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Invited Review

Current understanding of macrophage type 1 cytokine responses during intracellular infections

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Summary. Macrophages are important effector cells in cell-mediated immunity against intracelllular infection. Among cytokines that macrophages are able to release are IL-12 and TNFa. IL-12 is a critical linker between the innate and adaptive cell-mediated immunity, capable of Thl differentiation and IFNy release by T and NK cells. IFNy is critically required for the activation of macrophage bactericidal activities. Recently emerging evidence suggests that macrophages are able to release not only IL-12 and TNFa but also IFNy. However, the mechanisms that control the release of each of these type 1 cytokines in macrophages appear different. While macrophages release TNFa in an indiscriminate and IL-12-independent way, the release of IL-12, particularly bioactive IL-12 p70, and IFNy is under tight control. We are just beginning to understand what controls the release of IL-12 p70, a question of fundamental importance to understanding the mechanisms underlying the initiation of cell-mediated immunity. Our recent findings have shed more insights into the regulatory mechanisms of macrophage IFNy responses. It has become evident that IL-12 is required not only for Thl differentiation but also for IFNy responses by both T cells and macrophages during intracellular infection. In this overview, we have discussed about the current understanding of the regulation of macrophage type 1 cytokine responses during intracellular infection, based upon the recent findings from us and others.

Key words: Macrophages, Lung, Mycobacteria, Cytokines, Intracellular infection

Introduction

Many microbes are obligate intracellular pathogens of macrophages. It is generally believed that eradication of these pathogens lies in adequate activation of macrophages which is in turn dependent upon type 1 cytokines, particularly IFNy, released by activated antigen-specific T cells. However, recent evidence from us and others indicates that activated macrophages themselves can be a significant source of IFNy in addition to other type 1 cytokines, during intracellular infection. This thus suggests that macrophages may play a much greater role than previously thought in cell-mediated immunity. Furthermore, it also suggests that the critical role by T cells in host defense against intracellular infections goes beyond just the provision to macrophages of soluble activation signals such as IFNy, which we know now, can be at least in part fulfilled by rnacrophages themselves (Fenton et al., 1997; Munder et al., 1998; Wang et al., 1999). Emerging evidence supports a critical role of T cells in host resistance to intracellular infection via their cytotoxic activities (Bonecini-Almeida et al., 1998; Oddo et al., 1998). We are just in the beginning to understand the regulatory mechanisms of macrophage activation and the interaction between macrophages and T cells involved in anti-intracellular infection immune responses. Further understanding in this regard will undoubtedly foster the development of better vaccines and medicine for intracellular infectious diseases.

Type 1 and type 2 cytokines

Cytokines that are involved in adaptive immunity can be divided into Th1 and Th2 categories, depending on whether they are released primarily by murine Thl or Th2 clones (Mosmann and Sad, 1996). The Thl cytokines typically include IL-2 and IFNy whereas Th2 cytokines include IL-4 and IL-5. However, it is known that a number of cytokines can be released by both Thl and Th2 clones and these include IL-3, GM-CSF and TNFa. Furthermore, there are cytokines such as IL-12 and IL-18 that are not released primarily by either Th clone but are critical to Thl differentiation and/or Thl cytokine production. It is of help to group these immune-modulatory cytokines according to the type of tissue responses they mediate (Xing et al., 1999). The type 1 cytokines include IL-12, IFNy and TNFa which are

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involved in the development of cell-mediated immunity or type 1 tissue responses characterized by macrophage granuloma formation and T cell activation. Type 1 tissue responses are essential to host defense against intracellular infections by bacteria, protozoan parasites or fungi. The type 2 cytokine category encompasses IL-4, IL-5, IL-3 and GM-CSF which play an important role in the development of type 2 tissue responses. Type 2 tissue responses usually involve both humoral and cell immune components, characterized by increased antigen-specific IgE/IgG1, eosinophilia, and mast cell and T cell activation, and they underlie the pathogenesis of allergic diseases and play an important role in host responses to extracellular metazoan parasitic infection. In other instances, although the characteristic tissue responses may not be a feature, type 1 or type 2 cytokines constitute part of molecular mechanisms of host responses. These include organ-specific autoimmune diseases, allograft rejection and recurrent abortion (type 1 responses), and successful pregnancy (type 2 responses) (Romagnani, 1997).

