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出版情報：Self/Nonself - Immune Recognition and Signaling. 1 (4), pp.310-313, 2010-10. Landes Bioscience
バージョン：

Review

Self and nonself recognition through C-type lectin receptor, Mincle

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Running title

Mincle is a dual sensor for self- and nonself-ligand

Key words

Mincle

C-type lectin

FcRγ

Danger receptor

Dead cell

Mycobacterium

Inflammatory cytokine

Abbreviations

CLR	C-type lectin receptor
FcRγ	Fc receptor common γ-chain
Mincle	Macrophage-inducible C-type lectin
ITAM	immunoreceptor tyrosine-based activation motif

Abstract

Mincle (also called as Clec4e or Clecsf9) is a C-type lectin receptor expressed in activated macrophages. Recently, we have reported that Mincle transduces the activation signals through ITAM-containing adaptor protein, FcRγ, and induces the secretion of inflammatory cytokines. Furthermore, we and other groups have identified that Mincle recognizes a wide variety of ligands such as damaged cells, fungus, yeast and mycobacteria. These results indicate that Mincle acts as a multi-task danger receptor for both self and nonself ligands. This review summarizes the recent discoveries about the ligands and immunological roles of Mincle.

Introduction

The CLR (C-type lectin receptor) is a family of Ca^{2+}-dependent carbohydrate-binding lectins that contain at lease one C-type lectin-like domain (CTLD).[1] Recently, CLR was highlighted as a member of the innate immune receptor families, which contain TLRs (Toll-like receptors), NLRs (Nod-like receptors) and RLRs (RIG-I-like receptors). Some CLRs deliver their signals via an association with ITAM (immunoreceptor tyrosine-based activation motif)[2] bearing adaptors, which is used by acquired immune receptors, NK receptors and Fc receptors.[3]

Mincle (Macrophage-inducible C-type lectin) was originally identified as a transcriptional target of NF-IL6 (also called C/EBPβ) in macrophages.[4] Mincle is a type II transmembrane protein mainly expressed in myeloid cells, such as macrophages and neutrophils, and possesses a single carbohydrate-recognition domain (CRD) in the extracellular region. The expression level of Mincle in the steady-state condition is very low, however, it is strongly upregulated after exposure to various stimuli such as inflammatory cytokines and TLR ligands. Mincle is associated with an ITAM-containing Fc receptor γ chain (FcRγ) with a positively charged residue in the transmembrane region.[5] The ligation of ITAM leads to a signaling cascade that begins with phosphorylation of ITAM tyrosine residues by

Src-family kinases, followed by the recruitment and activation of Syk. Syk then activates a signaling cascade through CARD9, and this event leads to the induction of inflammatory cytokines such as TNFα, MIP-2 (CXCL2), KC (CXCL1) and IL-6. Therefore, Mincle may act as an inducible activating receptor for certain ligand(s).

Dead cell recognition by Mincle

To search for a specific ligand of Mincle, we first established a reporter cell line expressing Mincle, FcRγ and NFAT-GFP. When the cells were cultured alone without exchange of the medium, the number of GFP^+ cells was gradually increased. During this culture period, increasing numbers of dead cells paralleled the increase in the GFP^+ population, suggesting that a component derived from dead cells signals through Mincle. Indeed, we have identified SAP130 (spliceosome-associated protein 130) as a Mincle-binding protein.[5] SAP130 is a component of the U2 snRNP, and is localized in the nucleus in normal live cells[6], and is released from necrotic cells, but not from live or early apoptotic cells. These results suggest that SAP130 released from necrotic cells may act as a danger signal through Mincle.

Whole-body irradiation or administration of dexamethazone induces massive cell death in the thymus, and consequently elicits transient infiltration of neutrophils into the thymus.[7, 8] The administration of an anti-Mincle blocking antibody resulted in a considerable suppression of such neutrophil infiltration, thus suggesting that Mincle is a functional sensor for damaged cells *in vivo*.

It has been reported that other CLRs expressed on myeloid cells potentially bind to dead cells.[9] MBL (mannose-binding lectin), Lox-1 and MGL-1 are involved in the phagocytotic clearance of dead cells.[10-13] Human Dectin-1 (CLEC7A), DNGR1 (CLEC9A) and DEC205 (CD205) were also reported to bind to apoptotic or necrotic cells.[14-16] In most cases, however, the nature of the ligand(s) derived from dead cells is still unknown.

