Published in final edited form as:
Eur J Immunol. 2012 June ; 42(6): 1385–1387. doi:10.1002/eji.201242635.

Membrane Interleukin-18 Revisits Membrane IL-1α in T-helper Type 1 Responses

Charles A. Dinarello
University of Colorado Denver, Aurora, Colorado and University Medical Center Nijmegen, Nijmegen, The Netherlands

Abstract

Although all structural studies on cytokine-cytokine receptor interactions are based on a crystallized cytokine binding to its specific receptor, there is no dearth of evidence that membrane-embedded cytokines are biologically active by virtue of cell-cell contact. Clearly the orientation of the membrane cytokine is such that it allows binding to the receptor, as takes place with the soluble form of the cytokine. In this issue, Bellora et al. report that interleukin-18 (IL-18) exists as an integral membrane protein on M-CSF-differentiated human macrophages and that upon LPS stimulation, IL-18 induces IFN-γ from NK cells in a caspase-1-dependent fashion. The immunological and inflammatory implications for this finding are considerable because of the role of IL-18 as the primary IFN-γ inducing cytokine in promoting Th1 responses.

Interleukin-18 (IL-18), a member of the IL-1 family, was first characterized as an inducer of interferon-γ (IFN-γ) and initially thought to be IL-12. Only after the cloning of the cDNA coding for this IFN-γ-inducing factor [1] did it become clear that the factor belonged to the IL-1 family, and in particular, was closely related to IL-1β. Like IL-1β, IL-18 is first synthesized as an inactive precursor without a signal peptide, and requires cleavage by caspase-1 for processing and release of the active cytokine. But upon further investigation, the similarity to IL-1β became less apparent. First, unlike IL-1β, the IL-18 precursor is found constitutively present in mesenchymal cells and blood monocytes in healthy humans and mice [2]. For example, the IL-18 precursor is present in keratinocytes of the skin and in the epithelial cells of the entire gastrointestinal tract [3]. The IL-1α precursor is also constitutively present in mesenchymal cells in healthy humans and mice and also in the epithelial cells of the entire gastrointestinal tract. Since the IL-1α precursor is present in the same cells as IL-18, IL-18 is similar to IL-1α in this regard. However, the IL-1α precursor is active and therefore in a dying hypoxic cell, such as a keratinocyte [4], the IL-1α precursor is released and induces a proinflammatory response such as chemokine production and neutrophil infiltration [5]. Since the recombinant form of the IL-18 precursor is inactive, IL-18 released from a dying cell would not contribute to inflammation or act as an inducer of IFN-γ unless processed by a protease. Proteinase-3 (PR3) is such a protease that cleaves the IL-18 precursor and coverts the cytokine to an active molecule [6]. But, is it necessary to have cell death and the release of intracellular contents to see a role for IL-18?

In freshly obtained human blood monocytes, LPS stimulation does not result in a significant secretion of IL-1α [7]. In the same blood monocytes, the secretion of IL-18 following LPS stimulation is consistently low and, compared with IL-1β, negligible. By comparison, IL-1β is readily released following LPS stimulation in the absence of added ATP because caspase-1 is already active in fresh monocytes [8]. In contrast, macrophages require activation of caspase-1 with substantial concentrations of ATP [8]. Thus, the robust release of processed IL-1β compared with the weak release of processed IL-18 reveals that the mechanism of release from the post-caspase-1 cleavage step is not the same for these two cytokines. Indeed, a lingering question is why this difference exists. One possible

explanation is that the constitutive presence of the IL-18 precursor in monocytes remains in the cytoplasm whereas the newly synthesized IL-1β precursor enters the secretory lysosome where it is processed by caspase-1 and exported [9, 10].

With the report by Bellora et al. in this issue of the *European Journal of Immunology* [11], the similarity of IL-18 to IL-1α now becomes closer with the observation that a membrane form of IL-18 is found on a subset of monocyte-derived macrophages following exposure to macrophage colony stimulating factor (M-CSF). Similar to IL-1α, membrane IL-18 is an active cytokine only upon stimulation with TLR ligands such as LPS [12, 13]. This is an important similarity for IL-1α and IL-18 in that LPS stimulation triggers a step resulting in an active cytokine. Membrane cytokines are not new to cytokine biology. TNF-α can exist in a membrane form, and requires a protease for release. However, the first report of a functional membrane cytokine was that of IL-1α in 1985 [12]. This milestone was at first appreciated for its relevance to the biology of the IL-1 family, then questioned and finally resolved. The insertion of IL-1α into the membrane is possible because of myristoylation of the IL-1α precursor at lysines 82 and 83, a step that facilitates the insertion into the membrane [14]. There is a potential myristoylation site in the IL-18 precursor but it remains unclear if this site accounts for insertion into the membrane.

