2019台灣胸腔暨重症加護醫學會

2019 Taiwan Society of Pulmonary and Critical Care Medicine

The role of M2a monocyte polarization and formyl peptide receptor (FPR)1/2/3 expressions in the progression from latent TB infection to active pulmonary TB disease

血液第2a型單核球極化和第一、二、三型甲酰肽受器在潛伏性結核感染進展至活動性肺結核疾病所扮演的角色

陳永哲　醫師　高雄長庚醫院　胸腔內科

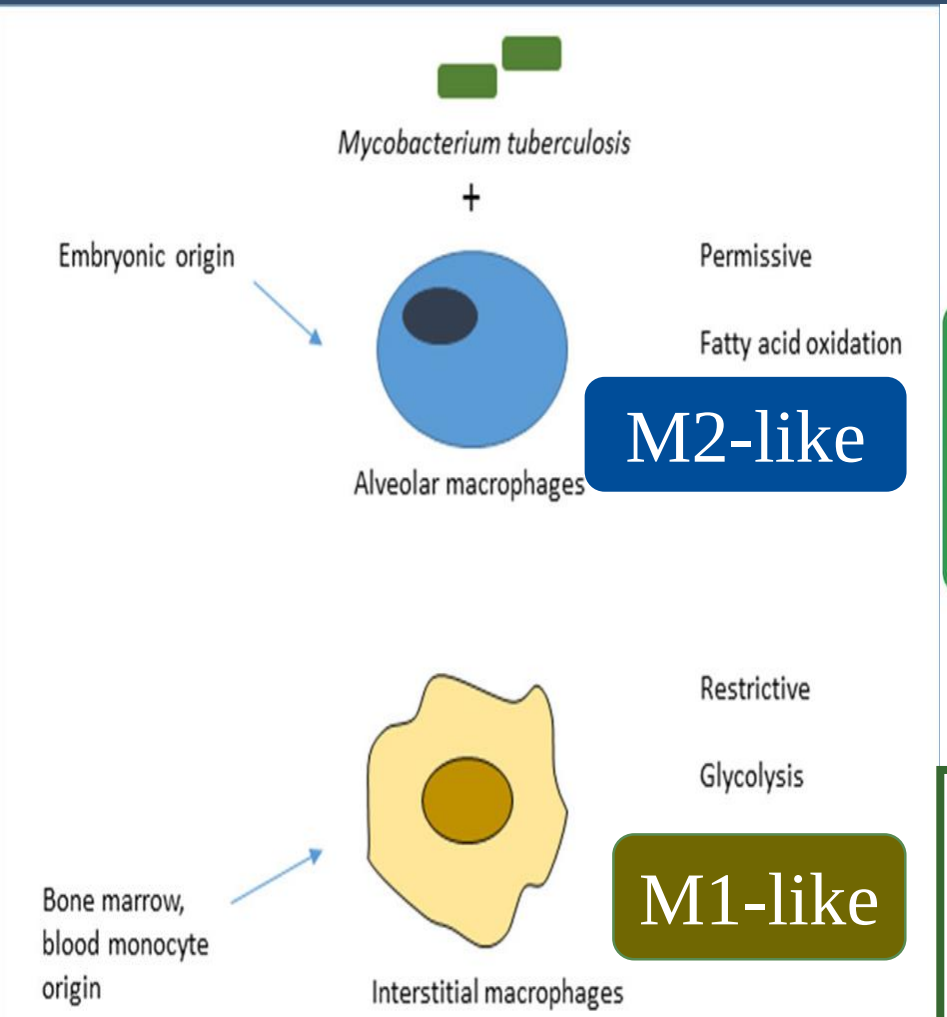
時間：2019/12/07 PM 16:40 ~ 16:50

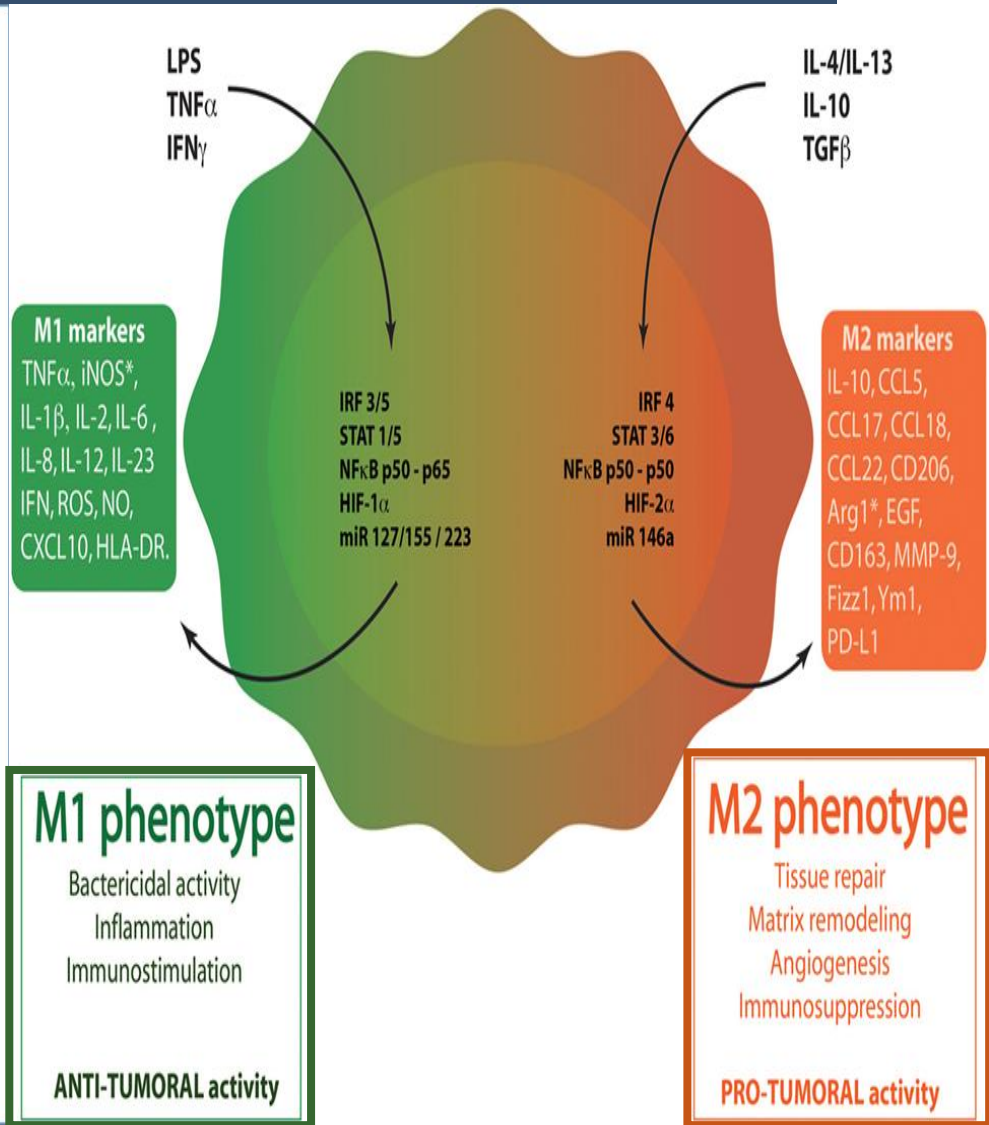
地點：高雄展覽館3樓 304A

長庚 高雄

In their naive stage, alveolar macrophages (AMs) that phagocytose the inhaled *M.tb* at alveoli exhibit an M2-like phenotype that is more permissive for the growth of M. tuberculosis.

