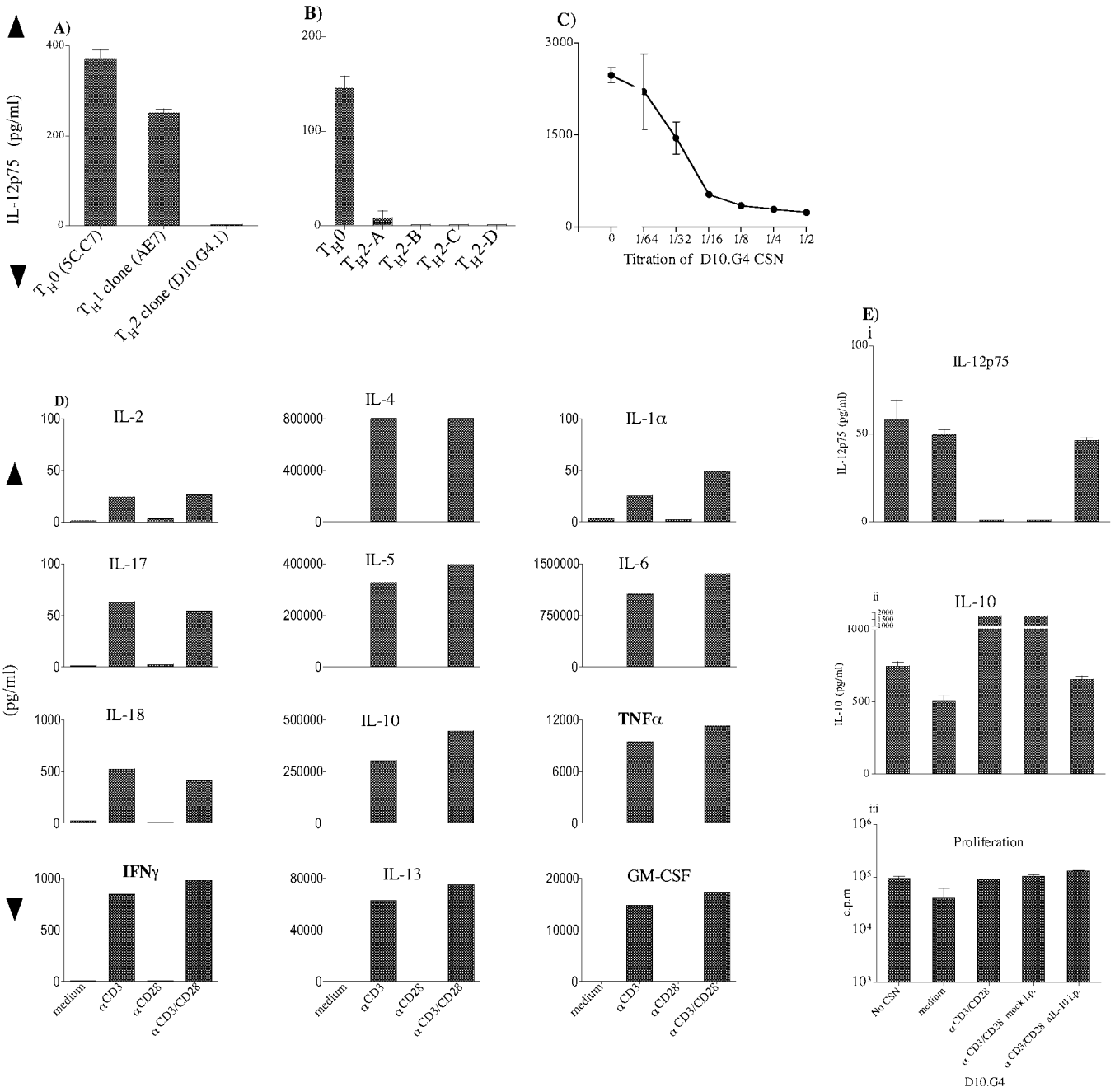

Figure 3. T_H0 and T_H1 cells induce IL-12p75 production but not T_H2 cells due to the presence of endogenous IL-10. (A) BM-DCs from B10.A-Rag2KO mice were pre-activated with 100ng/ml LPS for 24hr then washed and co-cultured at $2x10^4$ cells/well with $5X10^5$ cell/well 5C.C7 T cells (T_H0) AE7 (T_H1 clone, I-E^k) or D10.G4.1 (T_H2 clone, I-A^k) in the presence of 0.1μM MCC and 1μM conalbumin respectively for 48hr in a 96 well U-bottom plate. CSN were tested for the presence of IL-12p75 with specific ELISA. (B) Same as (A) except naïve 5C.C7 T cells were cultured under TH2 skewing conditions by adding 10μg/ml anti-IFNg, 10μg/ml anti-IL-12 and 100ng/ml rIL-4 plus 0.4μM MCC peptide for 3 days washed, Ficolled and maintained in 20U/ml rIL-2 plus100ng/ml rIL-4, these skewed T_H2-type T cells were sorted for coculture with BM-DCs. CSN were collected and tested for the presence of IL-12p75 with specific ELISA. (C) Same as (A) antigen-activated 5C.C7 T cells were co-cultured with LPS pre-activated BM-DCs plus 0.1μM MCC in the presence of titrated CSN obtained from APC free D10.G4.1 TH2 T cell clone that has been stimulated with plate bound anti-CD3 and soluble anti-CD28 for 48hrs in a 24 well plate. (D) Same CSN that was used in (C) was analyzed with the help of multiplex cytokine array for measuring various cytokines in CSN. (**E i-iii**) Same conditions as (C) except some wells received CSN from anti-CD3 stimulated D10.G4.1 or the same CSN in which endogenous IL-10 has been specifically depleted by incubating the D10.G4.1 CSN with protein-G beads coupled with Rat anti-IL-10 monoclonal antibodies or the isotype control. (i) After 48hr of co-culture assay, CSN was tested for the presence of IL-12p75 with specific ELISA. (ii) Same conditions as (i) except CSN were tested for the presence of IL-10 with specific ELISA. (iii) ^{3}H-Thymidine incorporation was measured during the last 12 hrs of the coculture assay. These data are expressed as the mean ± SD triplicates from a 96 well.

Discussion:

