the potent regulatory cytokine IL-35 (**chapter 2**). Notably, it appeared that this cytokine was not constitutively produced by tolDCs, but was inducible upon challenge with TLR agonists, CD40L and most strikingly, IFNγ. Considering that Ebi3 is part of IL-27 and IL-35 and that the tolDC we generated were completely devoid of IL-12p40, we silenced IL-12p35 as a means of silencing IL-35 and found that tolDCs required IL-35 to elicit their full tolerogenic potential. This opens up an exciting opportunity to study the expression of IL-35 in other regulatory APCs including type II anti-inflammatory macrophages and myeloid derived suppressor cells. Although limited to murine data it has been shown that intracellular Ebi3 expression can block LPS induced

M1 to M2 transition and IL-12p70 production[25]. We have also observed elevated basal Ebi3 levels in M-CSF generated human M2 compared to GM-CSF generated M1 macrophages (Fig.1, unpublished data), although this difference normalised upon activation. Therefore, it would be interesting to extrapolate on the possible role of Ebi3 in maintaining the regulatory functions of M2 macrophages.

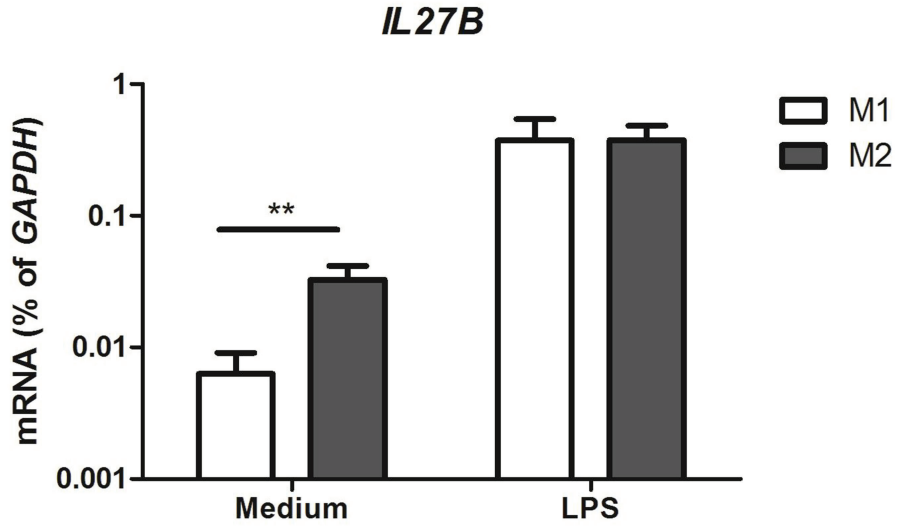

Figure 1: *IL27B* expression in M1 and M2 macrophages.

Advances in our understanding of the role of cytokines in inflammatory diseases has led to a growing field of cytokine driven therapies aimed to either block or restore the activity of a particular cytokine. We have seen through the use of Anakinra[26,27], Etanercept[28,29] and recombinant IFNα[30] and IFNβ[31,32] that cytokine based therapies have proven successful. However, one of the major pitfalls with cytokine therapy, and what has laboured progress, is that in healthy individuals there is equilibrium between inflammatory and regulatory cytokines. This balance becomes disturbed in chronic inflammatory states, such as autoimmunity and transplant rejection. However the required action to restore balance is not to just simply block inflammatory cytokines or treat systemically with anti-inflammatory cytokines. For the latter it is difficult to determine how much and what cocktail of anti-inflammatory cytokines to administer. Additionally, giving too much could render an individual susceptible to viral and bacterial infection and may even promote the development of tumour growth.

Ideally one would not want systemic suppression, but rather specific regulation directed against the offending antigen at the level of the affected organ or tissue. In this way tolDCs would be the ideal vehicle, not only have they the ability to directly suppress and induce antigen specific regulatory T cells, but the inducible

production of regulatory cytokines e.g. IL-10, IL-35, would infer tolDCs with a second wave of immunosuppressive potential e.g. through the action of regulatory T cells. IL-35 has already been proven to be a broad regulatory cytokine with the ability to promote tolerance and immune suppression in an array of disease models (Table I).

Table I: In-vivo studies evaluating the effect of IL-35 induction in disease settings

Disease Model	Mechanism of IL-35	Effect on disease progression	Reference
Autoimmune diabetes	Ectopic expression of rIL-35 in non-obese diabetic (NOD) RIP-IL35 transgenic mice.	IL-35 expression protected animals from AID by a decrease in islet infiltration and reduction in the number of $CD4^+$ and $CD8^+$ T cells.	Bettini et al.[33]
Collagen-induced arthritis (CIA)	1) Intraperitoneal injection of single-chain rIL-35 2) Intraperitoneal injection of rIL-35	1) IL-35 stimulation of CD39+ regulatory T cells reduced intensity and progression of CIA via the production of IL-10 2) IL-35 suppressed the proliferation of $CD4^+$ $CD25^-$ effector cells. Moreover, IL-35 inhibited the differentiation of Th17 cells in vitro thereby significantly reducing severity of CIA.	Kochetkova et al.[34] Niedbala et al.[35]
Inflammatory bowel disease (IBD)	1) Adoptive transfer of iTr35 cells into IBD-affected $Rag1^{-/-}$ mice 2) Gene therapy using plasmid DNA encoding single-chain IL-35 fusion protein	1) Transfer of iTr35 cells cured IBD 2) IL-35 gene therapy decreased symptoms of colitis and decreased colonic inflammatory markers	Collison et al.[36] Wirtz et al.[37]
Cancer	1) Ectopic expression of IL-35 in tumour cell lines 2) Adoptive transfer of iTr35 cells into tumour-bearing $Rag1^{-/-}$ mice	1) IL-35 expression increased infiltration of MDSCs and decreased the numbers and effector functions of $CD4^+$ and $CD8^+$TIL 2) iTr35 cells suppressed tumour immunity following adoptive transfer of $CD4^+$ and $CD8^+$ T cells	Olson et al.[38] Collison et al. [36]