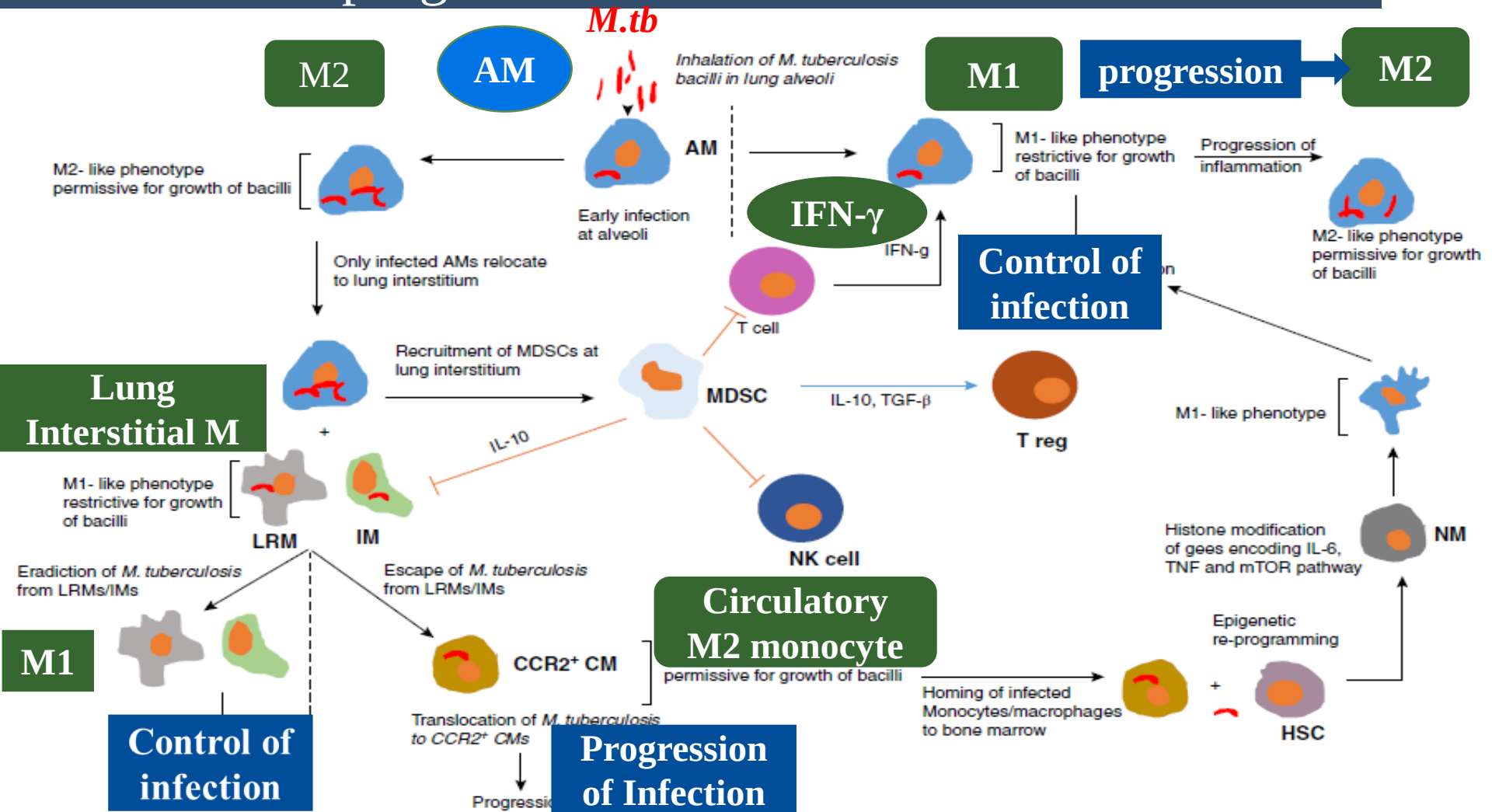
Front. Microbiol 2018. 9:1028.