There are unique findings in the study by Bellora et al. [11]. First, the appearance of membrane IL-18 is slow given the fact that the monocyte already contains the precursor. Second, its appearance is linked to the differentiation into an M2 type macrophage by exposure to M-CSF whereas differentiation into an M1 type macrophage by exposure to GM-CSF does not result in membrane IL-18. Third, although its presence on the membrane of the differentiated M2 macrophage is caspase-1 dependent, the cytokine is inactive. Activation requires LPS. In this regard, membrane IL-18 and membrane IL-1α are similar because they are both active. In the case of membrane IL-1α, the proof that the cytokine was truly acting as an integral membrane protein and not “leaking” out of the cell was a contentious issue. It was resolved by prolonged fixation of the cell demonstrating the absence of any IL-1α “leaking” into the supernatant [13]. In fact, the concept that cell-cell contact was a fundamental mechanism for inflammation as well as a specific immune response is derived from the studies initiated by Emil Unanue in 1985 [12]. In the case of active membrane IL-18, LPS is necessary for release of the active cytokine from its membrane residence and not for gene expression. Although the cleavage of the IL-1α precursor by the membrane cysteine protease calpain is known, a specific inhibitor of calpain did not prevent the release of active IL-18 from the cell. Therefore, the steps in the release of active IL-18 from M2 macrophages require caspase-1 plus an unknown protease induced by LPS. This protease is likely PR3, as published in studies such as [6], and because macrophages contain inactive (latent) PR3 in the membrane that requires an activation step.

In the article by Bellora et al. [11], the biological read-out for active IL-18 was not only IFN-γ induction from NK cells but also the expression of CCR7. Using a neutralizing anti-IL-18 antibody, these two effects established that IL-18 needed for the responses. It would be interesting to know what would have taken place if the IL-1 receptor antagonist were added to the NK-cell experiment in order to ascertain a role for IL-1α in IFN-γ production from NK cells. Furthermore, is the effect of mixing two cell populations and measuring an effect due to a single cytokine, or to a synergy of two or more cytokines? It is not uncommon in cytokine biology to have synergy such that the neutralization of one cytokine dismantles the synergy and there is no longer a biological effect.

Regardless of these currently unanswered questions, the study by Bellora et al. [11] contributes greatly to understanding the role of IL-18 in inflammation and immune responses; however, the role of IL-18 in either response is far more complicated than that of

IL-1α and IL-1β. IL-18 can be proinflammatory in some models and anti-inflammatory in others. IL-18 likely contributes to macrophage activation syndrome because of its capacity to induce IFN-γ [15]. In a dreaded disease called age-related macular degeneration, in which sight is lost, a requirement for caspase-1 was shown in a mouse model for this disease [16]. However, unexpectedly, the activation of caspase-1 provided a protective role for the disease and it was IL-18, not IL-1β, that was protective. IL-18 is protective in models of colitis but in the same models it can also be inflammatory [17]. Blocking IL-18 protects in several models of inflammation, as reviewed in [18], but mice deficient in IL-18 or deficient in IL-18Rα develop a severe form of metabolic syndrome [19]. Knowing that macrophages express a membrane form of IL-18 extends beyond NK-cell biology and into a broad spectrum of how we view and interpret IL-18.

Acknowledgments

The author thanks M. G. Netea and L. A. Joosten for helpful discussions in the preparation of this editorial. Supported by NIH Grants AI-15614, AR-45584 and CA-04 6934.