Type 1 cytokines and mycobacterial infection

IL-12 is released from activated macrophages or dendritic cells and drives a unique Th1 phenotypic differentiation and IFNγ release from antigen-specific T cells. IL-12 also stimulates NK cells to release IFNγ. Bioactive IL-12 (IL-12 p70) is a heterodimeric cytokine composed of p35 and p40 subunits, encoded by two separate genes (Trinchieri, 1997; Okamura et al., 1998). The p35 subunit is constitutively expressed and remains intracellular in a wide range of cell types including macrophages while it can be further induced under inflammatory and immune conditions. In comparison, expression of the p40 subunit is highly regulated and is expressed primarily by macrophages and dendritic cells. Normally, cells express the p40 subunit at a level 10 to 100 higher than the p35 subunit, thus resulting in a similarly greater secretion of IL-12 p40 monomers and/or homodimers than IL-12 p70 heterodimers. IL-12 p40 has been found to be, particularly in rodents, antagonistic to bioactive IL-12 p70 by competing for the IL-12 receptor. IFNγ has long been considered to be primarily a T cell-derived cytokine and is a key macrophage activator capable of markedly enhancing MHC molecule expression and bactericidal activities of macrophages in addition to its role in Th1 differentiation and activation (Orme et al., 1993). TNFα is released primarily from stimulated macrophages but can also be released from activated T cells. TNFα has broad stimulatory effects on a variety of cell types including macrophages and endothelial cells. We have found that all of these type 1 cytokines but not type 2 cytokines IL-4 and GM-CSF are elevated both in the lung and peripheral blood during pulmonary mycobacterial infection (Wakeham et al., 1998).

While the relative importance of type 1 cytokines IL-12, IFNγ and TNFα in host defense against mycobacterial infection still remains to be completely understood, we and others have recently provided experimental evidence to support a critical role of each of these cytokines in host defense against mycobacterial infection. Mice deficient in IL-12 lacked IFNγ and TNFα responses, granuloma formation and suffered an increased susceptibility to intracellular infections established via a systemic or local pulmonary route (Mattner et al., 1996; Cooper et al., 1997; Wakeham et al., 1998; Xing et al., 1998a). Mice deficient in IFNγ or TNFα suffered a similarly weakened immune protection from mycobacterial infection (Cooper et al., 1993; Flynn et al., 1993, 1995). Very recently, mice deficient in IL-18 have also been found to have impaired Th1 responses to mycobacterial infection (Takeda et al., 1998). Since unlike IL-12, IL-18 seems to have no direct effect on Th1 differentiation, the mechanisms by which IL-18 contributed to the overall Th1 responses remain fully understood. Of particular interest, recent findings from us (unpublished data) and others (Oxenius et al., 1999) suggest that in sharp contrast to intracellular bacterial infection, IL-12 is not required in a significant manner for type 1 immune responses to local lung or systemic viral infection in vivo.

The critical role of these type 1 cytokines in host resistance to intracellular infections by mycobacteria or other intracellular pathogens has been further supported by recent findings in humans. Patients with a genetic deficiency in the IFNγ or IL-12 receptor have increased susceptibility to atypical mycobacterial infections or developing systemic tuberculous infection following BCG vaccination (Jouanguy et al., 1996; Altare et al., 1998a). Likewise, hosts genetically deficient in the gene coding for IL-12 suffer disseminated BCG and *Salmonella* infection (Altare et al., 1998b).