Neutrophil infiltration induces acute inflammation and tissue damage, but in some cases, early, small-scale neutrophil-mediated tissue destruction eventually promotes tissue repair.[17] It has also been reported that infiltrating neutrophils assist macrophage-mediated clearance of stress-induced dead cells in the thymus.[18] Therefore, it is possible to speculate that Mincle may trigger the "beneficial" recruitment of inflammatory cells in response to tissue damage. However, the function of Mincle in the recovery process of tissue repair remains to be determined.

Fungus recognition by Mincle

Some C-type lectin receptors directly recognize specific fungi.[19] Dectin-1 recognizes β-glucans on the surface of a wide variety of fungal species, including *S. cerevisiae*, *C. albicans*, *C. posadasii*, *P. carinii* and *A. fumigatus*, and this recognition mediates fungal uptake and killing, and the production of inflammatory cytokines and chemokines.[20-24] FcRγ-coupled Dectin-2 recognizes *C. albicans*, *M. audouinii* and *T. rubrum*.[25, 26] Other CLRs, such as MR (CD206), DC-SIGN, MBL, surfactant protein A (SP-A) and surfactant protein D (SP-D) have been implicated in antifungal immunity.[27-34]

Wells et. al. reported that Mincle recognized *C. albicans* and promoted the production of TNFα by macrophages.[35] In addition, mice lacking Mincle showed a significantly increased susceptibility to systemic candidiasis. We established a Mincle reporter cell line and screened the cells for a response to 50 different kinds of fungi, including *C. albicans*. Three different strains of *C. albicans* were not recognized by Mincle in our reporter systems, although these strains differ from that used in the previous study. Therefore, Mincle may distinguish structural differences in the strain of *C. albicans*.

Instead, we found that Mincle recognized *Malassezia* species such as *M. pachydermatis*, *M. furfur* and *M. japonica*. *Malassezia*, an obligatory lipophilic organism commonly found on human skin, is a causative fungus of skin diseases and fatal sepsis, including intravascular catheter-associated sepsis.[36-38] However, the specific receptor for this organism on host cells has not been identified. *Malassezia*-induced production of cytokines such as TNFα, MIP-2, KC and IL-10 was significantly impaired in Mincle-deficient macrophages *in vitro*. Cytokine production and neutrophil infiltration against *Malassezia* injection were also impaired in Mincle-deficient mice *in vivo*. These results suggest that Mincle plays a crucial role in immune response to *Malassezia* fungi. Although *Malassezi*a species can live as commensal flora in normal skin, these fungi elicit an inflammatory response in the skin lesions of patients with atopic/eczema dermatitis syndrome and psoriasis.[36] Since Mincle expression is up-regulated by several stresses, Mincle-induced inflammation may contribute to the regulation of dermatitis or sepsis elicited by *Malassezia*. The identification of a Mincle ligand in *Malassezia* will provide valuable information for the development of an effective therapy against *Malassezia*-related diseases.

Mincle recognizes Mycobacterial glycolipid TDM

Heat-killed *Mycobacterium tuberculosis* is widely used as a component of complete Freund's adjuvant (CFA), because it contains various immunostimulatory components. Among such components, cell wall glycolipid TDM (trehalose-6,6'-dimycolate), also known as cord factor, has been extensively studied for decades, as it possesses an effective adjuvant activity.[39] One class of the receptors implicated in TDM recognition is the TLRs. MARCO (macrophage receptor with collagenous structure) is also reported to be a tethering factor of TDM to the macrophage, and is thought to activate the TLR2 signaling pathway.[40] Another group reported that TDM activated macrophages and DCs via the Syk–Card9–Bcl10–Malt1 signaling axis rather than TLR-MyD88 pathway.[41] Thus, the candidate receptor for TDM has been controversial.