Although some studies have directly utilised rIL-35[35] or induced local ectopic expression[33], successful suppression has also been obtained by transferring iTr35 cells[36]. As of yet, no data has been generated using tolDCs expressing IL-35, however Ebi3 expression in tolerised DCs has been implicated as a key mediator of allograft acceptance[39]. In this study the authors could not identify expression of either IL-27p28 or IL-12p35 but considering the powerful regulatory potential of IL-35, it would be of significant interest to investigate the role of IL-35 expressing

tolDCs in such disease settings.

7.3 Role of apoptotic cell clearance in the regulation of immune responses.

When one considers DC activation, the first idea that comes to mind is activation in response to invading pathogens and viruses. The general conjecture is that under steady state conditions, DCs are quiet sentinels, and that introduction of microbial products is required to evoke a response. However this simplified theory does not fully address the role and function of DCs under normal physiological conditions. In the body, cell death and turnover is an inevitable process and is essential to preserve normal homeostasis throughout all our tissues.

Extensive literature has shown that apoptosis is a controlled physiologic process resulting in the generation of "eat me" signals designed to promote efficient uptake of apoptotic cells by phagocytes during the early stage of cell death, thereby preventing the release of potentially immunogenic intracellular contents[40]. This so termed `silent removal` of apoptotic cells is well acknowledged, however there is an increasing body of evidence that apoptotic cell clearance is not just an immunologically inert event[41-43], and that active processes must be taking place in order to preserve immune tolerance to self and innocuous antigens[44-47]. In line with this, some studies have observed that uptake of apoptotic cells of different origins to the ingesting APC (i.e neutrophils, T cells etc) can inhibit up-regulation of co-stimulatory molecules on APCs upon subsequent LPS challenge[48]. In addition, murine studies have suggested that uptake of apoptotic cells, by DCs leads to up-regulation of TGF-β[49]. Until recently the only member of the IL-12 family to be investigated upon apoptotic cell uptake was IL-12. There have been conflicting reports about the regulation of IL-12 upon apoptotic cell ingestion however the greater body of literature points to decreased IL-12 production upon ingestion of apoptotic material. We explored the regulation of IL-12 compared to IL-27 and IL-35 and demonstrated (**chapter 3**) that uptake of apoptotic cells by viable DC, in the absence of any exogenous stimuli, led to enhanced transcription of *IL27B* and *IL12A*.

As we have mentioned, Ebi3 can also dimerise with IL-27p28 to yield IL-27, and an interesting study demonstrated that uptake of apoptotic tumour cells by DCs led to *IL27B* upregulation and IL-27 production[50]. While we did not observe upregulation of *IL27A* in our study it is intriguing that Ebi3 was up-regulated in both studies, and notably remained unaffected by necrotic cell uptake in our study. An additional hypothesis is that uptake of DCs by DCs[51,52] is the key determinant for the profound regulatory changes we observed (**chapter 3**). Work by Martin and colleagues has generated some interesting findings that cells undergoing apoptosis are capable of producing cytokines and chemokines which

they suggest influences the response of the ingesting phagocyte[53]. The cytokine profile of apoptotic cells of diverse lineages has not yet been fully elucidated. However, one could imagine that DCs would be particularly adept at secreting potent immune mediators that may specifically tailor the immune response in an immunogenic or tolerogenic fashion.
It would be of interest to fully investigate the mechanism of Ebi3 upregulation in our experimental setup and to additionally dissect whether pre-stimulated apoptotic cells would infer an even better regulatory response. Of course this will likely depend on the stimulus used but, based on our findings throughout the thesis, IFNγ could be an interesting target.

7.4 IFNγ and immune regulation.

IFNγ is a remarkable cytokine that orchestrates many distinct and complex networks through its transcriptional control over large numbers of genes[54]. Initially termed macrophage activating factor (MAF), IFNγ is more often expected to be an inflammatory molecule. However, in recent years IFNγ has proven to exert many regulatory functions, including the potent upregulation of B7H1[55] and IDO[56]. Though paradoxical to many, in a study mentioned earlier, Cuturi and colleagues demonstrated up to 3 fold higher levels of IFNγ in allograft tolerised rats[39]. In this study the authors investigated the use of autologous DCs as a tolerance inducing strategy and found a novel role for Ebi3 in transplant tolerance, whereby blockade of Ebi3 resulted in allograft rejection. Notably blockade of Ebi3 also resulted in ablation of IFNγ production, indicating a surprising link between these two molecules in the induction of allograft tolerance, which is noteworthy considering our findings with IFNγ induction of Ebi3 in tolDCs (**chapter 2**). In fact more and more evidence is gathering that IFNγ is an important mediator of tolerance induction in both cell and organ transplantation, and in autoimmunity. The immuno-modulatory activity of MSCs has been shown to be significantly augmented when the cells were first primed with IFNγ[57]. This presumably depends on the duration and concentration of IFNγ. Based on our findings (**chapter 2**) IFNγ treatment would also seem like a potentially useful conditioning strategy for tolDCs. We demonstrated that tolDCs expressed high levels of the IFNγ R and had higher basal levels of total STAT1 (**chapter 4**). Additionally it has been determined that the promoter region of Ebi3 has a high number of binding sites for IRF-1[58]. Together this resulted in a strong and sustained response upon IFNγ treatment and yielded many regulatory features including upregulation of co-inhibitory molecules B7H1 and B7DC and regulatory IL-12 family members IL-12p35 and Ebi3 (**chapter 2**).
We demonstrated an additional novel regulatory role for IFNγ in complement

