Chapter 1

# General introduction

## Contents

## 1.0 Summary.

Dendritic cells (DCs) are antigen-presenting cells (APCs) which play a key role in the regulation of immune responses. DCs are unique APCs and are often referred to as "professional" APCs, since their primary function is to present antigens, e.g. from pathogens or malignant cells. DCs have the ability to induce a primary protective immune response to these antigens in resting naïve T lymphocytes. Consequently, there is a great deal of interest in how DCs might be exploited as a form of immunotherapy e.g. to induce immunity to cancers. However, DCs are also thought to play an important role in directing regulatory immune responses to innocuous antigens, which are targeted in autoimmune disease or during transplantation. Although fulfilling different roles, both responses are vitally important to maintain immune homeostasis. Soluble factors secreted by DCs are crucial mediators in determining this balance between the immunogenic and regulatory arms of the immune system. One such group of factors is cytokines. These cytokines are small proteins which are often grouped into different families based on structural similarities. One family which is gaining increasing attention is the IL-12 family. It is composed of four members; two are immunogenic and their expression has been very well characterised in DCs. The other two are regulatory, but relatively little is known about their regulation and expression in DC populations. In this thesis we aim to give a comprehensive overview of the expression and regulation of IL-12 family members in human DCs, with a particularly emphasis on IL-12, IL-27 and IL-35. We will discuss how the local cellular environment and normal physiological processes, such as clearance of dying cells can affect cytokine production and DC function. We will give a detailed analysis of the regulation of IL-12 family members in DCs using sterile and non-sterile stimuli, and describe the production of a novel anti-inflammatory cytokine, IL-35, by tolerogenic DCs. We will demonstrate a previously unidentified role for complement components, properdin and fH, in the stimulatory capacity of DCs and describe a role for IL-27 in their regulation. Finally, we will touch on the expression IL-12 family members in human kidney and discuss changes in their expression during renal allograft rejection.

## 1.1 History of Dendritic cells.

Dendritic cells (DCs) represent a heterogeneous population of APCs that are found in virtually all tissues of the body[1]. They were first described by Paul Langerhans as a new epidermal cell in the paper published in 1868 entitled "On the nerves of the human skin", and these epidermal DCs still bear his name today. Over a hundred years later, cells of a comparable nature were first described in mouse peripheral lymphoid organs by Ralph Steinman and Zanvil Cohn in 1973[2].

Although their function at that time was not known, their name was derived from the surface projections they possessed which resembled dendrites of neurons, and so coined the term "dendritic cell". These days we know that these dendrite projections somewhat allude to the primary function of DCs, which is to constantly probe and sample their environment for signs of damage or infection, followed by ingestion and breakdown of antigen into peptide for presentation[3]. Interest in DCs gathered pace in the 1990s when a model was proposed that described two distinct subsets of DCs, immature and mature. It was thought that immature DCs were derived from bone marrow and had not yet encountered antigen, while the mature cells were derived from tissues and must have encountered Ag in order to have undergone maturation[4]. However, some debate was ongoing in the field to understand how a lymphocyte, upon engagement with a DC, determined when to proliferate and attack, and conversely, when not to, if antigen alone was the sole conveyor of this information. Research conducted a few years prior to the immature/mature findings demonstrated that although antigen was required, it was not sufficient to drive the adaptive immune response, and that accessory functions of DCs were crucial[5,6] (signal 2).

### 1.2 Dendritic cells initiate the immune response.

For much of this time the accepted idea was that the main goal of the immune system was to eliminate or attack foreign pathogens "non-self", while remaining nonreactive to endogenous proteins in the body "self". This theory of self-nonself, although compelling, contradicted again with the observations discussed earlier, that antigen alone although required, is not sufficient to induce an immune response. Unlike T cells which may be able to discriminate between self and foreign antigen, DCs cannot, so how does an APC regulate immunity versus tolerance, and what controls signal 2?

In 1989, Charles Janeway, reasoned that the adaptive immune system must rely on other receptors that unlike T and B cell receptors, are not randomly generated, and have been evolutionarily selected over time[7]. Janeway hypothesised the "Infectious-Nonself" model, which suggested that microbial components were recognised by innate cells and that this recognition led to signal 2 required for lymphocyte activation[8]. Remarkably, within the next decade Janeway`s theory would be confirmed.

Groundbreaking work initiated by the discovery of Toll by Hoffmann and colleagues in 1996[9], led Janeway and Medzhitov to describe LPS as a potent immune trigger to initiate NFκB signalling, leading to upregulation of co-stimulatory molecule CD80 (signal 2) and release of cytokines IL-1 and IL-6 in human cell lines[10]. Several publications followed, from various groups,

demonstrating a number of human receptors with Toll homology[11-13], which are now collectively known as the Toll-like receptor (TLR) family.
Janeway`s theory, although well articulated and for a large part proven, left some inexplicable phenomena, like allograft rejection and anti-tumour immunity, which both can occur in the absence of pathogens. His principle was further expanded on by Matzinger in 1994 in the article entitled "Tolerance, Danger and the Extended family"[14]. Matzinger reasoned that the immune system does not distinguish between self and non-self, but rather uses receptors, expressed on APCs, to recognise danger signals not only from pathogens, but also from injured or stressed cells.