One of the key questions in immunology is how immune responses are initiated. The current paradigm emphasizes that binding of microbes or their products to Toll-like receptors (TLRs) will lead to activation and secretion of IL-12p75 from the sentinel cells of the innate immune system (phagocytic and dendritic cells). In this model, IL-12p75 plays an essential role as an early signal to activate host immune responses. Even though various antigen-presenting cells have been shown to make IL-12p75, perhaps the most relevant source of this cytokine for initiating immune responses is the dendritic cell (DC). DCs are known as professional antigen-presenting cells that play an essential role in the initiation of the host immune response to various pathogens. The expression and secretion of IL-12p75 by DCs however is complicated by the fact that this cytokine is composed of two unrelated proteins, which are the products of two unrelated genes, namely p35 and p40 subunits (Trinchieri, 1995). Therefore, to obtain biologically active IL-12p75, a highly coordinated expression of these two subunits within the same cell is required.

This creates a problem because in many inflammatory conditions, the p40 subunit has been detected independently and in the absence of IL-12p75 (Chapter-I). Moreover, by using *in situ* hybridizations to detect p35 and p40 mRNA in the spleen of mice, it was demonstrated that there was a spatial dissociation of these two subunits within the LPS-challenged spleen (Bette et al., 1994). In contrast, p35 cannot be secreted alone and the data suggest that its secretion is only possible when it associates with the p40 subunit to

form the IL-12p75 heterodimer. As a result, the rate-limiting factor in IL-12p75 production is the p35 subunit (Hayes et al., 1995; Snijders et al., 1996), which is tightly regulated through posttranslational modifications (Babik et al., 1999; Carra et al., 2000; Murphy et al., 2000). It has been demonstrated that the p35 subunit can be elicited by T cell receptor (TCR)/antigen interactions in a dose-dependent manner (Yamane et al., 1999), by cross-linking MHC-II molecules on DCs with anti-I-A antibodies (Koch et al., 1996), or in the presence of IFN-γ plus microbial products such as LPS, which is commonly used during *in vitro* stimulation of DCs (Hayes et al., 1995; Liu et al., 2003; Snijders et al., 1996). Moreover, utilization of anti-CD40 monoclonal antibodies or cells that are transfected with CD40-Ligand is another way of inducing the IL-12p75 heterodimer form DCs (Cella et al., 1996; Koch et al., 1996); however, a priming step using microbes is an absolutely necessary step for IL-12p75 production (Schulz et al., 2000).