regulation (**chapter 4**). fH and fP are at opposite poles in terms of complement regulation with fH being an important negative regulator and fP being the only know positive regulator within the entire system. Surprisingly, IFNγ played a dual role, with the ability to markedly reduce fP secretion while simultaneously increasing fH. As such IFNγ appears to support complement regulation as opposed to activation. The combined efforts of DCs and complement in T cell activation has recently gained interest with the studies by Heeger and colleagues which demonstrated that DCs and T cells secrete complement proteins, express C3aR, C5aR and complement regulators on their surfaces[59,60]. The authors further demonstrated that locally produced C3a and C5a influences the strength and phenotype of T cell responses. In our work we have shown that DCs produce both fP and fH and thereby also possess the tools to potentially regulate the local complement activity when encountering T cells. Using RNA interference we found that inhibition of fP diminished the allostimulatory capacity of DCs. A combination of siRNA directed against fP and IFNγ stimulation, acting as a natural inhibitor of fP, resulted in an even more pronounced reduction in allostimulatory capabilities. Conversely interference of fH led to enhanced T cell activation by DCs thereby demonstrating that the active production of complement regulators by DCs does influence T cell activation and cytokine production. This has the potential to open up many exciting avenues of research. Information regarding the role of local complement synthesis by DCs in determining T helper cell skewing is virtually unknown. One could speculate that management of the two key regulators of complement activity by IFNγ could serve as a negative feedback loop to control cellular immune activation. In view of the established role of complement activation in lowering the threshold of B cell activation one may also question the contribution of local complement activity, or its absence, in lowering or elevating the threshold of naive T cell activation by DCs. Considering the many functions of IFNγ in DC activation it will prove difficult to address these specific questions in-vitro, but use of IFNγ$^{-/-}$ and IFNγ R$^{-/-}$ mice and BMDCs could prove useful.

7.5 Involvement of local tissues in DC regulation.

As mentioned earlier it is not yet fully clear whether regulatory DCs in vivo represent a terminally differentiated DC phenotype, or reflect a transient state subject to changes within distinct tissue microenvironments. There is an increasing body of evidence to strongly support the latter hypothesis whereby several studies focusing on stromal or mesenchymal stem cells[61-63] derived from various tissues, have been shown to modulate DC function. DC differentiation in the presence of tissue-dependent microenvironment leads to a more regulatory

cell population with lower expression of co-stimulatory molecules and poor induction of T cell proliferation. This process has been described for several organs[64,65] including the bone marrow, lung[66], thymus and intestine[67]. More recently the presence of kidney derived MSC like cells has been identified in mouse[68]. It has been suggested that renal fibroblasts may be a stromal lineage of kidney MSCs, thereby warranting further investigation into their immunological contribution. We demonstrated (**chapter 6**) that culture of monocyte derived DCs in the presence of renal fibroblasts led to the generation of a more regulatory DC population with diminished co-stimulatory molecules and enhanced expression of co-inhibitory molecules B7H1 and B7DC. Notably IL-12 was markedly reduced in this cell population while IL-23 and IL-27 remained largely unaffected. Together this resulted in a DC population with a significantly impaired ability to induce IFNγ production in allostimulated T cells. Although the ontogeny of renal DCs remains unknown, it is likely that under steady state conditions renal DCs do not arise from monocytes[69,70]. However during periods of stress or injury it is widely thought that monocytes function as tissue infiltrating DCs[3,4,71-73], thereby calling for a deeper understanding of how the local tissue network can influence and modulate the development of DCs. In line with some studies investigating the mechanisms of stromal cells and DC regulation, we found that IL-6 was at least partially responsible for the modulating effect of renal fibroblasts on DC generation and subsequent function. The cytokine IL-6 is highly pleiotropic possessing both pro[74,75] and anti-inflammatory[76,77] properties. Only recently a possible explanation for these discrepancies has come to light. All IL-6 family members signal via the beta receptor gp130. In addition some members also need to bind to a non-signalling alpha receptor in order to facilitate signal transduction. In the case of IL-6 this alpha receptor is IL-6R. While gp130 is ubiquitously expressed on most cells in the body, expression of the IL-6R is more finite. Cells which express both IL-6R and gp130 respond to IL-6 via "classic signalling". In contrast cells which do not typically express the IL-6R may still respond to IL-6 by engaging with a soluble IL-6 receptor and function via "trans-signalling"[78,79]. It is thought that responsiveness to IL-6 via "classic" signalling exerts anti-inflammatory functions whereas "trans-signalling" exerts pro-inflammatory functions. Still there are some caveats with this theory as addition of exogenous IL-6R has been shown to further enhance the regulatory potential of dermal fibroblast derived IL-6 on human monocyte-derived DCs[62].