Recently, we and another group have demonstrated that Mincle is the receptor for TDM.[42, 43] TDM activates macrophages to produce nitric oxide and inflammatory cytokines such as TNFα and MIP-2, and this effect was completely suppressed in Mincle-deficient macrophages. The *in vivo* TDM administration induced a robust elevation of inflammatory cytokines in sera and granuloma formation.[44, 45] Granulomas are complex aggregates of immune cells, and are widely believed to constrain mycobacterial infection by physically surrounding the infecting bacteria.[46] No TDM-induced lung granuloma was formed in Mincle-deficient mice, suggesting that Mincle is a critical receptor for TDM-induced granuloma formation, most likely through the production of inflammatory cytokines/chemokines to recruit inflammatory cells.[47] Schoenen et al. demonstrated that Mincle is essential for generation of Th1/Th17 cellular immunity to subunit vaccination using TDB (torehalose dibehenate; a synthetic analogue of TDM) as an adjuvant.[43] These results suggest that Mincle may contribute to innate- and acquired-immunity against mycobacteria. Furthermore, the identification of the host receptor for TDM/TDB will provide valuable information related to the design of vaccine adjuvants against not only tuberculosis, but also other infectious diseases and cancers.

Conclusion

In recent years, many reports have shown that C-type lectin receptors are fundamental mediators of diverse immune interactions, especially in the recognition of various self and non-self ligands.[9, 19, 48, 49] Mincle uniquely senses the “danger” derived from both damaged-self and non-self psthogen to induce inflammatory responses (Fig. 1). Recognition of these molecules by Mincle may lead to beneficial inflammation that promotes tissue repair or host defense responses.

From a pathological point of view, Mincle has been reported to be dramatically upregulated in patients with rheumatoid arthritis[50], and rat chromosome 4q42 (which includes the gene encoding Mincle) has been linked to arthritis.[51] These observations suggest that dysregulated expression of Mincle might elicit unwanted inflammation responsible for autoimmune or granulomatous diseases. The pathological risk, as well as the physiological advantage, of Mincle-mediated recognition of “danger” needs clarification, but is likely to provide information that could be useful for a variety of conditions.

Figure legend

Figure 1. Mincle is a dual sensor for self and non-self ligands. Mincle recognizes SAP130 released from necrotic cells (damaged self). Invading pathogens (non-self), such as Mycobacterium and the pathogenic fungus, *Malassezia*, are also recognized by Mincle. TDM (torehalose dimycolate) is Mycobacterial molecule identified as a ligand of Mincle. Sensing of these self and non-self ligands by Mincle leads to transduction of activation signals through FcRγ (ITAM-containing adapter protein), thus inducing the inflammatory responses that possess both physiological advantages and potential risks.

References

1. Zelensky AN, Gready JE. The C-type lectin-like domain superfamily. *FEBS J* 2005; 272: 6179-217.
2. Humphrey MB, Lanier LL, Nakamura MC. Role of ITAM-containing adapter proteins and their receptors in the immune system and bone. *Immunol Rev* 2005; 208: 50-65.
3. Kerrigan AM, Brown GD. Syk-coupled C-type lectin receptors that mediate cellular activation via single tyrosine based activation motifs. *Immunol Rev* 2010; 234: 335-52.
4. Matsumoto M, Tanaka T, Kaisho T, Sanjo H, Copeland NG, Gilbert DJ, *et al.* A novel LPS-inducible C-type lectin is a transcriptional target of NF-IL6 in macrophages. *J Immunol* 1999; 163: 5039-48.
5. Yamasaki S, Ishikawa E, Sakuma M, Hara H, Ogata K, Saito T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat Immunol* 2008; 9: 1179-88.
6. Das BK, Xia L, Palandjian L, Gozani O, Chyung Y, Reed R. Characterization of a protein complex containing spliceosomal proteins SAPs 49, 130, 145, and 155. *Mol Cell Biol* 1999; 19: 6796-802.
7. Uchimura E, Watanabe N, Niwa O, Muto M, Kobayashi Y. Transient infiltration of neutrophils into the thymus in association with apoptosis induced by whole-body X-irradiation. *J Leukoc Biol* 2000; 67: 780-4.
8. Li J, Scherl A, Medina F, Frank PG, Kitsis RN, Tanowitz HB, *et al.* Impaired phagocytosis in caveolin-1 deficient macrophages. *Cell Cycle* 2005; 4: 1599-607.
9. Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S, Reis e Sousa C. Myeloid C-type lectins in innate immunity. *Nat Immunol* 2006; 7: 1258-65.
10. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, *et al.* C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J Exp Med* 2001; 194: 781-95.
11. Nauta AJ, Raaschou-Jensen N, Roos A, Daha MR, Madsen HO, Borrias-Essers MC, *et al.* Mannose-binding lectin engagement with late apoptotic and necrotic cells. *Eur J Immunol* 2003; 33: 2853-63.
12. Oka K, Sawamura T, Kikuta K, Itokawa S, Kume N, Kita T, *et al.* Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. *Proc Natl Acad Sci U S A* 1998; 95: 9535-40.