Prior to these discoveries, DCs had been described as merely "natural adjuvants"[15]. In less than 10 years, the discovery of innate receptors revolutionised DCs as an inextricable link between innate and adaptive immunity. It was already known that after antigen uptake, DCs efficiently process antigens for presentation in the context of MHC. However, these later findings led to the understanding that before DCs can prime the adaptive immune response, they must complete a full maturation process induced by alarmins; pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). We now know that DCs are equipped with an impressive repertoire of innate PRRs including, TLRs, NLRs, RLRs and CTLRs, which together grants them the ability to decipher the nature of a given insult. This allows DCs to mediate a fully integrated immune response, to provide not only signal 1, but also 2 and 3, which are essential to completely direct the specificity,strength and class of T cell responses (Figure 1).

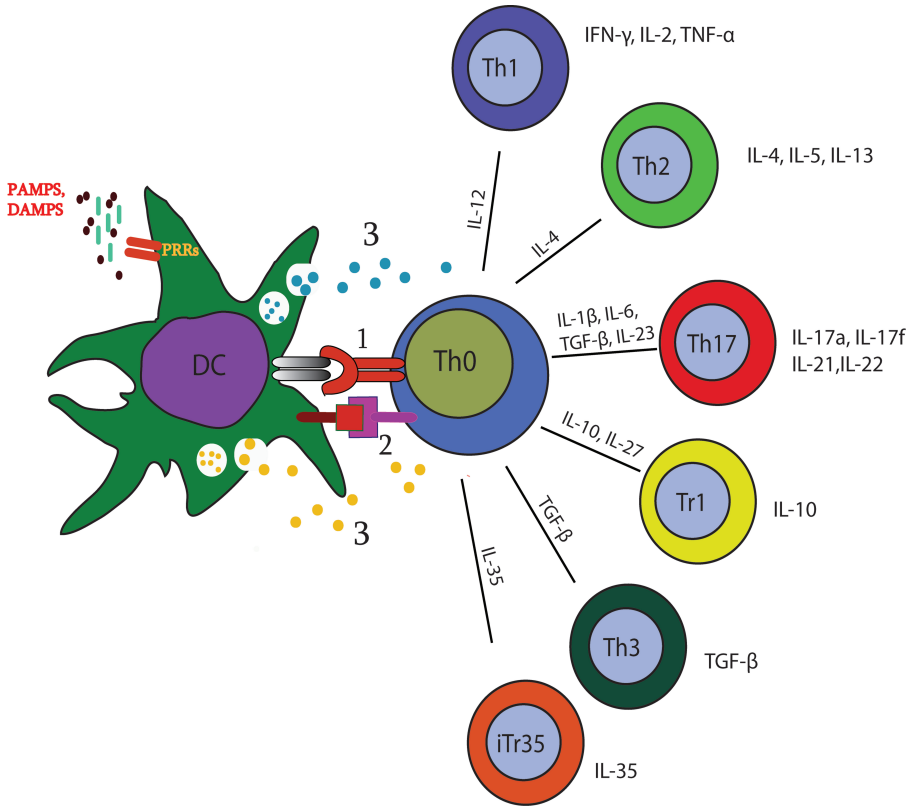


**Figure 1: Polarisation of naive T cells depends on 3 key signals from DCs.** Signal 1 is the antigen-specific signal mediated via peptide MHC:TCR interaction. Signal 2 DCs become activated upon recognition of PAMPs or DAMPs expressed by pathogens or local injured tissues/cells. This leads to upregulation of co-stimulatory molecules CD80 and CD86 which can trigger CD28 (on T cells) and leads to feedback stimulation of the DCs e.g. via ligation of CD40 on DCs by CD154. Signal 3 is the crucial polarising signal secreted by the activated DCs, and is mediated by various soluble factors such as IL-12 and IL-4 which promotes the development of Th1 or Th2 cells, respectively. The nature of signal 3 depends on the activation of particular PRRs (TLRs, CTLRs) by PAMPs or DAMPs and can lead to a diverse secretion of various cytokines. Depending on the local environment and cytokines produced, naive Th0 cells can differentiate into helper T cell populations ranging from the important inflammatory Th1 subset to the equally important Tr1 regulatory subset.