We investigated how healthy renal fibroblasts regulate DC generation. One could question what happens in organs undergoing chronic inflammation. Considering the central location of fibroblasts within the kidney it is reasonable to postulate that these cells themselves are functionally affected in periods of inflammation or upon injury. This is likely to impair their ability to regulate local and infiltrating

DC populations, thereby potentially exacerbating the inflammatory response. Little is known about the expression and responsiveness of renal fibroblast to DAMPs but research into this field could prove invaluable in fully understanding local DC responses. With a view to expand our knowledge on the immunological contribution of the kidney we investigated the expression of IL-12 family members within normal human kidney (**chapter 5**). Unlike other subunits of the IL-12 family whose expression is largely restricted to haematopoietic cells, Ebi3 expression has been demonstrated in placental synctiotrophoblasts[80], endothelial cells, intestinal mucosa[81,82] and aortic smooth muscle cells[83]. We observed a surprisingly abundant expression of Ebi3 within the glomeruli of normal kidney, and in distal tubuli. We could confirm the expression of *IL27B* transcripts within the glomeruli, but further work will be needed to establish whether the tubular cells are actively producing Ebi3 or are possibly endocytosing the protein from the lumen. We provided strong indications that podocytes were the source of Ebi3 in the kidney and observed a marked reduction in *IL27B* expression during acute allograft rejection. Ebi3 is known to have two isoforms and aside from IL-27p28 and IL-12p35, Ebi3 can interact with Calnexin[84], Golgi SNAP receptor complex member 1[85], MyoD family inhibitor, and SMAD family member 3[86]. It remains to be seen whether Ebi3 expressed within human kidney is functioning as a cytokine, or if it is remaining as an intracellular molecule and interacting with the aforementioned proteins. Additionally, the possibility that Ebi3 is functioning as a homodimer cannot be excluded. Nevertheless is it intriguing that Ebi3 expression was so abundant in human kidney and further investigation into the role of this molecule may shed light on previously unidentified regulatory pathways within the kidney.

7.6 Looking ahead...

As we look towards the future of immune regulation and tolerance, it is imperative to focus not only on identifying novel mediators of tolerance, but also how these regulatory cell populations can be identified. This is particularly relevant with the generation of tolerogenic or regulatory DCs, as the plastic nature of DCs makes them challenging to identify and track over time. Even in vitro generated tolDCs, which we and others have shown to be a highly stable regulatory cell population, would undoubtedly prove challenging to identify once injected in vivo. If we want to try to identify populations of endogenous regulatory DCs expressing IL-35 in vivo then a daunting task lies ahead. As we have demonstrated in this thesis, the extensive chain sharing within the IL-12 family means that one must assess all 5 chains of the family before beginning to interpret any data. The use of knockout mice for studies in this area are limited as mice deficient in one

chain can obviously lack multiple cytokines. As we have already seen in the early data regarding IL-12 and IL-23[87-89], this complicates data interpretation and often leads to false conclusions.

Unfortunately IL-35, unlike IL-23, does not express a unique chain so the generation of knockout mice specific for IL-35 will be challenging. Perhaps the most feasible way will be through the generation of specific neutralising antibodies that target a unique epitope only found in the Ebi3: IL-12p35 heterodimer. These antibodies would also prove invaluable for in-vitro assays, as currently one must combine anti-Ebi3 and anti IL-12p35 to try and block IL-35. This works relatively well for T cell populations[36,90], however considering DCs express all chains this method is not useful for investigating the presence and function of IL-35 in DC populations and further complicates an already complex relationship that exists between the opposing roles of IL-12 and IL-35. In our experience there is also a degree of difficulty in generating rhu-IL-35 that is functionally active in terms of T cell suppression and iTr35 induction. In time these reagents will surely be more robustly developed which will open up many avenues in IL-35 research.

In light of the function of IL-35 in the generation of a unique inducible regulatory T cell population, it is possible that IL-35 may also generate a regulatory DC population, as DCs do express relatively abundant transcript levels of the IL-35R (Fig. 2). IL-35 is promiscuous in that it can exert its activity via three different receptor combinations, thus potentially facilitating IL-35 with a wide berth of activity. As mentioned earlier in this thesis, IL-27 can up regulate the expression of IL-12Rβ2 and thereby sensitise naive T cells not only to IL-12 but also IL-35. It may well be that some of suppressive action of IL-27 is in fact mediated by the priming of iTr35 cells, though this remains to be proven experimentally.