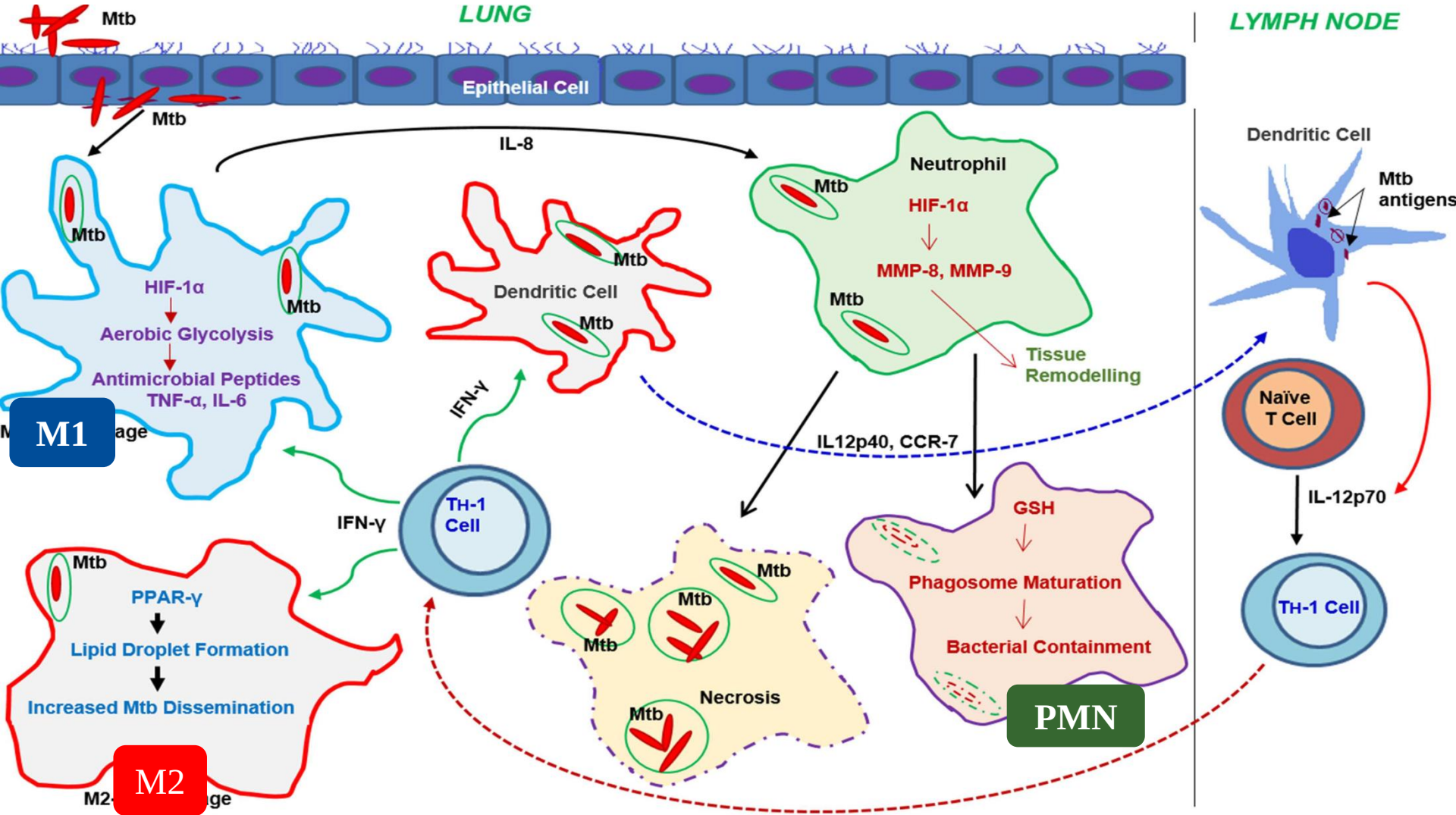
With IFN-γ-mediated priming through T cells, AMs could exhibit the M1-like phenotype at the early stage of infection and restrict bacterial growth.
AMs eventually acquire an M2-like phenotype after inflammation progresses.

J Leukoc Biol. 2019;106:275–282.



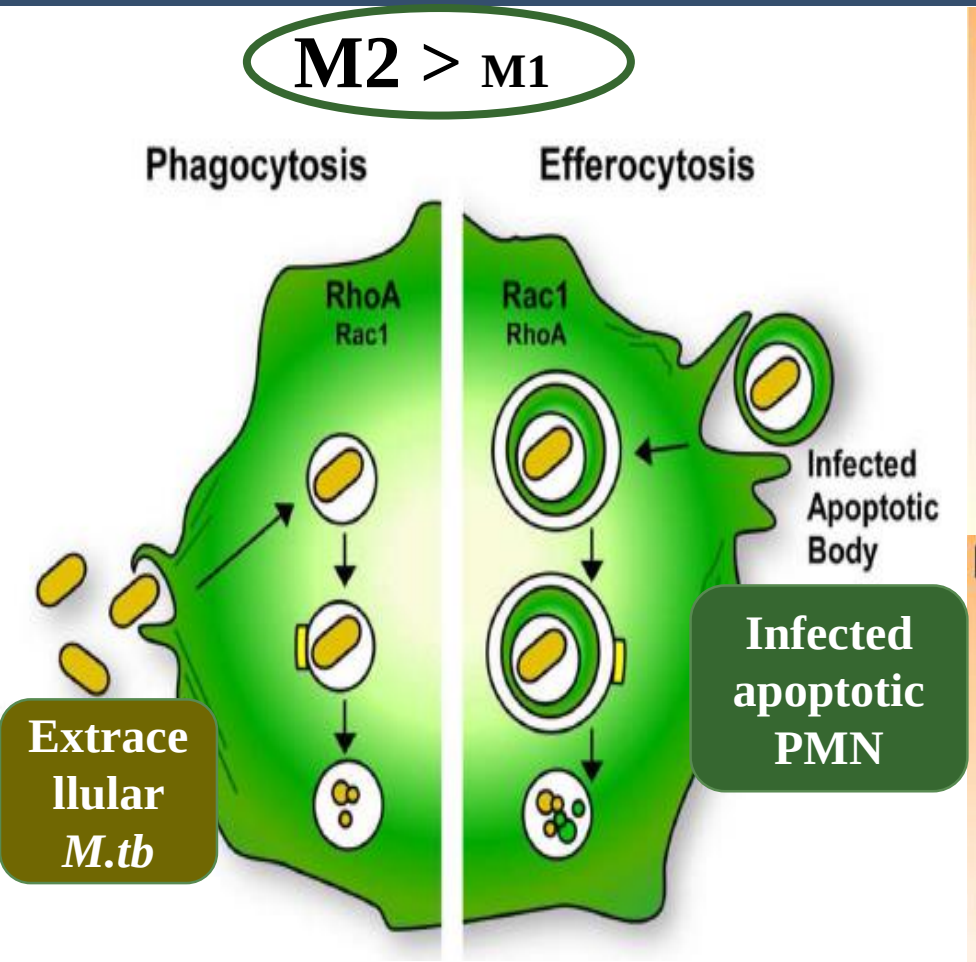
During the advanced disease stage TB, the macrophages adapt to an M2- polarization state, whereby metabolic reprogramming leads to the expression of anti-inflammatory cytokines such as IL-4, IL-10, and TGF-b, and increased phagocytosis.