Collectively, these data indicate that induction and secretion of the IL-12p75 heterodimer is tightly controlled to ensure that enough safeguards are in place to prevent over-production of this potentially dangerous cytokine. Furthermore, the requirement for cell-cell contact for its secretion (Miro et al., 2006) guarantees its delivery to the appropriate cell during the formation of an immunological synapse between antigen-bearing DCs and antigen specific T cells. Therefore, in Chapter-III we have argued that secretion of IL-12p75, and thus control of the effector class of an immune response, is not solely in the hand of DCs or pathogens but rather that secretion of

IL-12p75 takes place during the cognate interaction between an antigen-activated T cell and antigen-bearing DCs (Chapter-III).

It is currently thought that IL-12p75 is one of the key bridges between the innate and adaptive immune system. This idea has led to the establishment of a conceptual frame-work in the field of immunology positing that IL-12p75 is the initial start-up signal for the polarization of naïve $CD4^+$ T cells toward a T_H1-type cytokine pattern by instructing NK and T cells to produce IFN-γ (Trinchieri, 1998). Although it is true that addition of recombinant IL-12p75 to the culture of naïve T cells will skew their cytokine profile toward a T_H1-type response, our seminal findings in Chapters-III and -IV suggest that only in the presence of antigen-activated (but not naïve) T cells, DCs are capable of secreting IL-12p75 in an antigen-specific manner. These data are also consistent with results in the human system, where it has been demonstrated that only purified human T cells with a memory phenotype ($CD45RO^+$), but not a naïve phenotype ($CD45RA^+$), are capable of inducing IL-12p75 when these cells were co-cultured with syngeneic DCs in the presence, but not in the absence, of Superantigen (Miro et al., 2006). This creates a conundrum: if naïve T cells are not the inducer of IL-12p75 during the primary immune response, then how is T_H1-type mediated immunity initiated? These findings are not in agreement with the current paradigm. Therefore, one important question needs to be addressed: under natural physiological conditions, how and where is IL-12p75 produced from DCs?

Our *in vitro* data strongly suggest that naïve T cells cannot be the instigator of IL-12p75 production when these cells engage antigen-bearing DCs. Thus, naïve T cells cannot skew the immune response toward a T_H1-type cytokine pattern. The kinetic studies have demonstrated that at no point in time could IL-12p75 be detected from the co-culture of naïve T cells with LPS-activated antigen-bearing DCs, pointing to the importance of regulatory mechanism(s) that are in place for the induction and secretion of the IL-12p75 heterodimer (Chapters-III & -IV). In contrast, under similar conditions, both in mouse and in human, antigen-activated effector or memory T cells were potent inducers of IL-12p75 from DCs. Its induction required cell-cell contact and the presence of the antigen (Chapters-III & -IV) (Miro et al., 2006).

The idea that signaling through TLRs would lead to the secretion of IL-12p75 and the skewing of the immune response toward the development of a T_H1-type cytokine pattern (Brightbill et al., 1999; Trinchieri, 2003) is mostly based on *in vitro* experiments. In general, regulation of IL-12p75 *in vivo* is poorly understood. Based on the current paradigm, it is not clear when DCs encounter antigens in the peripheral tissue, which is the site of infection, where does these cells secrete IL-12p75 and how this cytokine would reach the T or NK cells. A contributing factor that led to the establishment of the current paradigm is the mouse model of the intracellular protozoa, Toxoplasma *gondii,* or injection of its extract Soluble Tachyzoite antigen (STAg). This model emphasizes that IL-12p75 plays a crucial role during the early host response to this pathogen (Aliberti et al., 2004). The majority of studies that used this mouse model measured