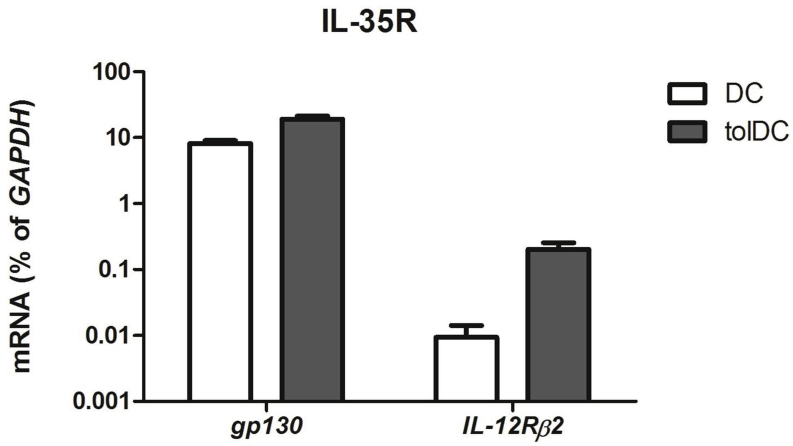

Figure 2: IL-35 receptor expression on DC and tolDC

With all of this in mind it makes the finding of the abundant expression of Ebi3 in the kidney very intriguing. This is one area where Ebi3 knockout mice could prove very useful. Expression of *IL27B* or Ebi3 protein has not been previously explored in murine kidney but if it is indeed expressed, then Ebi3$^{-/-}$ mice would be a useful tool to fully investigate the role of Ebi3 in renal immunology. During periods of inflammation, one could wonder if local IL-35 production helps to maintain a regulatory environment for resident DCs. Although cytokine mRNA transcripts are typically transient it would also be interesting to utilise in-situ hybridisation to explore the expression of IL-12 family transcripts within interstitial DCs of the kidney to evaluate if these cells may be primed to produce the regulatory cytokines IL-27 or IL-35. Concerning the IL-12 family as a whole, many fundamental questions remain unanswered. For example what dictates the heterodimerisation of IL-12p35 with IL-12p40 in some conditions, and IL-12p35 with Ebi3 in others? This competition extends beyond the chains themselves as the receptor subunits are also shared between cytokines. For example IL-12 and IL-23 both utilise IL-12Rβ1.Can one cytokine out-compete the other? Or if IL-23 is bound by its receptor does this affect availability of IL-12Rβ1 for IL-12 and thus reduce the potency of IL-12?

It is clear that the IL-12 family and DCs together provide a fertile ground for future research but more importantly together have the potential to expand our understanding of regulatory cytokines beyond IL-10 and TGF-β and enable us to get a wider and deeper look into the regulation of immunity and immunological tolerance.

References

1. Banchereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. Nature 392: 245-52
2. Reis e Sousa C. 2006. Dendritic cells in a mature age. Nat Rev Immunol 6: 476-83
3. Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP, Jeffrey KL, Anthony RM, Kluger C, Nchinda G, Koh H, Rodriguez A, Idoyaga J, Pack M, Velinzon K, Park CG, Steinman RM. 2010. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. Cell 143: 416-29
4. Segura E, Touzot M, Bohineust A, Cappuccio A, Chiocchia G, Hosmalin A, Dalod M, Soumelis V, Amigorena S. 2013. Human inflammatory dendritic cells induce Th17 cell differentiation. Immunity 38: 336-48
5. Quaratino S, Duddy LP, Londei M. 2000. Fully competent dendritic cells as inducers of T cell anergy in autoimmunity. Proc Natl Acad Sci U S A 97: 10911-6
6. Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, Steinman RM. 2003. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J Exp Med 198: 235-47
7. Matzinger P. 2007. Friendly and dangerous signals: is the tissue in control? Nat Immunol 8: 11-3
8. Matzinger P, Kamala T. 2011. Tissue-based class control: the other side of tolerance. Nat Rev Immunol 11: 221-30
9. Coates PT, Duncan FJ, Colvin BL, Wang Z, Zahorchak AF, Shufesky WJ, Morelli AE, Thomson AW. 2004. In vivo-mobilized kidney dendritic cells are functionally immature, subvert alloreactive T-cell responses, and prolong organ allograft survival. Transplantation 77: 1080-9
10. Rock J, Schneider E, Grun JR, Grutzkau A, Kuppers R, Schmitz J, Winkels G. 2007. CD303 (BDCA-2) signals in plasmacytoid dendritic cells via a BCR-like signalosome involving Syk, Slp65 and PLCgamma2. Eur J Immunol 37: 3564-75
11. Bauer M, Redecke V, Ellwart JW, Scherer B, Kremer JP, Wagner H, Lipford GB. 2001. Bacterial CpG-DNA triggers activation and maturation of human CD11c-, CD123+ dendritic cells. J Immunol 166: 5000-7
12. Pacanowski J, Kahi S, Baillet M, Lebon P, Deveau C, Goujard C, Meyer L, Oksenhendler E, Sinet M, Hosmalin A. 2001. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. Blood 98: 3016-21
13. Steinman RM. 2007. Dendritic cells: understanding immunogenicity. Eur J Immunol 37 Suppl 1: S53-60