13. Yuita H, Tsuiji M, Tajika Y, Matsumoto Y, Hirano K, Suzuki N, *et al.* Retardation of removal of radiation-induced apoptotic cells in developing neural tubes in macrophage galactose-type C-type lectin-1-deficient mouse embryos. *Glycobiology* 2005; 15: 1368-75.
14. Weck MM, Appel S, Werth D, Sinzger C, Bringmann A, Grunebach F, *et al.* hDectin-1 is involved in uptake and cross-presentation of cellular antigens. *Blood* 2008; 111: 4264-72.
15. Sancho D, Joffre OP, Keller AM, Rogers NC, Martinez D, Hernanz-Falcon P, *et al.* Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. *Nature* 2009; 458: 899-903.
16. Shrimpton RE, Butler M, Morel AS, Eren E, Hue SS, Ritter MA. CD205 (DEC-205): a recognition receptor for apoptotic and necrotic self. *Mol Immunol* 2009; 46: 1229-39.
17. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006; 6: 173-82.
18. Iyoda T, Nagata K, Akashi M, Kobayashi Y. Neutrophils accelerate macrophage-mediated digestion of apoptotic cells in vivo as well as in vitro. *J Immunol* 2005; 175: 3475-83.
19. Willment JA, Brown GD. C-type lectin receptors in antifungal immunity. *Trends Microbiol* 2008; 16: 27-32.
20. Herre J, Marshall AS, Caron E, Edwards AD, Williams DL, Schweighoffer E, *et al.* Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. *Blood* 2004; 104: 4038-45.
21. Saijo S, Fujikado N, Furuta T, Chung SH, Kotaki H, Seki K, *et al.* Dectin-1 is required for host defense against Pneumocystis carinii but not against Candida albicans. *Nat Immunol* 2007; 8: 39-46.
22. Viriyakosol S, Fierer J, Brown GD, Kirkland TN. Innate immunity to the pathogenic fungus Coccidioides posadasii is dependent on Toll-like receptor 2 and Dectin-1. *Infect Immun* 2005; 73: 1553-60.
23. Steele C, Marrero L, Swain S, Harmsen AG, Zheng M, Brown GD, *et al.* Alveolar macrophage-mediated killing of Pneumocystis carinii f. sp. muris involves molecular recognition by the Dectin-1 beta-glucan receptor. *J Exp Med* 2003; 198: 1677-88.
24. Steele C, Rapaka RR, Metz A, Pop SM, Williams DL, Gordon S, *et al.* The beta-glucan receptor dectin-1 recognizes specific morphologies of Aspergillus

fumigatus. *PLoS Pathog* 2005; 1: e42.

25. McGreal EP, Rosas M, Brown GD, Zamze S, Wong SY, Gordon S, *et al.* The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* 2006; 16: 422-30.
26. Sato K, Yang XL, Yudate T, Chung JS, Wu J, Luby-Phelps K, *et al.* Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses. *J Biol Chem* 2006; 281: 38854-66.
27. Mansour MK, Schlesinger LS, Levitz SM. Optimal T cell responses to Cryptococcus neoformans mannoprotein are dependent on recognition of conjugated carbohydrates by mannose receptors. *J Immunol* 2002; 168: 2872-9.
28. Dobozy A, Szolnoky G, Gyulai R, Kenderessy AS, Marodi L, Kemeny L. Mannose receptors are implicated in the Candida albicans killing activity of epidermal cells. *Acta Microbiol Immunol Hung* 1996; 43: 93-5.
29. Ezekowitz RA, Williams DJ, Koziel H, Armstrong MY, Warner A, Richards FF, *et al.* Uptake of Pneumocystis carinii mediated by the macrophage mannose receptor. *Nature* 1991; 351: 155-8.
30. Cambi A, Gijzen K, de Vries JM, Torensma R, Joosten B, Adema GJ, *et al.* The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. *Eur J Immunol* 2003; 33: 532-8.
31. Serrano-Gomez D, Dominguez-Soto A, Ancochea J, Jimenez-Heffernan JA, Leal JA, Corbi AL. Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of Aspergillus fumigatus conidia by dendritic cells and macrophages. *J Immunol* 2004; 173: 5635-43.
32. Kilpatrick DC. Mannan-binding lectin: clinical significance and applications. *Biochim Biophys Acta* 2002; 1572: 401-13.
33. Madan T, Eggleton P, Kishore U, Strong P, Aggrawal SS, Sarma PU, *et al.* Binding of pulmonary surfactant proteins A and D to Aspergillus fumigatus conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. *Infect Immun* 1997; 65: 3171-9.
34. Yong SJ, Vuk-Pavlovic Z, Standing JE, Crouch EC, Limper AH. Surfactant protein D-mediated aggregation of Pneumocystis carinii impairs phagocytosis by alveolar macrophages. *Infect Immun* 2003; 71: 1662-71.
35. Wells CA, Salvage-Jones JA, Li X, Hitchens K, Butcher S, Murray RZ, *et al.* The macrophage-inducible C-type lectin, mincle, is an essential component of the innate