exclusively the p40 subunit of IL-12p75 as their biological readout, which can easily be detected by ELISA in the serum of mice injected with STAg (LaRosa et al., 2008). However, the conclusions from these studies usually referred to the IL-12p75 heterodimer that was not actually measured in this mouse model. Therefore, it has been reasoned that since p40 can be secreted and detected in this model, it is reasonable to presume that IL-12p75 is also secreted, but that the levels are too low to be measured by conventional methods (Alan Sher personal communications). Therefore, based on this supposition, it has been concluded that IL-12p75 is a major initiation signal for host resistance to this parasite (Aliberti et al., 2004; Gazzinelli et al., 1994; Liu et al., 2006; Yap and Sher, 1999; Yarovinsky, 2008). This thesis argues against the presumption that IL-12p75 is the early instigator of protective immune responses against various infectious agents. The theoretical basis for this issue has been discussed in detail in Chapter I with various references to specific studies that have used T. *gondii*.

A highly referenced paper, (Scopus September 2008; 455 references) published in 1997 in the Journal of Experimental Medicine, used T. *gondii* and led to two important conclusions that in turn led to establishment of the idea that secretion of IL-12p75 in response to STAg is independent of signals from T lymphocytes, specifically, IFN-γ and CD40L (Sousa et al., 1997), two important factors that are absolutely necessary for IL-12p75 secretion. Even though this study only measured the p40 subunit and the authors were unable to measure the IL-12p75 in the mice that were challenged with STAg, conclusions were still made concerning IL-

12p75 (Sousa et al., 1997). Moreover, in another mouse study that has also used T. *gondii* and measured p40, it was concluded that secretion of IL-12 from Neutrophils is independent of IFN-γ (Bliss et al., 1999).

In Chapter-III we argue against the belief that IL-12p75 can be secreted in the absence of IFN-γ in direct response to TLR agonists. We show how bone marrow-derived DCs and splenic $CD11c^{+}$ cells were used to test the role of IFN-γ in IL-12p75 production from these cells when stimulated with Lipopolysaccride (LPS). We have specifically chosen LPS because it has been established in immunology textbooks that LPS induces IL-12p75 during the innate immune response. The study in Chapter III demonstrate that DCs exposed to LPS alone, in the absence of T cells or T cell products, will not lead to IL-12p75 production and that mice deficient in the IFN-γ gene will not make IL-12p75 when they are challenged with LPS. We have used this mouse model of sepsis to test the role of IFN-γ in IL-12p75 production, although this model does not truly represent an immune-regulation. But rather, it is a manifestation of a full-blown, systemic activation of the host immune system accompanied by a markedly imbalanced cytokine response (which can be called an immune dysregulation), commonly known as a"cytokine storm." We have chosen this model to show that, even under such extreme conditions, when many cytokines are simultaneously secreted, the induction of IL-12p75 is IFN-γ-dependent. This study also demonstrated that under both *in vitro* and *in vivo* conditions, where IL-12p75 cannot be detected, secretion of p40 is easily detectable, supporting the original hypothesis that initial signals to DCs, in the absence of T cells or T cell products such as IFN-

γ, will only lead to the secretion of p40 and not the secretion of IL-12p75. Therefore, we concluded that, the presence of IFN-γ is an essential factor in the induction of IL-12p75 when DCs are stimulated with LPS (Chapter-I).

It has been argued that IFN-γ plays a role as a cofactor to amplify the already low level of IL-12p75 secreted (which is hard to measure by conventional ELISA) in response to engagements of a single TLR ligand (Edwards et al., 2002; Ma et al., 1996). Therefore, based on this premise, it has been concluded that usually a small amount of IL-12p75 can be secreted in response to a single TLR agonist and that co-stimulation with IFN-γ will increase its levels (Trinchieri and Sher, 2007). To address this issue and further substantiate that LPS alone cannot induce IL-12p75, we developed a novel enzyme-linked immunosorbent spot assay (ELISPOT) with a detection frequency of 1 cell in a million, to specifically measure the IL-12p75 heterodimer at the single cell level. By using this sensitive assay, it was shown that the signal from LPS alone is inadequate to induce secretion of IL-12p75 from DCs, and again, the presence of IFN-γ was absolutely necessary for its induction (Chapter-III).
Thus, the data from the ELISPOT assay argue against the notion that low levels of IL-12p75 can be secreted by engagement of a single TLR agonist. These data demonstrated that, even at the single cell level, in the absence of T cells or IFN-γ, LPS and a host of other bacterial products could not induce IL-12p75 (Chapter-III). In contrast, under the same conditions, the p40 subunit could be easily secreted from these cells, further supporting the hypothesis behind this thesis, which emphasizes that it is the p40 protein that is secreted in