Regulation of IL-12 responses in macrophages and dendritic cells during intracellular infection

IL-12 is released primarily by macrophages and dendritic cells and has been believed to act primarily upon T and NK cells. IL-12 thus serves as an important linker between the innate and adaptive cell-mediated immunity. However, macrophages or dendritic cells constitute the first defense line confronting almost any invading organisms/antigens or stimuli and yet, the host does not always mount a cell-mediated immune response. Thus, IL-12 and the nature of antigens likely operate as a crucial check point to determine whether a type 1 immune response should result. So little is known about exactly how the host makes such a decision at this point. Conceivably, whether IL-12 is released upon encounter of macrophages/dendritic cells with organisms or antigens or any other stimuli, which form of IL-12, p40 or bioactive p70, is released, how sustained the level of IL-12 p70 is, and whether there is active antigen presentation by these cells, all play a part in host's decision-making (Fig. 1). Therefore, it is of particular importance to understand the regulation of IL-12,

particularly IL-12 p70 in macrophages and dendritic cells.

The majority of studies have been carried out in vitro or in vivo by examining either gene expression or total IL-12 protein (p40 and p70) or only p40 protein release by cells, and little is known about the relative level of IL-12 p40 and bioactive IL-12 p70. IL-12 is elevated at the tissue sites or by peripheral blood monocytes in patients with tuberculosis or leprosy (Zhang et al., 1994). We and others have observed markedly released or expressed IL-12 during pulmonary or systemic mycobacterial infection in mice (Cooper et al., 1997; Wakeham et al., 1998; Xing et al., 1998a). We have further demonstrated that lung macrophages are a significant source of IL-12 during pulmonary mycobacterial infection and these macrophages release further increased amounts of IL-12 upon stimulation by LPS or mycobacterial antigens PPD (Wang et al., 1999). Most, but not all, of intracellular pathogens including both bacteria and viruses or microbial products have been found capable of releasing IL-12 from macrophages in vitro. Live *Mycobacterium* tuberculosis or BCG, live *Listeria*, LPS and peptidoglycans from certain types of bacteria are inducers of total IL-12 release from macrophages (Flesch et al., 1995; Skeen et al., 1996; Ladel et al., 1997; Lawrence and Nauciel, 1998). In comparison, dead *Listeria*, latex beads and *Leishmania* amastigotes cannot effectively induce IL-12 release (Skeen et al., 1996; Weinheber et al., 1998). These findings convey a clear message that unlike other monokines such as TNFα, IL-1 and IL-6, IL-12 release is under tight control in macrophages/monocytes (Fig. 1). It is worth noting that other macrophage agonists have not been tested for comparison and whether IL-12 p70 is released under all of these conditions is still unclear. In this regard, a very recent study carried out by using human monocytes and alveolar macrophages has demonstrated that while LPS up-regulates expression of both IL-12 p35 and p40 genes, it induces the release of only IL-12 p40 but not IL-12 p70 and, IL-10 release stimulated by LPS is found accountable for such an inhibition of IL-12 p70 release (Isler et al., 1999). These important findings suggest that whether endogenous IL-10 release is induced is one mechanism of inhibitory nature that controls the form of IL-12 released by macrophages. We are currently investigating the regulation of IL-12 release in models of LPS- or Gram-negative bacterium-induced acute lung inflammation.