### 1.3 Tolerogenic DCs- paving the way for immunological tolerance.

Despite the identification of DCs in the early 1970s, research into this cell type, compared to T cells for example, was vastly under-represented. This stemmed from the difficulty in isolating DCs from whole blood, and the low cell yield when one succeeded. It was understood that DCs found in the periphery arose

from proliferating precursors within the bone marrow compartment, so several groups sought to generate DCs ex vivo, with the aim to obtain larger cell numbers for experiments. Steinman and colleagues demonstrated the generation of "large number" of DCs from murine bone marrow cultured in the presence of GM-CSF[16] and many laboratories were also trying to generate human DCs from similar precursors. As a reward for the efforts of several groups[17], often utilising HSCs as the starting population[18,19], finally in 1994 a landmark paper from Sallusto and Lanzavecchia, described the in-vitro generation of human DCs from blood derived monocytes, maintained in GM-CSF and IL-4[20]. Although not 100% homologous with blood derived myeloid DC (mDCs), monocyte- and HSC-derived DCs became a widely used tool to increase our understanding of human DC biology. Despite the identification of specific mDCs markers for isolation from peripheral blood, (BDCA1-4)[21], monocyte derived DCs are still extensively used today as a model to study interstitial mDCs. This is distinct from the field of skin DC biology where innovative culture systems have facilitated the migration of native DCs directly from their environment. This remains the ultimate goal for studying interstitial DCs found in other human organs, but due to their scarcity and the lack of reliable techniques, this remains elusive.

Nevertheless using these in-vitro systems the concept of tolerogenic DCs (tolDCs) first arose when it was observed that treatment of HSC derived DCs with IL-10, resulted in a DC population with impaired allostimulatory ability[22]. In the years that followed many groups explored ways of generating tolDCs, from HSCs and monocytes in vitro using various anti-inflammatory and immunosuppressive agents. These agents included IL-10[23,24], TGF-β[25], adenosine and the vitamin D3 metabolite 1α, 25-dihydroxyvitamin D3 (1α, 25(OH) $2D_3$)[26]. Particularly in the field of transplantation, clinically approved immunosuppressive drugs such as corticosteroids[27-29], cyclosporine[30], tacrolimus and rapamycin[31,32] have been used in vitro to target DC differentiation and function, with the latter molecule leading the greatest debate. It was found that although rapamycin interfered with monocyte-derived DC generation, it did not suppress mDC differentiation in vivo, and in fact increased their IL-12 production and T cell stimulatory capacity[33]. This study highlights the important of careful consideration before choosing an immunomodulatory strategy. The ability to induce tolerance in an antigen specific manner is the ultimate goal clinically, in fields such as transplantation, autoimmune disease and allergy[34-38]. The synthetic glucocorticoid, dexamethasone (Dex), amongst others, is already approved as a broad immunosuppressant for the treatment of a variety of autoimmune diseases including chronic idiopathic thrombocytopenic purpura[39], systemic lupus erythematosus, and Graves' disease. In these patients higher levels of regulatory T cells have been identified[40], indicating that Dex is a promising tool clinically. More recently, exciting

studies have been performed using Dex as a tolerogenic adjuvant in a model of suppressed immunisation[41]. The authors showed that administration of Dex in combination with peptide antigen resulted in expansion of Ag specific T regs that persisted in vivo and prevented the development of autoimmune diabetes. The exact mechanism was further elucidated to be dependent on the Dex treatment which led to enrichment of tolerogenic APCs in vivo[42], indicating that Dex does not only target T cells but also APCs. Additionally, extensive in vitro studies, performed with human DCs, showed that Dex altered the phenotype and function of DCs, rendering them tolerogenic, with absent production of IL-12 and a poor allostimulatory capacity[27,28,43,44].

In the field of transplantation there are two major hurdles to overcome immunologically, namely direct and indirect allorecognition, which can both result in graft injury and rejection. Depending on their origin (donor or recipient), tolDCs can be exploited to potentially treat both phenomena and prolong allograft survival. For the control of indirect alloreactivity, one could exploit tolDCs (of recipient origin) in vitro by loading the cells with antigen of donor origin. Although this has proved successful in preliminary murine experiments[45], it will be important to identify which type of donor material would be most efficiently ingested by tolDCs, and to identify what source of dying material would be most effectively presented. Kuswah et al. suggested that specifically uptake of apoptotic murine DCs by DCs potently promotes immune tolerance[46], but unfortunately they did not compare their findings to necrotic cells. Considering that immune tolerance remains the most promising, yet elusive, strategy for treating autoimmunity and preventing transplant rejection, much can be gained from understanding the exact mechanism of how apoptotic cells are able to promote immune tolerance. Finally, it will be very important to demonstrate that uptake of such material does not alter the tolerogenic nature of these cells and that their regulatory cytokine profile is maintained.