immune response to Candida albicans. *J Immunol* 2008; 180: 7404-13.

36. Ashbee HR. Recent developments in the immunology and biology of Malassezia species. *FEMS Immunol Med Microbiol* 2006; 47: 14-23.

37. Scheynius A, Johansson C, Buentke E, Zargari A, Linder MT. Atopic eczema/dermatitis syndrome and Malassezia. *Int Arch Allergy Immunol* 2002; 127: 161-9.

38. Devlin RK. Invasive fungal infections caused by Candida and Malassezia species in the neonatal intensive care unit. *Adv Neonatal Care* 2006; 6: 68-77; quiz 78-9.

39. Azuma I, Seya T. Development of immunoadjuvants for immunotherapy of cancer. *Int Immunopharmacol* 2001; 1: 1249-59.

40. Bowdish DM, Sakamoto K, Kim MJ, Kroos M, Mukhopadhyay S, Leifer CA*, et al.* MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and Mycobacterium tuberculosis. *PLoS Pathog* 2009; 5: e1000474.

41. Werninghaus K, Babiak A, Gross O, Holscher C, Dietrich H, Agger EM*, et al.* Adjuvanticity of a synthetic cord factor analogue for subunit Mycobacterium tuberculosis vaccination requires FcRgamma-Syk-Card9-dependent innate immune activation. *J Exp Med* 2009; 206: 89-97.

42. Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O*, et al.* Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J Exp Med* 2009; 206: 2879-88.

43. Schoenen H, Bodendorfer B, Hitchens K, Manzanero S, Werninghaus K, Nimmerjahn F*, et al.* Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. *J Immunol* 2010; 184: 2756-60.

44. Yamaguchi M, Ogawa Y, Endo K, Takeuchi H, Yasaka S, Nakamura S*, et al.* [Experimental formation of tuberculous cavity in rabbit lung. IV. Cavity formation by paraffine oil extract prepared from heat killed tubercle bacilli.]. *Kekkaku* 1955; 30: 521-4.

45. Hunter RL, Olsen MR, Jagannath C, Actor JK. Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavitary tuberculosis, including a revised description of the pathology of secondary disease. *Ann Clin Lab Sci* 2006; 36: 371-86.

46. Adams DO. The granulomatous inflammatory response. A review. *Am J Pathol* 1976; 84: 164-92.

47. Welsh KJ, Abbott AN, Hwang SA, Indrigo J, Armitige LY, Blackburn MR, *et al.* A role for tumour necrosis factor-alpha, complement C5 and interleukin-6 in the initiation and development of the mycobacterial cord factor trehalose 6,6'-dimycolate induced granulomatous response. *Microbiology* 2008; 154: 1813-24.
48. Cambi A, Figdor CG. Dual function of C-type lectin-like receptors in the immune system. *Curr Opin Cell Biol* 2003; 15: 539-46.
49. Geijtenbeek TB, van Vliet SJ, Engering A, t Hart BA, van Kooyk Y. Self- and nonself-recognition by C-type lectins on dendritic cells. *Annu Rev Immunol* 2004; 22: 33-54.
50. Nakamura N, Shimaoka Y, Tougan T, Onda H, Okuzaki D, Zhao H, *et al.* Isolation and expression profiling of genes upregulated in bone marrow-derived mononuclear cells of rheumatoid arthritis patients. *DNA Res* 2006; 13: 169-83.
51. Ribbhammar U, Flornes L, Backdahl L, Luthman H, Fossum S, Lorentzen JC. High resolution mapping of an arthritis susceptibility locus on rat chromosome 4, and characterization of regulated phenotypes. *Hum Mol Genet* 2003; 12: 2087-96.