response to microbes and not, as is commonly believed, the IL-12p75 heterodimer (Chapter-I).

After the recent publication of Chapter III, proponents of the idea that IL-12p75 is the key bridge between innate and adaptive immunity have argued that high levels of IL-12p75 can be secreted when DCs are stimulated with multiple TLR ligands, and the idea that individual TLRs might have evolved to recognize their distinct pattern has been abandoned. Emerging data suggest that broad recognition of microbial products are mediated by many of the TLRs as opposed to a single TLR (Trinchieri and Sher, 2007).

Consequently, the idea that engagement of multiple TLR will induce IL-12p75 production has led to the conclusion that the synergism between multiple TLRs is an important contributor for IL-12p75 production from DCs during the early host response to pathogens (Gautier et al., 2005; Napolitani et al., 2005; Trinchieri and Sher, 2007). I do not subscribe to the above notion for two reasons. First, teleologically speaking, if each time a host encounters pathogens and the immune sentinel cells are engage with PAMPs should results in the activation of multiple TLRs causing IL-12p75 production, this would always lead to the skewing of immune responses toward a T_H1-type cytokine pattern. In this case, there would be no place for any other type of response (T_H2, T_H3, T_H17, T_HX). Furthermore, this suggests that the control of the effector class of an immune response lies with the DCs or with the pathogen. Second, it seems to me that secretion of IL-12p75 from DCs responding to TLR signals during an *in vitro* culture is an artifact. Since many of these *in vitro* studies (mouse or human) that

employed either bone marrow cells or monocytes to generate DCs did not specifically remove the T cells from the bone marrow that was used to generate DCs, they created a source for endogenous IFN-γ or other T cell products that are not normally present within the peripheral tissues where DCs are residing. Moreover, culture conditions that are usually used for the stimulation of DCs with various TLR ligands contain recombinant IL-4 and GM-CSF (DC growth/differentiation factors) both of which have been shown to be important contributors IL-12p75 production from DCs (Hochrein et al., 2000). There is also evidence that physical manipulation and adherence of DCs to the tissue culture vessels will lead to IL-12p75 production (Abdi K. unpublished observations).

It has been shown that recombinant IL-12 is an important cofactor in the generation of cytotoxic T lymphocytes (CTL) from Con-A activated naïve thymocytes (Bertagnolli et al., 1992). In Chapter-II, it was demonstrated that among various cytokines tested, recombinant IL-4 was the only cytokine that could be added alone to the culture of Con-A activated naïve thymocytes, leading to the generation of strong CTL responses. In addition, this IL-4-dependent CTL response ((Bertagnolli et al., 1991) was shown to be driven by the induction of endogenous IL-12 (Chapter-II). This CTL response could be blocked in the presence of recombinant mouse p40 homodimer, a known antagonist of IL-12 biological activities (Chapter II). The study demonstrated that endogenous IL-12p75 synergized with recombinant exogenous IL-4 to support the development of a strong CTL activity (Chapter-II). This supports the idea that IL-4, a T_H2-type cytokine, is instrumental in the secretion of

IL-12 from APCs, and points to the fact that *in vitro* culture conditions that are usually used to generate DCs are conducive for IL-12p75 production. Once these cells are harvested, washed, and re-cultured in medium that does not contain any of the growth factors (IL4 & GM-CSF), then engagement of single TLRs (Hochrein et al., 2000) or multiple TLRs are inadequate signals to induce IL-12p75 production (Abdi K. manuscript in preparation).