### 1.4 Dendritic cells and immune homeostasis.

Historically the maintenance of immunity versus tolerance has been attributed to T cells, and loss of tolerance, e.g. during autoimmunity, has been considered to be a "T cell problem". However, there is an increasing body of evidence to support the idea that DCs are the master regulators of these processes, and as already mentioned, can be exploited therapeutically, for on the one hand boosting immunity to tumours or viruses, and on the other, restoring tolerance to innocuous antigens[1,47]. Until recently, immature DCs characterised as having low MHC and B7 molecules were believed to induce T-cell anergy or T regs, while mature

DCs expressing high levels of MHC and B7 molecules were the immunogenic DCs. This paradigm has been challenged by the observation that T regs can be induced by fully mature, antigen bearing DCs[48,49]. Similarly, semi-mature DCs[50] with a distinctive cytokine profile of IL-10$^{+}$IL-12$^{-}$ have been shown to possess tolerogenic functions[43], supporting the notion that the maturation status of DCs should no longer be the only distinguishing feature of immunogenic, as opposed to tolerogenic DCs (tolDCs)[51]. As such, the only true way to identify a regulatory DC compared to an immunogenic DC is to identify functional differences. Cytokines and chemokines receptors[52,53] have proven to be very useful in differentiating helper T cell subsets. Greater understanding into the cytokine profile of different DC subsets would significantly enhance our understanding of disease pathogenesis and may aid identification of these subsets in vivo.

### 1.5 Dendritic cells and soluble mediators of immunity.

A key mechanism of how DCs communicate with other cells, and ultimately orchestrate the nature and intensity of a given immune response is through the production of soluble mediators.

#### 1.5.1 Cytokines and Dendritic cells.

The most widely studied mechanism of how immune cells communicate with one another focuses on a large group of proteins called cytokines. These cytokines are low-molecular-weight proteins that regulate the nature, intensity and duration of the immune response. Historically cytokines have been classified into groups based on their cellular secretion or activity with regard to a given cell population. More recently cytokines have been grouped based on the cytokine receptors they utilise, which has led to the establishment of several major cytokine groups including:

- **Haematopoietin family** (utilises class I cytokine receptors) which includes; IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, IL-23, IL-27, IL-35, GM-CSF, G-CSF, OSM, LIF, CNTF and prolactin.
- **Interferon family** (utilises class II cytokine receptors) which includes; IFN-α, IFN-β, IFN-γ and cytokines IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 (IFN-λ 2/3), IL-29 (IFN-λ1).
- **TGF-β and Growth Factor family** (utilises tyrosine kinase cytokine receptors) which includes TGF-α, TGF-β, M-CSF, EGF, FGF, FLT3.
- **TNF family** (utilises family TNF receptors) which includes; TNF-α, LT-a, LT-b, BAFF, APRIL, FASL, TRAIL, CD40L, OX40L, 4-1BBL, CD70/

CD27L and CD30L.

- **Chemokine family** (utilises G protein coupled chemokine receptors) which includes; CCL5, MIP-1, MCP-1, IL-8, CXCL-12, IP-10.
- **IL-1/TLR family** (utilise TIR domains): which includes IL-1α/β, IL-18, IL-33, IL-37

The cytokine network is a highly integrated and complex system which is generated in response to immune challenge. The overall balance between immunogenic and regulatory cytokines is what determines the nature of a particular immune response. Cytokines are not generally pre-stored proteins, but rather are transiently produced in response to stimuli. As such, their mRNAs are typically short lived. The net effect of any cytokine is critically dependent on the timing of its release, the local milieu in which it acts, the presence of competing or synergistic elements, cytokine receptor density on target cells, and tissue responsiveness to each cytokine.

Many cytokines can exert similar functions, and this redundancy is often attributed to the nature of cytokine receptors, whereby one receptor chain can often be used by multiple cytokine receptors e.g. gp130. These common structural features make it possible to further group cytokines within families. For example the class I receptor family listed above can be further subdivided into 3 further groups:

**GM-CSF receptor family** (common beta subunit): IL-3, IL-5, GM-CSF
**IL-6 receptor family** (common gp130 subunit): IL-6, IL-11, LIF
**IL-2 receptor family** (common gamma chain): IL-2, IL-4, IL-7, IL-9, IL-15

Despite the structural similarities between the members, cytokines within a particular family can often have surprisingly divergent functions[54]. One such family with a remarkable degree of chain sharing, yet entirely distinct functions is that of the IL-12-family.

### 1.5.2 The IL-12 family.

The IL-12 family is a further subdivision of the IL-6 superfamily and is composed of four heterodimeric complexes each consisting of an alpha chain (IL-12p35, IL-23p19 and IL-27p28) and a beta chain (IL-12p40 and Ebi3). The IL-12p40 subunit can pair with either IL-12p35 or IL-23p19 to yield IL-12 and IL-23 respectively. The second beta chain subunit Ebi3, can pair with 2 other alpha chain subunits, IL-27p28 and IL-12p35, to yield IL-27 and IL-35 respectively. Chain sharing has

become a quintessential feature of this cytokine family and their receptors, yet despite this, each member possesses a unique and distinct biological function.