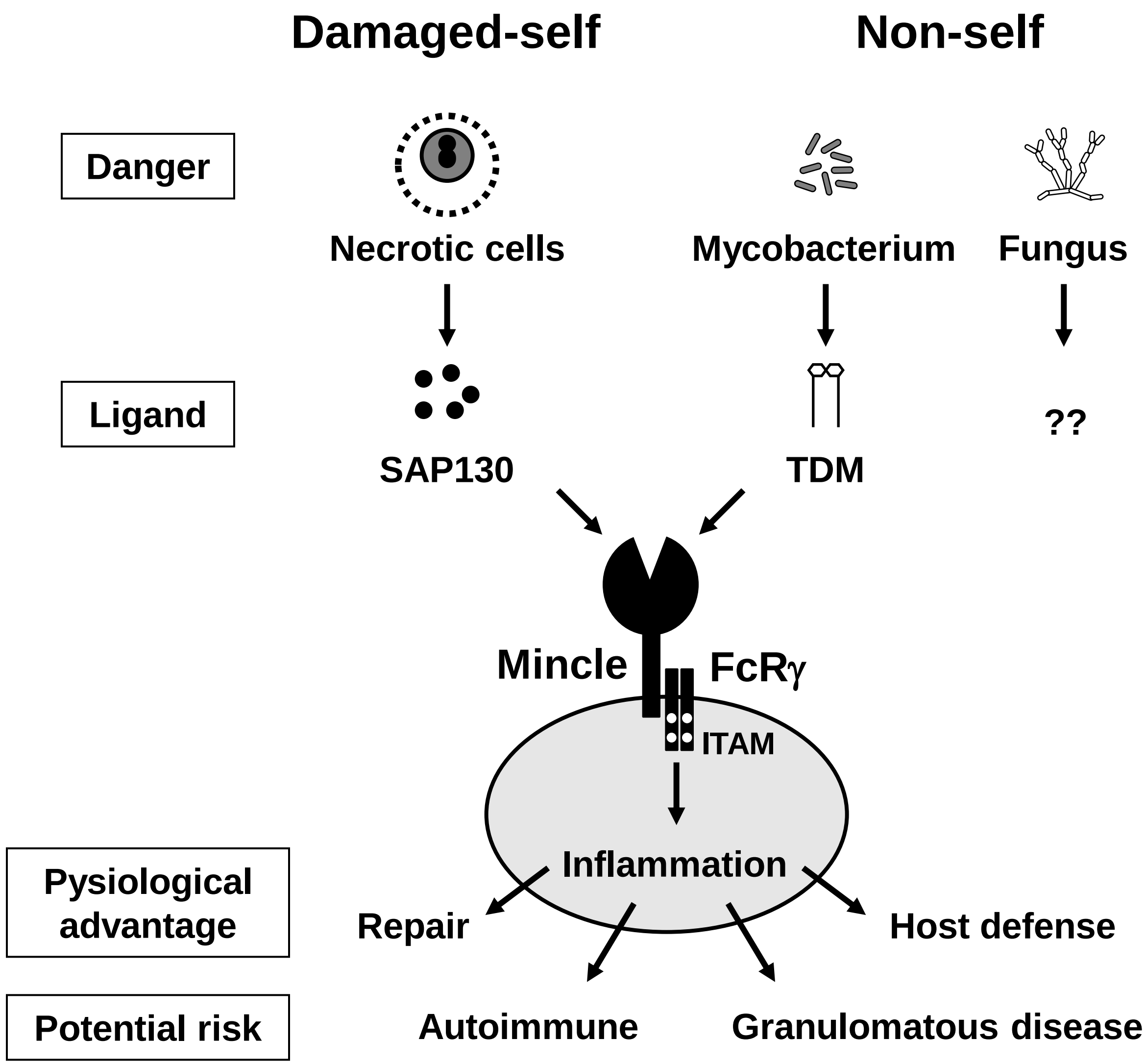
Damaged-self
Non-self
Danger
Necrotic cells
Mycobacterium
Fungus
Ligand
SAP130
TDM
??
Mincle
FcRγ
ITAM
Inflammation
Pysiological advantage
Repair
Host defense
Potential risk
Autoimmune
Granulomatous disease

Figure.1