References

1. Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M. Cloning of a new cytokine that induces interferon-γ. Nature. 1995; 378:88–91. [PubMed: 7477296]
2. Puren AJ, Fantuzzi G, Dinarello CA. Gene expression, synthesis and secretion of IL-1β and IL-18 are differentially regulated in human blood mononuclear cells and mouse spleen cells. Proc Natl Acad Sci (USA). 1999; 96:2256–2261. [PubMed: 10051628]
3. Pizarro TT, Michie MH, Bentz M, Woraratanadharm J, Smith MF Jr, Foley E, Moskaluk CA, Bickston SJ, Cominelli F. IL-18 a novel immunoregulatory cytokine is up-regulated in Crohn's disease: expression localization in intestinal mucosal cells. J Immunol. 1999; 162:6829–6835. [PubMed: 10352304]
4. Cohen I, Rider P, Carmi Y, Braiman A, Dotan S, White MR, Voronov E, Martin MU, Dinarello CA, Apte RN. Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. Proc Natl Acad Sci U S A. 2010; 107:2574–2579. [PubMed: 20133797]
5. Rider P, Carmi Y, Guttman O, Braiman A, Cohen I, Voronov E, White MR, Dinarello CA, Apte RN. IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. J Immunol. 2011; 187:4835–4843. [PubMed: 21930960]
6. Sugawara S, Uehara A, Nochi T, Yamaguchi T, Ueda H, Sugiyama A, Hanzawa K, Kumagai K, Okamura H, Takada H. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. J Immunol. 2001; 167:6568–6575. [PubMed: 11714826]
7. Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC, Dinarello CA. Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. Blood. 1990; 75:40–47. [PubMed: 2294996]
8. Netea MG, Nold-Petry CA, Nold MF, Joosten LA, Opitz B, van der Meer JH, van de Veerdonk FL, Ferwerda G, Heinhuis B, Devesa I, Funk CJ, Mason RJ, Kullberg BJ, Rubartelli A, van der Meer JW, Dinarello CA. Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. Blood. 2009; 113:2324–2335. [PubMed: 19104081]
9. Andrei C, Dazzi C, Lotti L, Torrisi MR, Chimini G, Rubartelli A. The secretory route of the leaderless protein interleukin 1beta involves exocytosis of endolysosome-related vesicles. Mol Biol Cell. 1999; 10:1463–1475. [PubMed: 10233156]
10. Andrei C, Margiocco P, Poggi A, Lotti LV, Torrisi MR, Rubartelli A. Phospholipases C and A2 control lysosome-mediated IL-1 beta secretion: Implications for inflammatory processes. Proc Natl Acad Sci U S A. 2004; 101:9745–9750. [PubMed: 15192144]

11. Bellora F, Castriconi R, Doni A, Cantoni C, Moretta L, Mantovani A, Moretta A, Bottino C. M-CSF induces the expression of a membrane-bound form of IL-18 in a subset of human monocytes differentiating in vitro toward macrophages. Eur J Immunol. 2012 in press.
12. Kurt-Jones EA, Beller DI, Mizel SB, Unanue ER. Identification of a membrane-associated interleukin-1 in macrophages. Proc Natl Acad Sci USA. 1985; 82:1204–1208. [PubMed: 3919388]
13. Kaplanski G, Farnarier C, Kaplanski S, Porat R, Shapiro L, Bongrand P, Dinarello CA. Interleukin-1 induces interleukin-8 from endothelial cells by a juxacrine mechanism. Blood. 1994; 84 :4242–4248. [PubMed: 7994038]
14. Stevenson FT, Bursten SL, Fanton C, Locksley RM, Lovett DH. The 31-kDa precursor of interleukin 1 alpha is myristoylated on specific lysines within the 16-kDa N-terminal propiece. Proc Natl Acad Sci U S A. 1993; 90:7245–7249. [PubMed: 8346241]
15. Mazodier K, Marin V, Novick D, Farnarier C, Robitail S, Schleinitz N, Veit V, Paul P, Rubinstein M, Dinarello CA, Harle JR, Kaplanski G. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood. 2005; 106:3483–3489. [PubMed: 16020503]
16. Doyle SL, Campbell M, Ozaki E, Salomon RG, Mori A, Kenna PF, Farrar GJ, Kiang AS, Humphries MM, Lavelle EC, O'Neill LA, Hollyfield JG, Humphries P. NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. Nat Med. 2012 in press.
17. Siegmund B. Interleukin-18 in intestinal inflammation: friend and foe? Immunity. 2010; 32:300–302. [PubMed: 20346770]
18. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol. 2007; 27:98–114. [PubMed: 17336692]
19. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, Tack CJ, van Krieken H, Kim SH, Stalenhoef AF, van de Loo FA, Verschueren I, Pulawa L, Akira S, Eckel RH, Dinarello CA, van den Berg W, van der Meer JW. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med. 2006; 12:650–656. [PubMed: 16732281]