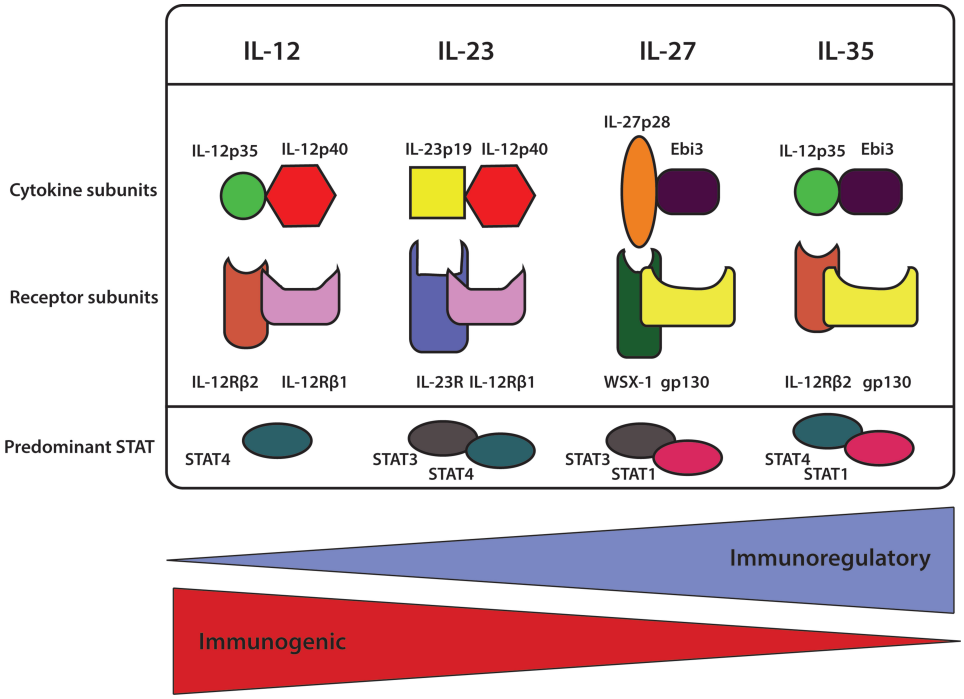


**Figure 2: IL-12 family members.** Illustration of the IL-12 family members together with their receptors and the predominant STATs used by the individual cytokines. Note: IL-35 can also signal via two alternative receptors, a homodimer of gp130 or a homodimer of IL-12Rβ2. It has been reported that the heterodimer of gp130 and IL-12Rβ2 yields the most potent suppressive abilities of IL-35[55] and is therefore the receptor illustrated in the diagram above.

**IL-12** is a pro-inflammatory cytokine produced by activated antigen presenting cells, including DCs, and its main role is in the initiation and commitment of naive $CD4^+$ T cells to IFNγ producing Th1 cells[56]. This induction of IFNγ, results in a feedback loop, whereby additional APCs, in response to the IFNγ, are primed to produce IL-12 which further feeds naive $CD4^+$ T cells towards Th1 commitment[57-59]. IL-12 signals via IL-12Rβ1 and IL-12Rβ2 and is produced by DCs activated with microbial products including poly I:C, zymosan and most notably LPS. This production is potently enhanced upon ligation of CD40[60] or when microbial stimuli are combined e.g. poly I:C + LPS[61]. IL-12p40 is produced vastly in excess to IL-12p70, and p40 subunits can homodimerise to yield IL-12p80[62]. This molecule has been proposed to act as a negative regulator for IL-12p70 due to its ability to bind to IL-12Rβ1. In addition to this, several cytokines have been identified as negative regulators of IL-12, namely, the Th2 cytokines

IL-4 and IL-13, IL-10 and type I IFNs, most notably IFN-β[63].

**IL-23**, like IL-12, utilises the beta chain IL-12p40, and is an immunogenic cytokine with an important role in the commitment and maintenance of Th17 cells. IL-23 signals via IL-12Rβ1 and the IL23R[64] and was first identified by Kastelein and colleagues in 2000[65]. Its functional importance came to light when studies were performed to investigate the role of IL-12 in experimental models for encephalomyelitis (EAE) and arthritis. It was found that while IL-12p40$^{-/-}$ mice were protected from disease, IFNγ$^{-/-}$ , IFNγR$^{-/-}$ or STAT-1$^{-/-}$ mice were still susceptible, thereby suggesting a Th1 independent mechanism of disease pathogenesis. Further work identified that both IL-12p40$^{-/-}$ and IL-23p19$^{-/-}$ mice were protected from EAE while IL-12p35$^{-/-}$ mice were highly susceptible, and had elevated levels of IL-17[66-70]. These leading studies paved the way for the identification of a novel and distinct new lineage of helper T cells, Th17[71]. Although not critical for the initiation of Th17 differentiation from naive T cells, IL-23 seems to play a crucial role in the stabilisation of Th17 cells[72], and more recently has been identified as a playing an important role in the generation of highly pathogenic, IL-23R expressing, Th17 cells[73,74]. In line with IL-12, microbial products, are potent induces of IL-23 production by DCs, and interestingly stimulation of DCs with IL-12 increases transcription of IL-23p19.