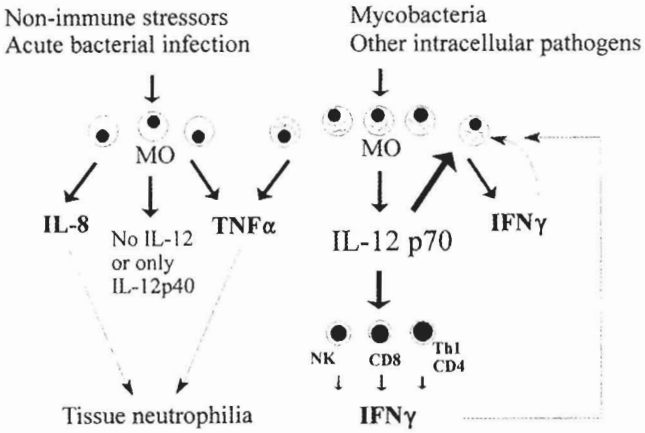

Fig. 1. Schematic presentation of the mechanisms that determine when a type 1 cell-mediated immune response will result. Upon encounter with non-immune stressors or bacteria that normally cause an acute inflammatory response, macrophages do not release bioactive IL-12 p70. Released neutrophil chemokines such as IL-8 and pro-inflammatory cytokine TNFα will orchestrate the development of tissue neutrophilia and macrophage/neutrophil activation. Such a tissue response is transient, only involving the innate immune components. However, upon encounter with intracellular pathogens and perhaps when co-stimulated by IFNγ that is released from NK cells via an IL-12-independent manner, macrophages release bioactive IL-12 p70. On one hand, this macrophage-derived IL-12 drives Th1 differentiation and IFNγ release from Th1 cells and on the other hand, it stimulates IFNγ release from macrophages that have been infected with intracellular pathogens. This macrophage IFNγ response is tightly controlled by both IL-12 and intracellular infection and may represent the most effective auto-stimulatory mechanism to activate macrophages. During these responses, TNFα is released in an IL-12-independent manner and its level may be in check by both IL-10 and IL-12.

The positive signals, in addition to microbial agents, that are required for active IL-12 release by macrophages, have remained to be completely understood. It has been believed that IL-12 is the first cytokine released from macrophages which ignites the downstream events of cascade including IFNγ responses in cell-mediated immunity. However, recently emerging experimental evidence has challenged this notion and suggested far more complicated regulatory mechanisms involved in the up-regulation of IL-12. It has been noticed that the presence of NK cells or activated T cells facilitates IL-12 release or gene expression in human macrophages/monocytes infected with BCG or *Cryptococcus neoformans* (Matsumoto et al., 1997; Retini et al., 1999). In addition to potential soluble molecules such as IFNγ that may have played a role, the interaction between CD40 on macrophages and CD40 ligand on T cells was found to play a role (Retini et al., 1999). The role of IFNγ in IL-12 induction in macrophages has been speculated for quite some time. While the data are still conflicting, IFNγ has been shown to have either potent priming effects (Matsumoto et al., 1997; Jiang and Dhib-Jalbut, 1998) or stimulatory effects on IL-12 release from macrophages (Isler et al., 1999; Retini et al., 1999). Priming by rIFNγ was found to be required for BCG- or LPS-induced IL-12 p40 release by bone-marrow-derived macrophages (Flesch et al., 1995). Furthermore, in mice deficient in receptors for either IFNγ or TNFα, IL-12 p40 release was found dependent upon the function of either IFNγ or TNFα (Flesch et al., 1995). Although all of these studies have restrictions in various ways, they collectively suggest a role of IFNγ in active IL-12 release. Since IFNγ release by T or NK cells is believed largely dependent up the action of IL-12 and/or IL-18, the intriguing question is whether there is early IFNγ release independently of IL-12 or IL-18. Much less is known about the regulation of IL-18 during intracellular infection and it is known that IL-18 can be released,

compared to IL-12, by a much wider spectrum of cell types including tissue structural cells. It is possible that IL-18 is regulated in different ways from IL-12 and it accounts for such an early IL-12-independent IFNγ release from NK cells. Apparently, the full understanding of these processes entails vigorous studies ahead. Of particular interest and importance is the recent finding that it is dendritic cells but not macrophages that rapidly produce IL-12 in the early stage of *Toxoplasma gondii* infection (Sher and Reis e Sousa, 1998) and this early IL-12 production is IFNγ-independent. This finding suggests an alternative mechanism for macrophage IL-12 release, which is that dendritic cell-derived IL-12 serves as an ignition switch for type 1 cytokine responses in macrophages by stimulating NK cells to release IFNγ which will in turn trigger massive IL-12 release from macrophages.