14. Adorini L. 2003. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmune diabetes. Ann N Y Acad Sci 987: 258-61
15. Stoop JN, Harry RA, von Delwig A, Isaacs JD, Robinson JH, Hilkens CM. 2010. Therapeutic effect of tolerogenic dendritic cells in established collagen-induced arthritis is associated with a reduction in Th17 responses. Arthritis Rheum 62: 3656-65
16. Woltman AM, van Kooten C. 2003. Functional modulation of dendritic cells to suppress adaptive immune responses. Journal of Leukocyte Biology 73: 428-41
17. Morelli AE, Thomson AW. 2007. Tolerogenic dendritic cells and the quest for transplant tolerance. Nat Rev Immunol 7: 610-21
18. Vignali DA, Kuchroo VK. 2012. IL-12 family cytokines: immunological playmakers. Nat Immunol 13: 722-8
19. Collison LW, Vignali DA. 2008. Interleukin-35: odd one out or part of the family? Immunol Rev 226: 248-62
20. Diveu C, McGeachy MJ, Boniface K, Stumhofer JS, Sathe M, Joyce-Shaikh B, Chen Y, Tato CM, McClanahan TK, de Waal Malefyt R, Hunter CA, Cua DJ, Kastelein RA. 2009. IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells. J Immunol 182: 5748-56
21. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, Wu C, Kleinewietfeld M, Kunder S, Hafler DA, Sobel RA, Regev A, Kuchroo VK. 2012. Induction and molecular signature of pathogenic TH17 cells. Nat Immunol 13: 991-9
22. Mascanfroni ID, Yeste A, Vieira SM, Burns EJ, Patel B, Sloma I, Wu Y, Mayo L, Ben-Hamo R, Efroni S, Kuchroo VK, Robson SC, Quintana FJ. 2013. IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39. Nat Immunol 14: 1054-63
23. Woltman AM, Fijter JWd, Kamerling SWA, C. Paul L, Daha MR, Kooten Cv. 2000. The effect of calcineurin inhibitors and corticosteroids on the differentiation of human dendritic cells. European Journal of Immunology 30: 1807-12
24. Torres-Aguilar H, Aguilar-Ruiz SR, Gonzalez-Perez G, Munguia R, Bajana S, Meraz-Rios MA, Sanchez-Torres C. 2010. Tolerogenic dendritic cells generated with different immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory CD4+ T cells. J Immunol 184: 1765-75
25. Zheng XF, Hong YX, Feng GJ, Zhang GF, Rogers H, Lewis MA, Williams DW, Xia ZF, Song B, Wei XQ. 2013. Lipopolysaccharide-induced M2 to M1 macrophage transformation for IL-12p70 production is blocked by Candida albicans mediated up-regulation of EBI3 expression. PLoS One 8: e63967
26. Dinarello CA. 2011. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 117: 3720-32
27. Dinarello CA. 2011. Blocking interleukin-1beta in acute and chronic autoinflammatory diseases. J Intern Med 269: 16-28
28. Haraoui B, Bykerk V. 2007. Etanercept in the treatment of rheumatoid arthritis. Ther Clin Risk Manag 3: 99-105
29. Mease PJ, Kivitz AJ, Burch FX, Siegel EL, Cohen SB, Ory P, Salonen D, Rubenstein J, Sharp JT, Tsuji W. 2004. Etanercept treatment of psoriatic arthritis: safety, efficacy, and effect on disease progression. Arthritis Rheum 50: 2264-72
30. Kamal SM, Ismail A, Graham CS, He Q, Rasenack JW, Peters T, Tawil AA, Fehr JJ, Khalifa Kel S, Madwar MM, Koziel MJ. 2004. Pegylated interferon alpha therapy in acute hepatitis C: relation to hepatitis C virus-specific T cell response kinetics. Hepatology 39: 1721-31
31. Niemela J, Ifergan I, Yegutkin GG, Jalkanen S, Prat A, Airas L. 2008. IFN-beta regulates CD73 and adenosine expression at the blood-brain barrier. Eur J Immunol 38: 2718-26
32. Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, Hartung HP, Havrdova E, Selmaj KW, Weiner HL, Fisher E, Brinar VV, Giovannoni G, Stojanovic M, Ertik BI, Lake SL, Margolin DH, Panzara MA, Compston DA, investigators C-MI. 2012. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. Lancet 380: 1819-28
33. Bettini M, Castellaw AH, Lennon GP, Burton AR, Vignali DA. 2012. Prevention of autoimmune diabetes by ectopic pancreatic beta-cell expression of interleukin-35. Diabetes 61: 1519-26
34. Kochetkova I, Golden S, Holderness K, Callis G, Pascual DW. 2010. IL-35 stimulation of CD39+ regulatory T cells confers protection against collagen II-induced arthritis via the production of IL-10. J Immunol 184: 7144-53
35. Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, Liew FY. 2007. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol 37: 3021-9
36. Collison LW, Chaturvedi V, Henderson AL, Giacomin PR, Guy C, Bankoti J, Finkelstein D, Forbes K, Workman CJ, Brown SA, Rehg JE, Jones ML, Ni HT, Artis D, Turk MJ, Vignali DA. 2010. IL-35-mediated induction of a potent regulatory T cell population. Nat Immunol 11: 1093-101
37. Wirtz S, Billmeier U, McHedlidze T, Blumberg RS, Neurath MF. 2011. Interleukin-35 mediates mucosal immune responses that protect against T-cell-dependent colitis. Gastroenterology 141: 1875-86
38. Olson BM, Jankowska-Gan E, Becker JT, Vignali DA, Burlingham WJ, McNeel DG. 2012. Human prostate tumor antigen-specific CD8+ regulatory T cells are inhibited by CTLA-4 or IL-35 blockade. J Immunol 189: 5590-601
39. Hill M, Thebault P, Segovia M, Louvet C, Beriou G, Tilly G, Merieau E, Anegon I, Chiffoleau E, Cuturi MC. 2011. Cell therapy with autologous tolerogenic dendritic cells induces allograft tolerance through interferon-gamma and epstein-barr virus-induced gene 3. Am J Transplant 11: 2036-45
40. Green DR, Ferguson T, Zitvogel L, Kroemer G. 2009. Immunogenic and tolerogenic cell death. Nat Rev Immunol 9: 353-63
41. Ravishankar B, Liu H, Shinde R, Chandler P, Baban B, Tanaka M, Munn DH, Mellor AL, Karlsson MC, McGaha TL. 2012. Tolerance to apoptotic cells is regulated by indoleamine 2,3-dioxygenase. Proc Natl Acad Sci U S A 109: 3909-14
42. Getts DR, Turley DM, Smith CE, Harp CT, McCarthy D, Feeney EM, Getts MT, Martin AJ, Luo X, Terry RL, King NJ, Miller SD. 2011. Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1+ and IL-10-producing splenic macrophages and maintained by T regulatory cells. J Immunol 187: 2405-17
43. Wang Z, Shufesky WJ, Montecalvo A, Divito SJ, Larregina AT, Morelli AE. 2009. In situ-targeting of dendritic cells with donor-derived apoptotic cells restrains indirect allorecognition and ameliorates allograft vasculopathy. PLoS One 4: e4940
44. Steinman RM, Turley S, Mellman I, Inaba K. 2000. The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 191: 411-6