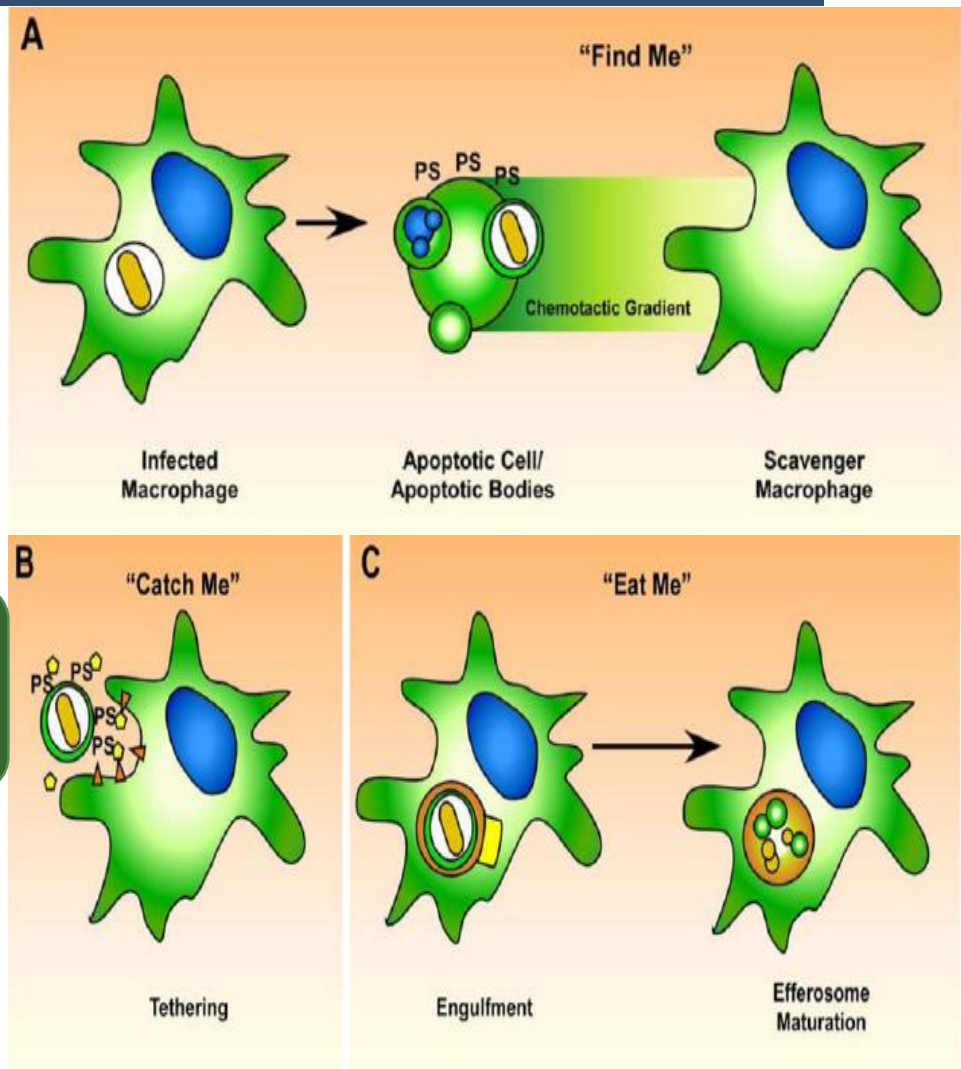
Front Mol Biosci. 2019 Oct 14;6:105.