Therefore, neither a single TLR agonist nor multiple TLR agonists is capable of inducing IL-12p75 when culture conditions are devoid of these factors. This is clearly demonstrated in Chapter III. By using ELISPOT assay, it was shown that in the presence of Mycobacterium whole cell lysate which contains multiple TLR ligands, only p40 could be secreted, even at the single cell level, IL-12p75 could not be detected. These findings further substantiate the hypothesis behind this thesis that proposes that T cells or T cell factors are playing an important role in induction and secretion of IL-12p75 and that sole engagement of TLRs would not have the same effect (Chapter-I).

One important observation from Chapter-III is the demonstration that only antigen-activated effector and/or memory but not naïve T cells are capable of inducing IL-12p75 from DCs. This finding is in conflict with the prevailing view that emphasizes that IL-12p75 is secreted during the primary immune response and that it is instrumental in the initiation and polarization of T_H1-type immunity (Paul 2008, Article in Pillars of Immunology) (Hsieh et al., 1993). We followed up on this observation and since induction of IL-12p75 in the presence of effector/memory but not that of naïve T cells co-cultured

with DCs correlated with the presence of IFN-γ, we asked if addition of recombinant IFN-γ to the co-culture of naïve T cells with DCs would reconstitute IL-12p75 production. This was not the case. When IFN-γ was added to the co-culture of naïve T cells and DCs, it had no effect on the secretion of IL-12p75 (Chapter-IV). To our surprise, neutralization of IFN-γ with the help of monoclonal antibodies in the co-culture of effector/memory T cells with DCs did not lead to a significant reduction in IL-12p75 production either.

These data suggest that, in the presence of antigen-activated T cells, secretion of IL-12p75 from DCs is independent of IFN-γ (Chapter-IV). Furthermore, a kinetic study of IL-12p75 secretion from the co-culture of naïve T cells with DCs demonstrated that at no time during the six-day assay, were naïve T cells capable of inducing IL-12p75 production from DCs. This finding is not in agreement with the common belief that IL-12 is the initiator of a T_H1 response. In contrast, in parallel experiments, antigen-activated T cells were demonstrated to be potent inducer of IL-12p75 from DCs. Its induction was rapid and declined by over time (Chapter-IV).

This was an unanticipated finding because collectively our data and the data from the literature are all in support of IFN-γ being an important contributor of IL-12p75 production when TLR agonists are used. Thus, in order to have a better understanding of if endogenous IFN-γ secreted from T cells plays any role in induction of IL-12p75 when antigen-activated T cells interact with DCs, we generated 5C.C7 transgenic mice that are deficient in the IFN-γ gene. Splenic T cells from these mice were used in our co-culture assay to test what effect the absence of IFN-γ from T cells would have on IL-12p75 production

by DCs. We found that antigen-activated T cells from IFN-γ-deficient mice had absolutely no problem inducing IL-12p75 from LPS-activated DCs in comparison to their wild-type counterpart.

We thought, possibly, that the initial secretion of IL-12p75 might be IFN-γ-independent, but at a later time point, IFN-γ would be a necessary factor for its secretion. When we compared the kinetic of antigen-activated T cells obtained from wild-type mice with IFN-γ-deficient mice, we found that that IFN-γ was dispensable in this co-culture system. Both wild-type and IFN-γ-deficient T cells rapidly induced IL-12p75 from DCs with almost the same kinetics and its induction declined over time. We have found that the same applies *in vivo*. When IFN-γ-deficient mice were challenged with Superantigen, we could detect IL-12p75 in their serum (Chapter-IV); however, as it had been demonstrated, injection of LPS did not resulted in serum IL-12p75 (Chapter-III). These data suggest that induction and secretion of IL-12p75 from DCs co-cultured with antigen-activated T cells is IFN-γ-independent. Moreover, induction and secretion of IL-2p75 from DCs in response to bacteria or their products is different when T cells are interacting with DCs as opposed to microbes. It seems that in the presence of antigen-activated T cells, DCs do not require an IFN-γ signal to induce IL-12p75 secretion. In contrast, when DCs encounter a TLR agonist, in the absence of T cells, the presence of the IFN-γ is required to produce IL-12p75 (Chapter-IV). Furthermore, data from Chapter-IV resolves one of the outstanding questions concerning IL-12p75 immunobiology: that during an immune response, when an antigen-bearing DCs interacts with T cells, which comes first, IL-12p75 or IFN-γ? One could deduct from these data that, early during the