45. Johansson U, Walther-Jallow L, Hofmann A, Spetz AL. 2011. Dendritic cells are able to produce IL-12p70 after uptake of apoptotic cells. Immunobiology 216: 251-5
46. Martin SJ, Henry CM, Cullen SP. 2012. A perspective on mammalian caspases as positive and negative regulators of inflammation. Mol Cell 46: 387-97
47. Gleisner MA, Rosemblatt M, Fierro JA, Bono MR. 2011. Delivery of alloantigens via apoptotic cells generates dendritic cells with an immature tolerogenic phenotype. Transplant Proc 43: 2325-33
48. Stuart LM, Lucas M, Simpson C, Lamb J, Savill J, Lacy-Hulbert A. 2002. Inhibitory effects of apoptotic cell ingestion upon endotoxin-driven myeloid dendritic cell maturation. J Immunol 168: 1627-35
49. Kushwah R, Wu J, Oliver JR, Jiang G, Zhang J, Siminovitch KA, Hu J. 2010. Uptake of apoptotic DC converts immature DC into tolerogenic DC that induce differentiation of Foxp3+ Treg. Eur J Immunol 40: 1022-35
50. Sekar D, Hahn C, Brune B, Roberts E, Weigert A. 2012. Apoptotic tumor cells induce IL-27 release from human DCs to activate Treg cells that express CD69 and attenuate cytotoxicity. Eur J Immunol 42: 1585-98
51. Kushwah R, Hu J. 2010. Dendritic cell apoptosis: regulation of tolerance versus immunity. J Immunol 185: 795-802
52. Chen M, Wang YH, Wang Y, Huang L, Sandoval H, Liu YJ, Wang J. 2006. Dendritic cell apoptosis in the maintenance of immune tolerance. Science 311: 1160-4
53. Cullen SP, Henry CM, Kearney CJ, Logue SE, Feoktistova M, Tynan GA, Lavelle EC, Leverkus M, Martin SJ. 2013. Fas/CD95-induced chemokines can serve as "find-me" signals for apoptotic cells. Mol Cell 49: 1034-48
54. Schroder K, Hertzog PJ, Ravasi T, Hume DA. 2004. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 75: 163-89
55. Lee SJ, Jang BC, Lee SW, Yang YI, Suh SI, Park YM, Oh S, Shin JG, Yao S, Chen L, Choi IH. 2006. Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274). FEBS Lett 580: 755-62
56. Taylor MW, Feng GS. 1991. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 5: 2516-22
57. Sheng H, Wang Y, Jin Y, Zhang Q, Zhang Y, Wang L, Shen B, Yin S, Liu W, Cui L, Li N. 2008. A critical role of IFNgamma in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1. Cell Res 18: 846-57
58. Li X, Mai J, Virtue A, Yin Y, Gong R, Sha X, Gutchigian S, Frisch A, Hodge I, Jiang X, Wang H, Yang XF. 2012. IL-35 is a novel responsive anti-inflammatory cytokine--a new system of categorizing anti-inflammatory cytokines. PLoS One 7: e33628
59. Heeger PS, Lalli PN, Lin F, Valujskikh A, Liu J, Muqim N, Xu Y, Medof ME. 2005. Decay-accelerating factor modulates induction of T cell immunity. J Exp Med 201: 1523-30
60. Strainic MG, Liu J, Huang D, An F, Lalli PN, Muqim N, Shapiro VS, Dubyak GR, Heeger PS, Medof ME. 2008. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. Immunity 28: 425-35
61. Chiesa S, Morbelli S, Morando S, Massollo M, Marini C, Bertoni A, Frassoni F, Bartolomé ST, Sambuceti G, Traggiai E, Uccelli A. 2011. Mesenchymal stem cells impair in vivo T-cell priming by dendritic cells. Proceedings of the National Academy of Sciences 108: 17384-9
62. Chomarat P, Banchereau J, Davoust J, Palucka AK. 2000. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol 1: 510-4
63. Melief SM, Schrama E, Brugman MH, Tiemessen MM, Hoogduijn MJ, Fibbe WE, Roelofs H. 2013. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. Stem Cells 31: 1980-91
64. Lech M, Grobmayr R, Weidenbusch M, Anders HJ. 2012. Tissues use resident dendritic cells and macrophages to maintain homeostasis and to regain homeostasis upon tissue injury: the immunoregulatory role of changing tissue environments. Mediators Inflamm 2012: 951390
65. Owens BM, Kaye PM. 2012. Stromal cell induction of regulatory dendritic cells. Front Immunol 3: 262
66. Li Q, Guo Z, Xu X, Xia S, Cao X. 2008. Pulmonary stromal cells induce the generation of regulatory DC attenuating T-cell-mediated lung inflammation. European Journal of Immunology 38: 2751-61
67. Owens BM, Simmons A. 2013. Intestinal stromal cells in mucosal immunity and homeostasis. Mucosal Immunol 6: 224-34
68. Huang Y, Chen P, Zhang CB, Ko GJ, Ruiz M, Fiorina P, Hussain MA, Wasowska BA, Rabb H, Womer KL. 2010. Kidney-derived mesenchymal stromal cells modulate dendritic cell function to suppress alloimmune responses and delay allograft rejection. Transplantation 90: 1307-11
69. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, Becker CD, See P, Price J, Lucas D, Greter M, Mortha A, Boyer SW, Forsberg EC, Tanaka M, van Rooijen N, Garcia-Sastre A, Stanley ER, Ginhoux F, Frenette PS, Merad M. 2013. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 38: 792-804
70. Merad M, Sathe P, Helft J, Miller J, Mortha A. 2013. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu Rev Immunol 31: 563-604
71. Ifergan I, Kebir H, Bernard M, Wosik K, Dodelet-Devillers A, Cayrol R, Arbour N, Prat A. 2008. The blood-brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. Brain 131: 785-99
72. Siddiqui KR, Laffont S, Powrie F. 2010. E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis. Immunity 32: 557-67
73. Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. 1999. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. Immunity 11: 753-61
74. Nowell MA, Richards PJ, Horiuchi S, Yamamoto N, Rose-John S, Topley N, Williams AS, Jones SA. 2003. Soluble IL-6 receptor governs IL-6 activity in experimental arthritis: blockade of arthritis severity by soluble glycoprotein 130. J Immunol 171: 3202-9
75. Nowell MA, Williams AS, Carty SA, Scheller J, Hayes AJ, Jones GW, Richards PJ, Slinn S, Ernst M, Jenkins BJ, Topley