**IL-27**, unlike IL-12 and IL-23, plays duals roles possessing both immunogenic and immunoregulatory properties and is composed of IL-27p28 and Ebi3. Initially identified as an EBV induced gene in EBV infected B cells, *EBI3* was found to encode a 34kDa protein bearing structural homology to IL-12p40[75]. In the initial paper describing the *EBI3* gene, very high levels were found in placental tissue which highlights the diverse nature of *EBI3* expression compared to other IL-12 family member subunits. Since then, although Ebi3 protein expression has been mostly described in haematopoietic cells[75-78], several studies have accumulated data describing expression of this protein in placental synctiotrophoblasts[79], endothelial cells, intestinal mucosa[80,81] and aortic smooth muscle cells[82]. This expression of Ebi3, often in the absence of its typical pairing subunits has fuelled speculation that Ebi3 may possess biological functions in its own right or may dimerise with as yet unidentified partners.
First identified in 2002[83] IL-27 signals via gp130 and WSX-1 and was initially classified as a proinflammatory cytokine due to its ability to synergise with IL-12 and enhance IFNγ production by naive T cells[83,84]. It was also later found to up-regulate expression of IL-12Rβ2 on naive T cells. However IL-27 alone cannot induce IFNγ production by naive T cells and, in more recent years IL-27 has been demonstrated to possess many immunoregulatory functions. The first

report to highlight the regulatory properties of IL-27 showed that mice infected with Leishmania died from excessive immune responses if they were deficient in the IL-27R[85]. Following on from this it was demonstrated that IL-27 could convert activated Th1 cells into IL-10 producing Tr1 cells[86-88]. This was in part found to be mediated via c-MAF[89,90]. IL-27 has been gaining increasing attention in the field of autoimmunity due to many reports demonstrating that IL-27 can suppress Th17 responses[91-94], and it has shown particular promise in the treatment of EAE in murine models[95,96]. IL-27 does not only exert its activity on T cells but has also been demonstrated to up-regulate the inhibitory molecule B7H1 on DCs[97,98] and limit ATP mediated NALP3 activation via upregulation of CD39[95]. Inducers of IL-27 production by APCs include TLR agonists LPS, Poly I:C and Loxoribine. Notably, IFNβ, currently in clinical trials to limit the pathogenesis of MS has been shown to mediate its effects through the induction of IL-27[99]. An interesting feature of IL-27 is that the alpha chain IL-27p28 is in its own right a cytokine, namely IL-30. To date IL-30 had been shown to inhibit Th17 differentiation induced by IL-6 and TGF-β[94] and has also been shown to act as a natural antagonist to gp130 signalling[100]. As such IL-30 can limit IL-27 signalling as well as members of the IL-6 family including IL-6 an IL-11. Interestingly, over expression of IL-30 has been shown to disrupt germinal centre formation in mice[100] which is in line with reports that IL-27 can facilitate germinal centre formation via induction of IL-21[101].

**IL-35** is the most recently identified member of the IL-12 family and is a potent anti-inflammatory cytokine produced by regulatory T cells in mouse and man. It is composed of the alpha chain of IL-12 (IL-12p35) and the beta chain of IL27 (Ebi3). In 1997 it was first reported that Ebi3 and IL-12p35 could heterodimerise[102], however it was almost a decade later before this protein was named and its function elucidated[103]. Remarkably despite the knowledge of the dimerisation of IL-12p35 and Ebi3, the identification and characterisation of IL-27 came first. This perhaps alludes to the challenges in studying the expression and function of IL-35.

To date IL-35 has been shown to induce a regulatory T cell population, iTr35, which suppresses via IL-35 secretion but does not express Foxp3, IL-10 or TGF-β[104]. This regulatory T cell subset possesses a profound anti-inflammatory phenotype with the ability to reduce inflammation in animal models of IBD and EAE, and mediate infectious tolerance[105]. Interestingly activated B cells expressing IL-35 have also been demonstrated as key negative regulators of immunity in an animal model of EAE[106]. Ectopic expression of IL-35 in pancreatic beta cells prevents autoimmune diabetes[107], and administration of recombinant IL-35 protects against collagen-induced arthritis[108]. In humans, iTr35 cells can be

induced by the addition of IL-35 to activated naive CD4$^+$ T cell cultures[109] or by exposure to virus-infected DCs in a B7H1 and sialoadhesin dependent manner[110]. Conversely to the protective role of IL-35 in inflammatory conditions, transfer of in vitro generated iTr35 cells prevents the development of CD8$^+$ anti-tumour responses and accelerates B16 melanoma development in mice[104]. Additionally a novel population of CD8$^+$ IL-35-secreting tumour Ag-specific T regs have been shown to arise spontaneously in some prostate cancer patients[111].