Phagocytosis is a specific form of endocytosis by which cells internalise solid matter (microbial pathogens). Efferocytosis involves the regulated uptake & degradation of apoptotic bodies.

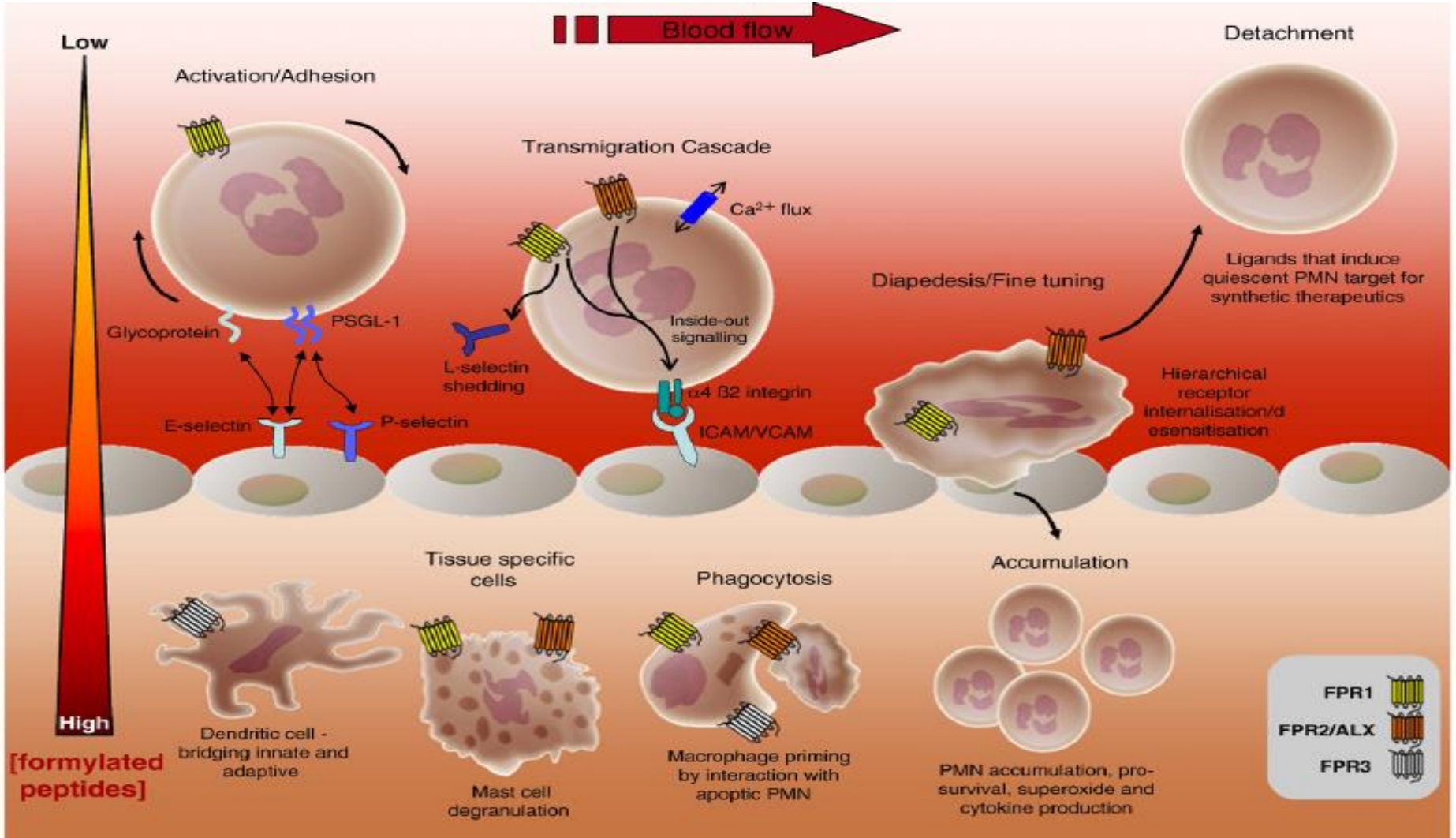
Curr Opin Microbiol. 2014 February ; 0: 17–23