N, Rose-John S, Jones SA. 2009. Therapeutic targeting of IL-6 trans signaling counteracts STAT3 control of experimental inflammatory arthritis. J Immunol 182: 613-22
76. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. 2009. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 15: 103-13
77. Park SJ, Nakagawa T, Kitamura H, Atsumi T, Kamon H, Sawa S, Kamimura D, Ueda N, Iwakura Y, Ishihara K, Murakami M, Hirano T. 2004. IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation. J Immunol 173: 3844-54
78. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1813: 878-88
79. Garbers C, Hermanns HM, Schaper F, Muller-Newen G, Grotzinger J, Rose-John S, Scheller J. 2012. Plasticity and cross-talk of interleukin 6-type cytokines. Cytokine Growth Factor Rev 23: 85-97
80. Coulomb-L'Hermine A, Larousserie F, Pflanz S, Bardel E, Kastelein RA, Devergne O. 2007. Expression of interleukin-27 by human trophoblast cells. Placenta 28: 1133-40
81. Maaser C, Egan LJ, Birkenbach MP, Eckmann L, Kagnoff MF. 2004. Expression of Epstein-Barr virus-induced gene 3 and other interleukin-12-related molecules by human intestinal epithelium. Immunology 112: 437-45
82. Gehlert T, Devergne O, Niedobitek G. 2004. Epstein-Barr virus (EBV) infection and expression of the interleukin-12 family member EBV-induced gene 3 (EBI3) in chronic inflammatory bowel disease. J Med Virol 73: 432-8
83. Kempe S, Heinz P, Kokai E, Devergne O, Marx N, Wirth T. 2009. Epstein-barr virus-induced gene-3 is expressed in human atheroma plaques. Am J Pathol 175: 440-7
84. Devergne O, Hummel M, Koeppen H, Le Beau MM, Nathanson EC, Kieff E, Birkenbach M. 1996. A novel interleukin-12 p40-related protein induced by latent Epstein-Barr virus infection in B lymphocytes. J Virol 70: 1143-53
85. Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, Hibbert L, Churakova T, Travis M, Vaisberg E, Blumenschein WM, Mattson JD, Wagner JL, To W, Zurawski S, McClanahan TK, Gorman DM, Bazan JF, de Waal Malefyt R, Rennick D, Kastelein RA. 2002. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. Immunity 16: 779-90
86. Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N, Klitgord N, Simon C, Boxem M, Milstein S, Rosenberg J, Goldberg DS, Zhang LV, Wong SL, Franklin G, Li S, Albala JS, Lim J, Fraughton C, Llamosas E, Cevik S, Bex C, Lamesch P, Sikorski RS, Vandenhaute J, Zoghbi HY, Smolyar A, Bosak S, Sequerra R, Doucette-Stamm L, Cusick ME, Hill DE, Roth FP, Vidal M. 2005. Towards a proteome-scale map of the human protein-protein interaction network. Nature 437: 1173-8
87. Becher B, Durell BG, Noelle RJ. 2002. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. J Clin Invest 110: 493-7
88. Gran B, Zhang GX, Yu S, Li J, Chen XH, Ventura ES, Kamoun M, Rostami A. 2002. IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. J Immunol 169: 7104-10
89. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD. 2003. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 421: 744-8
90. Collison LW, Pillai MR, Chaturvedi V, Vignali DA. 2009. Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner. J Immunol 182: 6121-8