innate immune responses, IFN-γ can be secreted from a population of innate leukocytes (NK, NKT or gamadelta cells), independent of T cells, in a non-antigen specific manner in the absence of IL-12p75. However, if this early IFN-γ is insufficient to contain and clear the pathogen then, IL-12p75 is produced during the interaction of antigen-activated T cells with antigen-bearing DCs to maintain an ongoing T_H1 response once it has been established by other means. The current data are in support of this presumption (K. Abdi manuscript in preparation) (Feng et al., 2005; Stobie et al., 2000). Thus, one important conclusion that could be taken away from chapter-IV is that during cognate antigen-driven responses, IL-12p75 can be secreted from DCs in the presence of antigen-activated but not naïve T cells and this IL-12p75 production is independent of IFN-γ.

During the study discussed in Chapter-III we also noticed that LPS-preactivated DCs no longer responded to LPS plus IFN-γ, which is normally a strong signal for IL-12p75 production. However, these cells were perfectly fine in secreting IL-12p75 when they were co-cultured with antigen-activated memory or effector T cells (Chapter-III). This was an unexpected result; it did not corroborate the phenomenon known as DCs "exhaustion"! It has been suggested that LPS-preactivated DCs become refractory to restimulation with LPS or CD40L and loose their IL-12p75 production. This results in a switch from a T_H1- to T_H2-inducing cytokines (Langenkamp et al., 2000). This study concluded that LPS-preactivated DCs have "exhausted" their IL-12p75 production. This conclusion led to a model that emphasizes two independent states for DCs. Early DCs, that synthesize cytokine, have been termed "active" and late-DCs that

have lost cytokine production, in which it has been referred to as "exhausted" DCs (Reiner et al., 2007)

We thought perhaps our LPS-activated DCs were not "exhausted" and that this might be the reason why they were still making IL-12p75 when they encounter T cells. For this reason, we stimulated DCs under conditions that are known to be optimal for IL-12p75 production. We have shown that IFN-γ is a critical co-factor for IL-12p75 production (Chapter-III). Therefore, we stimulated DCs with LPS alone or LPS plus IFN-γ and then re-stimulated them after >24-h with LPS alone, LPS plus IFN-γ or co-cultured them with effector/memory T cells. We found that the combination of LPS plus IFN-γ induced IL-12p75 only when DCs had no prior exposure to LPS (Chapter-V). It seems that LPS- or LPS plus IFN-γ-activated DCs were rendered refractory to further re-stimulation with LPS or LPS plus IFN-γ, since no IL-12p75 was detected after the second stimulation. However, effector/memory T cells induced high levels of IL-12p75 from both LPS- and/or LPS plus IFN-γ-activated DCs. Unlike IL-12p75, irrespective of prior LPS- or LPS plus IFN-γ activation in the same culture supernatant, DCs secreted high levels of the p40 subunit in response to LPS alone. As a result we have concluded that these DCs are not "exhausted;" they are viable and they are capable of actually making copious amount of p40, but not IL-12p75. However, if they receive appropriate signal(s), in this case antigen-activated T cells, these cells are perfectly capable of making IL-12p75 (Chapter-V).

We also found that T_H0 and T_H1 clones are potent inducers of IL-12p75 production from DCs. In contrast, T_H2 clones were unable to induce IL-12p75 production (Chapter-V). These data collectively

suggest that the exchange of information between antigen-bearing DCs and antigen-specific T cells and their cytokine milieu constitute a crucial determinant in the outcome of immune responses. Thus, once DCs encounter pathogenic bacteria, they shut down their response toward further stimulation by microbes or their products even if a strong stimulus such as LPS plus IFN-γ that usually leads to IL-12p75 production is used. They retain the information and wait, these cell are not "exhausted" but perfectly capable of making IL-12p75 when they encounter appropriate antigen-activated T cells.