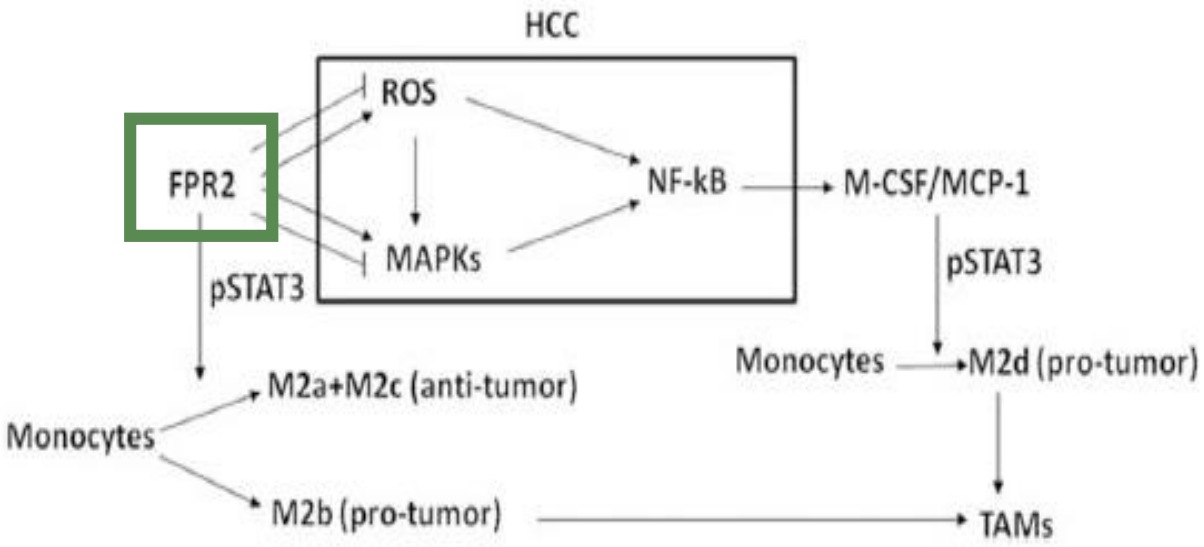
As a marker of efferocytosis, Formyl Peptide Receptors (FPRs) 1/2/3 play a distinct role in myeloid cell adhesion /activation, and macrophage phagocytosis.

Pharmacology & Therapeutics 127 (2010) 175–188



FPR1 maintains chemotaxis and superoxide production of resting and pro-inflammatory M1 macrophages, while FPR2 skews monocyte into M2a/c/b/d.

- M1 Polarization of macrophages with IFN-γ, LPS and with the TLR8 ligand 3M-2
  - further increases FPR1 mRNA levels
  - not consistently increase protein expression or chemotaxis towards the FPR1 ligand fMLF.
- M2 polarization of primary human macrophages with IL-4 and IL-13 leading to the alternative activated macrophages
  - reduces FPR1 cell surface expression and abolishes chemotaxis towards fMLF