Much less is known about how IL-12 p70 release is regulated in macrophages or dendritic cells (DC) during intracellular infection. Recent evidence suggests that examination of only IL-12 p35 and p70 mRNA expression can be misleading (Isler et al., 1999). Based upon limited information available to date, it appears that much less IL-12 p70 is released than IL-12 p40 in both in vitro and in vivo systems. A recent study by using human macrophages has provided evidence to suggest that IFNγ and/or activated lymphocytes are required for enhanced mycobactericidal activities and the release of IL-12 p70 but not p40 (Bonecini-Almeida et al., 1998). A different study has compared the release of IL-12 p70 by human bone marrow-derived macrophages, peripheral blood monocytes and monocyte-derived dendritic cells (Smith et al., 1998). The overall message from this study is that LPS stimulation alone releases lots of IL-12 p40, particularly from dendritic cells but no or little IL-12 p70 unless IFNγ is present. A tight control of IL-12 p70 release by both macrophages and dendritic cells was further demonstrated in a murine in vitro system. While both dendritic cells and macrophages readily release IL-12 p40 upon stimulation by *Leishmania* amastigotes, LPS+IFNγ or LPS+IFNγ+amastigotes, they release IL-12 p70 only when stimulated with amastigotes+IFNγ (DC) or LPS+IFNγ (macrophages), regardless of significant TNFα released under all of these stimulatory conditions. Of importance, IFNγ alone has no effect on IL-12 p70 release by dendritic cells. Even lesser information is available regarding the level of IL-12 p70 protein in vivo. In a model of *Listeria* or *Brucella* infection, IL-12 p70 protein is detected for a brief time post-infection in serum or spleen homogenates. This IL-12 p70 release appears TNFα-dependent but IFNγ-independent (Zhan and Cheers, 1998). And dead bacteria are ineffective in inducing IL-12 p70 release.

Regulation of IFNγ responses in macrophages during intracellular infection

It has been believed that macrophages are incapable of active IFNγ release until recently. Indeed, macrophages do not release IFNγ under conventional stimulatory conditions that result in the release of a wide array of other cytokines (Fultz et al., 1993; Marzio et al., 1994; Fenton et al., 1997). However, there has been evidence that human alveolar macrophages express IFNγ mRNA during pulmonary tuberculosis (Robinson et al., 1994). Perhaps, the first authentic evidence that macrophages are able to release IFNγ protein has been provided by Puddu et al. (1997). They found that murine peritoneal macrophages cultured in sustained presence of recombinant IL-12 could release IFNγ whereas freshly isolated cells released little in response to IL-12 stimulation. This finding suggests that macrophage IFNγ response requires multiple signals. Subsequent to this finding, Fenton et al. (1997) reported that human alveolar macrophages could release IFNγ in response to *Mycobacterium tuberculosis* infection but not LPS. Recently, by using murine bone marrow cell-derived macrophages, Munder et al. (1998) have reported that IL-12 or IL-18 alone only induces IFNγ mRNA expression but not protein release whereas stimulation with both IL-12 and IL-18 can induce the release of IFNγ protein. While these studies have established that macrophages are able to produce IFNγ under certain conditions, they involved artificial in vitro manipulations and the precise regulatory mechanisms governing IFNγ release in macrophages still remain to be fully understood.

Role of IL-12 and intracellular pathogens

We have been interested in dissecting the regulatory role of macrophages in cell-mediated immunity to pulmonary mycobacterial infection. Following the demonstration that the lack of IL-12 led to a lack of IFNγ and severely impaired TNFα responses in the lung and by T cells during pulmonary mycobacterial infection (Wakeham et al., 1998), we set out to examine the contribution of pulmonary macrophages to type 1 cytokine responses with the expectation that macrophages would be a primary source of IL-12 and TNFα but not IFNγ. We found, however, that lung macrophages freshly isolated from infected immune-competent mice spontaneously released not only IL-12 and TNFα but also IFNγ (Wang et al., 1999). The release of these type 1 cytokines was remarkably further enhanced by exposure to mycobacterial antigens PPD or LPS. To our surprise, while lung macrophages from infected IL-12-/- mice released no IFNγ at all, they released, spontaneously or upon stimulation, high levels of TNFα. These findings represent the first in vivo evidence that macrophages are able to release IFNγ and that IL-12 is required, directly or indirectly, for macrophage IFNγ but not TNFα release, during pulmonary mycobacterial infection. These findings also help interpret our in vivo observations that IFNγ was almost completely absent in the lung whereas TNFα response was severely, but not completely, inhibited over a course of 71 days