The idea that early DCs are active and synthesize proinflammatory cytokines while late DCs are "exhausted" and have lost the ability to produce such cytokines has recently been extended to macrophages (Foster et al., 2007). The study in chapter-V demonstrates for the first time that DCs are not "exhausted," that they are perfectly capable of making IL-12p75 when they encounter the right signal(s). These cells are simply waiting for an appropriate signal(s) to deliver IL-12p75 to the right cells, in the right place and at the right time to ensure the careful and specific delivery of this potent cytokine.

What role does IL-12p75 play during the host response to pathogens? Collectively, data from this thesis and the literature suggest that IL-12p75 may not be the "bridge" that initiates the proinflammatory response to pathogens, but is rather a positive feedback signal that can maintain an ongoing T_H1-dependent response once it has been established by other factors. This idea has been supported by various data (Mullen et al., 2001; Park et al., 2000; Stobie et al., 2000). Recently, in an *in vivo* study that used a

mouse model of *Mycobacterium tuberculosis*, it was shown that IL-12p75 played a major role in the maintenance of IFN-γ-producing effector $CD4^+$ T cells and its continuous production is required for maintenance of T_H1 responsiveness to this pathogen (Feng et al., 2005). A recent review by Cooper and Khader (Cooper and Khader, 2007) supports the principle behind this thesis that argues against an early role for IL-12p75 as an initiator of immune responses and emphasizes that it is the p40 protein that is secreted early and possibly plays an important role during the host response to pathogens (Chapter-I).

Previously, it was shown that p40-deficeint mice have drastically reduced antigen-specific DTH responses and IFN-γ production in comparison to p35-deficient mice, and it was shown that IL-12p75 is not essential in initiating these responses, in particular in the mouse model of *Mycobacterium tuberculosis* (Cooper et al., 2002). The most recent data further support p40's role as an important player during the early host response to pathogens; it has been suggested that p40 plays a role in DCs migration from lung to lymph node (Khader et al., 2006). In the mouse model of *Mycobacterium tuberculosis* DCs lacking the p40 gene failed to migrate toward CCR7 ligands when these cells were stimulated with bacteria. This migration was p40 but not IL-12p75-dependent (Khader et al., 2006). These observations were extended to the *Yersinia pestis* model showing that potent and rapid production of p40 correlated with the migration of DCs, suggesting that p40 plays an agonist role in initiation of immunity to bacteria (Robinson et al., 2008). Furthermore, it was demonstrated in gene-

targeted mice that express p40 with fluorescent reporters YFP or GFP that DCs migrate to the draining lymph nodes after s.c. vaccination with *Listeria monocytogenes,* promoting a T_H1 mediated differentiation (Reinhardt et al., 2006). These findings are in support of the original hypothesis suggesting that p40 is not simply a component of IL-12p75 but an important molecule in its own right, and its early secretion in responses to pathogens plays an essential role during the innate immune response (Chapter-I).

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Acknowledgements:

This work is dedicated to my late sister Maryam

I would like to thank my brother Kambiz for his unconditional support, without it, I would not have been able to move from Boston to the National Institute of Health in Bethesda Maryland to do this work. I am much obliged to Professor Hidde Ploegh for initiating the spark in me to follow up on this idea and his encouragements, support and collaboration to search for novel protein-protein interactions and also for the Dutch translation of the abstract. He read the very first draft of my hypothesis in 1999 and took it seriously in a climate that IL-12 dominated most immunologist view of the early host response to pathogens. I am grateful to Dr. Polly Matzinger who also read my hypothesis early on and immediately recognized its implication and provided me with an environment to do the work. I am thankful to Dr. Ronald Schwartz for his support throughout my appointment in the Laboratory of Cellular and Molecular Immunology at the NIH and also for critical reading of the thesis. I would like to thank Dr. Nevil Singh for collaboration and help in sorting and skewing of T cells that were used in the chapter IV and V. I would also like to thank Dr. Quirijin Vos for helping me with the Dutch translation of abstract and Mrs. Marcia Davis for editing the entire thesis.