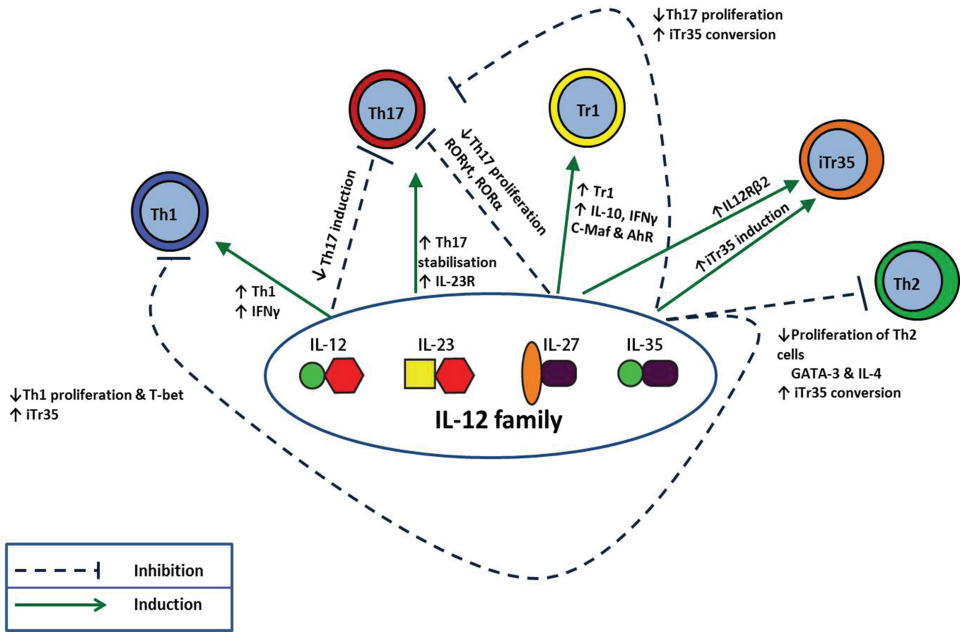


**Figure 3: IL-12 family mediated generation and cross regulation of Th subsets.** Illustration representing the most well described helper T cell populations directly regulated by IL-12 family members. The green arrows depict induction of a response; either inflammatory or regulatory. The dashed blue lines indicate inhibition of a response. This diagram does not represent indirect mechanisms of how IL-12 family members regulate immune responses e.g. Tr1 cells induced by IL-27 can continue to exert their regulatory potential without further requirement for IL-27.

Only recently the receptor for IL-35 was identified to be gp130 and IL-12Rβ2[55]. Intriguingly this means that IL-27 can not only sensitise naive CD4$^+$ T cells to IL-12 but also IL-35, highlighting the local cytokine network as being a crucial determinant in orchestrating the immune response. In many ways the IL-12 family could easily be argued as the cytokine family with the greatest influence in determining local T cell populations. Our full understanding of the newest member of this cytokine family is still in its infancy and unlike the other 3

members of the IL-12 family, IL-35 has not yet been shown to be produced by myeloid cells in mouse or human. Considering its potent regulatory potential the possible secretion of IL-35 from tolerogenic or regulatory DCs could be a new previously unexplored mechanism into how these cells can tailor immune responses.



### 1.5.3 Complement: A role in regulating the immunogenicity of DCs?

As mentioned earlier the discovery of innate PRRs transformed our appreciation for the innate arm of the immune system as being a key player in the regulation of adaptive immune responses. This re-ignited interest in innate immunity has sparked a renaissance in an even older innate system: Complement.

First discovered by its ability to assist or "complement" the bactericidal activity of blood, the complement system has established itself as an important mediator of apoptotic cell and immune complex clearance, pathogen eradication and is involved in lowering the threshold of B cell activation and antibody production. However more recently, complement activation has been shown to influence local T cell responses. Largely limited to murine data, the alternative pathway of complement has been demonstrated to play a role in the DC: T cell synapse[112-114]. Both APC and T cells have been shown to increase C3a and C5a receptors on their surface, while a surface regulator, DAF, has been shown to be decreased, allowing for further complement activation[115,116]. The link to the AP is particularly interesting. Unlike most other complement components, the key positive regulator of the AP, properdin (fP), is mainly produced by white cells. Furthermore, the key negative regulator, fH, although largely produced by the liver, has also been shown to be produced by extra-hepatic sources[117-120]. Limited data has shown by RT-PCR and western blot that DCs can function as a source for several complement components[118,121]. Despite their unique ability to prime and direct T cell responses, little is known about the contribution of local DC derived complement factors in regulating the potency of T cell activation. Additionally, studies investigating the regulation of complement factor production by DCs are even sparser. Interestingly, some complement components have been shown to possess GAS and ISRE elements in their promoters[122], and fP has been shown to be subject to IFNγ regulation in THP-1 cell lines[123]. IL-27 is a unique member of the IL-12 family, in that it has been shown to share some functional properties with IFNγ[97,124,125]. In view of the link between complement activation and autoimmunity and the therapeutic potential of IL-27 in such disease settings, it would be interesting to evaluate the effect of IL-27 on DC complement production. Considering the central role of the AP of complement in many human diseases

much can be gained from fully investigating the regulation of AP components in DCs and the functional contribution in DC T cell interactions.