(Wakeham et al., 1998). IL-12 is apparently required for IFNγ release from both T cells and macrophages the lack of which resulted in impaired macrophage accumulation. However, a minimum number of macrophages accumulating in the lung that we observed likely remained capable of releasing TNFα since TNFα release by macrophages is not dependent upon IL-12 (Wang et al., 1999).

The limitation to our in vivo/ex vivo observations is that they would not allow us to dissect the factors that were directly required for macrophage IFNγ responses since macrophages in the lung of IL-12-/- mice were infected already by mycobacteria and lacked the stimulation not only by IL-12 but also by other cytokines including IFNγ. In order to investigate the regulatory mechanisms, we undertook an in vitro approach by using freshly isolated lung macrophages from non-infected naive IL-12-/- mice. We found that IL-12-/- macrophages released no IFNγ but lots of TNFα in response to LPS stimulation or *M. bovis* BCG infection (Wang et al., 1999). IL-12 alone, even when used at high doses, was only a weaker stimulator of IFNγ, which supports the findings by Munder et al. (1998). However, large quantities of IFNγ were released by these macrophages when co-stimulated with LPS+IL-12 or BCG+IL-12. The latter released the greatest amount of IFNγ from macrophages. These findings indicate that both IL-12 and microbial agents are critically required for active release of IFNγ, but not TNFα, and suggest that IFNγ, but not TNFα, response in macrophages is under tight control. Furthermore, these findings provide the explanation regarding why macrophages, a cell type of the first defense line, are built up with armamentaria to respond to all kinds of encounters and yet, the host does not always mount a type 1 immune response. It is plausible to speculate that the key is the nature of stimulus which in turn determines the form of IL-12, p40 or p70 or both, released by macrophages. Once sufficient IL-12 p70 is released, this IL-12 p70 will not only activate T cells but also, together with intracellular pathogens, macrophages, to release IFNγ (Fig. 1). Apparently, the question still remains regarding whether the second signal that acts together with IL-12, has to be an intracellular pathogen in vivo since in our in vitro system, LPS+IL-12 also released IFNγ. It is plausible to speculate that since LPS alone does not seem able to stimulate IL-12 p70 release from macrophages (Smith et al., 1998), it is unlikely that macrophages will be turned on to release IFNγ during endotoxic pneumonia although they produce many other pro-inflammatory cytokines (Xing et al., 1994, 1998b) (Fig. 1).

Since the above experimental design does not allow to address whether dead intracellular organisms or various types of antigens, in the presence of IL-12, can also stimulate IFNγ release from macrophages, we tested the effect of heat-inactivated *M. bovis* BCG, mycobacterial antigens PPD or ovalbumin. Of interest, in the presence of exogenous IL-12, ovalbumin failed to induce any IFNγ release whereas heat-inactivated BCG or PPD induced moderate release of IFNγ, similar to that induced by IL-12+LPS (Wang et al., 1999). We found that ovalbumin did activate macrophages as demonstrated by increased TNFα release. These findings suggest that in addition to IL-12, the second signal must be microbial in nature, live or dead intracellular pathogens or intracellular pathogen-derived antigens.

Role of IFNγ, IL-18 and TNFα

IFNγ has been shown to be able to induce its own release from macrophages (Marzio et al., 1994). We therefore investigated whether exogenously added IFNγ, in doses similar to IL-12 that we used, could replace IL-12 to induce endogenous IFNγ release in the presence of live BCG. Without BCG, IFNγ, particularly in moderate doses, induces low levels of its own secretion. With BCG, IFNγ induced only a small increase in the secretion of endogenous IFNγ, thus suggesting that IFNγ cannot replace the role of IL-12 (Wang et al., 1999).