### 1.6 Dendritic Cells and the role of the local environment.

Although it is widely acknowledged that regulatory DCs exist in vivo, we know surprisingly little about how they develop and how to identify them. Recently an interesting idea, pioneered by Matzinger has surfaced, suggesting that the organs in which immune cells reside, may not be just passive hosts, but play a crucial role in actively promoting immune regulation at the level of the tissue[126,127]. Evidence to support this notion has been already gathering over the last decade. Many groups have identified stromal networks in lymphoid organs as being a key source of growth factors, cytokines and chemokines, in addition to their traditional role as a support network[128]. Most notably, stromal cells from the bone marrow (MSCs) have recently been under intense investigation due their ability to modulate T cell activity and promote induction of regulatory T cells[129], and a regulatory phenotype in monocyte derived DCs[130,131]. Concerning individual tissues, stromal cells including fibroblasts, and epithelial cells have come to light as exerting important biological functions in the control of local immune responses, and depending on their origin may exert their modulatory effects through different mechanisms[132-134]. In this regard, DCs residing in non-lymphoid organs are particularly intriguing. DCs have been identified in virtually all tissues, including the kidney, and definitive experimental proof has shown that in mice they form an extensive lattice like network within the renal parenchyma[135]. Also in human, DC subsets can be identified in pre-transplant biopsies of normal kidneys[136], and their frequency strongly increases during renal allograft rejection[137].

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. There are many open questions in renal DC (rDC) biology, although in mouse it has been shown that mobilised rDCs, *in-vivo*, are functionally immature with a poor allostimulatory capacity and most promisingly, have been shown to prolong allograft survival[138]. Understanding into how the renal stroma influences DC phenotype and function is limited to murine data, but has yielded interesting results. Huang et al demonstrated that DCs generated in the presence of renal MSC like cells led to the generation of a regulatory DC population with diminished allostimulatory capacity[139,140]. The ontogeny of different tissue resident DCs has not yet been fully elucidated, but

knowledge of how the local environment influences the local APC population, and how this changes during stromal cell perturbation, would certainly contribute significantly to our understanding of organ specific disease pathogenesis and prognosis. It is plausible that individual tissues imprint a particular functional signature in their resident DCs, e.g. cytokine profile or upregulation of particular chemokine receptors. This is important to fully investigate, for a potential use of tolDC therapy would be to navigate them towards the affected target organ, where they should exert their regulatory function in a way that preserves organ function. Additionally identification of unique functional markers on tissue resident rDCs would provide an invaluable method to distinguish between resident and infiltrating DCs in transplantation, or potentially discriminate between immunogenic versus regulatory DCs.



### 1.7 Scope of the thesis.

The current thesis was dedicated to understanding the expression, regulation and production of IL-12 family members in DCs and tolDCs and is divided into five chapters: **Chapter 2** presents data regarding the ability of human tolDCs to produce IL-12p35 and Ebi3, the alpha and beta chain of IL-35, respectively. In this chapter we demonstrate for the first time that abrogating the production of IL12A in tolDCs diminishes the immunosuppressive capability of these cells and points to IL-35 as being a novel mediator of immune suppression by tolDCs. **Chapter 3** details how phagocytosis of apoptotic or necrotic cells by immature DCs or tolDCs modulates the release and expression of IL-12 cytokine family members, particularly the IL-12/IL-35 axis. Importantly, we also describe that tolDCs maintain a stable regulatory cytokine profile upon ingestion of necrotic material. **Chapter 4** describes the production of two key regulators of the alternative pathway of complement by DCs and tolDCs. We show that IL-27 and members of the Interferon family differentially regulate the expression of fH and properdin and we demonstrate how alteration in the balance between properdin and fH can alter the allostimulatory capabilities of DCs. Considering the abundant expression of Ebi3 in tolDC populations, and the potent regulatory properties of IL-35, in **Chapter 5** we sought to investigate whether the local renal DC network would be positive for Ebi3 expression in normal human kidney. While we did observe very prominent Ebi3 expression in normal kidney, this was found to be particularly abundant in podocytes and not the renal DC network. We further demonstrated in a patient cohort, that *EBI3* expression is decreased during renal allograft rejection. **Chapter 6** describes how the local cellular network can influence the cytokine repertoire of DCs. In this chapter we demonstrate the ability of human renal fibroblasts to generate a regulatory DC population, in part

through the secretion of IL-6. Finally in **Chapter 7** we discuss and conclude our findings and describe potential areas of interest that warrant further investigation.

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