IL-18 is also called IFNγ-inducing factor. Not only can macrophages release this cytokine but they respond to this cytokine (Munder et al., 1998; Okamura et al., 1998). At this point, although we have not been able to measure the level of IL-18 release by stimulated lung macrophages, it is likely that this cytokine was released. If this was the case, our observation that without IL-12, BCG infection alone cannot release IFNγ already indicates that IL-18 cannot compensate for the function of IL-12. However, we could not rule out that endogenous IL-18 may have potentiated the effect of IL-12 on macrophage IFNγ release, as it does on NK and T cells (Trinchieri, 1997). We thus investigated the role of endogenous IL-18 by using an anti-IL-18 monoclonal antibody. We found that endogenous IL-18, if any, played a marginal role in IFNγ responses in IL-12/BCG-stimulated macrophages, thus establishing a unique role of IL-12 in macrophage type 1 cytokine responses (Wang et al., 1999) (Fig. 1).

Since we found that TNFα was released upon stimulation by any macrophage agonists, independent of IL-12, we examined whether this cytokine may have potentiated the effect of IL-12 on macrophage IFNγ release by using a TNFα neutralizing polyclonal antibody. Similar to the results with anti-IL-18 antibodies, endogenous TNFα played a marginal, if any, role (unpublished data).

Regulation of TNFα responses in macrophages during intracellular infection

We have provided unequivocal evidence that TNFα release represents a non-specific response of macrophages to almost any stimuli including non-microbial antigen (ovalbumin), microbial products (LPS) or antigens (PPD) or live or dead mycobacteria (BCG) in an IL-12-independent manner (Wang et al., 1999). These findings fit well the role of this cytokine in host responses to "stress" or "alarm" signals (Xing et al., 1999). This non-selective nature of macrophage TNFα responses contrasts the tight control of macrophage IFNγ

responses which require con-current stimulation by IL-12 and intracellular pathogens/antigens (Wang et al., 1999) (Fig. 1).

Since IL-12, IFNγ and TNFα as well as mycobacteria are all present in the lung during mycobacterial infection, we have further investigated the role of type 1 cytokines IL-12 and IFNγ in macrophage TNFα responses (unpublished data). Contrast to our expectation, IL-12 alone had little effect on TNFα release from IL-12-/- lung macrophages whereas IFNγ released TNFα in a dose-dependent fashion. These findings suggest that IL-12 has a specialized effect on macrophages and is involved primarily in macrophage IFNγ responses whereas IFNγ has a global macrophage activation effect. Thus, that lung macrophages from infected IL-12-/- mice lacked surface activation markers MHC II and CD11b (Wang et al., 1999), was likely a result of the lack of IFNγ but not IL-12.

Not only is IL-12 alone unable to directly induce TNFα release from macrophages, but we have recently found that it inhibits TNFα release by macrophages when co-stimulated with both IL-12 and live BCG mycobacteria but not LPS. These findings suggest that while stimulating IFNγ release from macrophages during intracellular infection, IL-12 operates to put the level of TNFα responses by macrophages under check. This may reflect an active mechanism by which the host attempts to minimize the toxic effect of TNFα since this cytokine tends to be over-produced.

Concluding remarks

IL-12 plays a unique role in host defense against intracellular infections. Its function cannot be compensated for by other IFNγ-inducing factors such as IL-18 during pulmonary mycobacterial infection. IL-12 is a critical type 1 cytokine, critically required not only for Th1 differentiation but also for IFNγ responses in both T cells and macrophages. While it by itself does not induce TNFα release from macrophages, it may serve to modulate the level of TNFα release during intracellular infection. Further studies are warranted to investigate the regulation of IL-12 p70 release by macrophages and dendritic cells, the mechanisms by which IL-12 controls the level of TNFα release by macrophages, and how the nature of antigens and the genetic background dictate the dichotomy of types 1 and 2 immune responses.

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