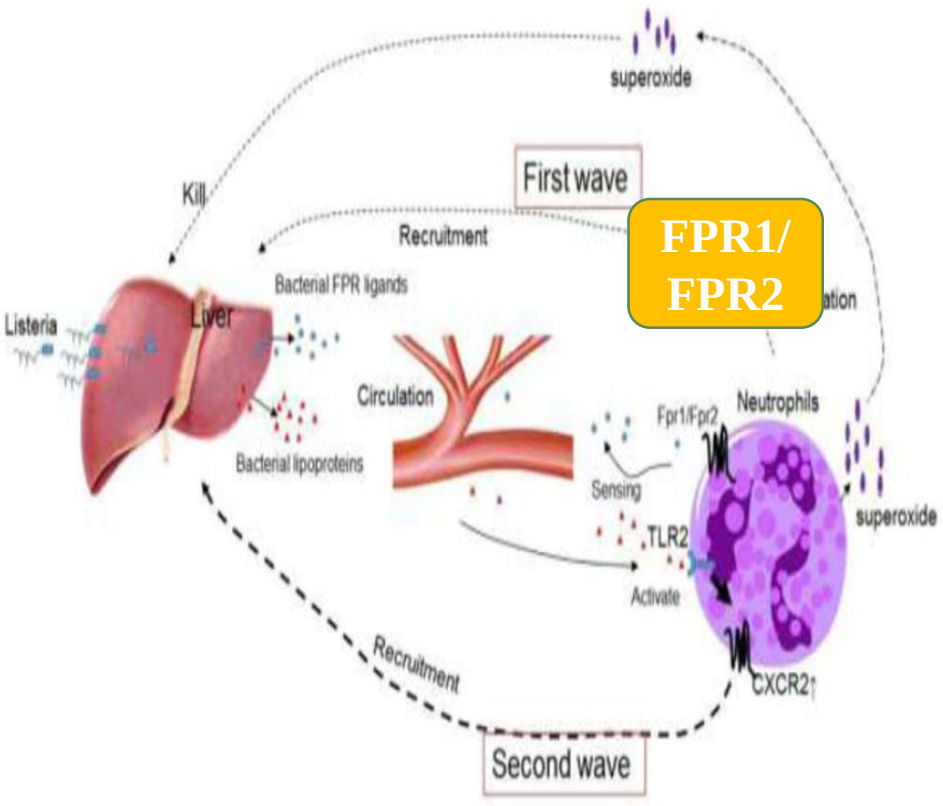


- FPR2 stimulation (AnxA1)
- decreased neutrophil-endothelial interactions by 25-45%
- stimulated neutrophil apoptosis and macrophage efferocytosis by 45%.

PLoS One. 2012;7(11):e50195 **Oncogene (2011) 30, 3887–3899** **J Immunol. 2013;190:6478-87**

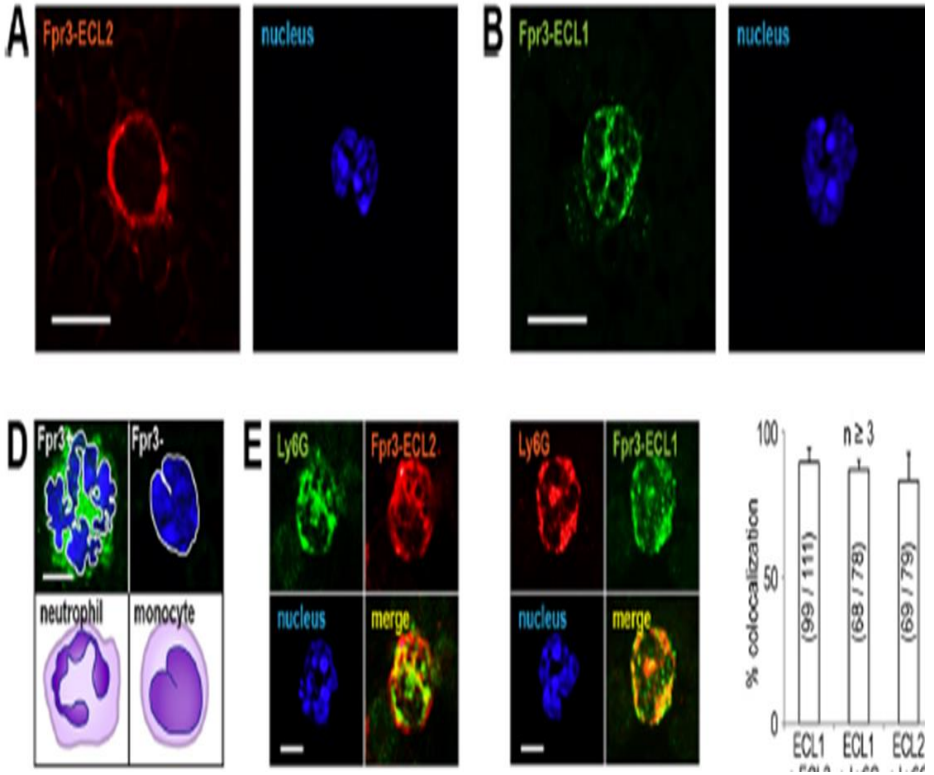
FPR1/FPR2 on neutrophils mediate a rapid neutrophil influx in response to Listeria infection, while FPR3 in neutrophils is enhanced by LPS stimuli.

Both Fpr1 and Fpr2 expressed by mouse neutrophils sense bacteria-derived chemotactic PAMPs to mediate a rapid neutrophil influx into the liver of listeria-infected mice.



J Autoimmun. 2017 December ; 85: 64–77

Fpr3 (a decoy receptor) Expression in Neutrophils Is Enhanced by LPS Stimulation in mice



THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 291, pp. 9762–9775, 2016

Little is known about the role of FPR1/2/3 in human immune responses against *M.tb*. Mycobacteria can activate FPR1 on monocyte and PMN.

- Mycobacteria contain formyl peptides, which are released during bacterial lysis.
- Increased FPR1 gene expression of blood monocytes in active TB patients vs. LTBI subjects.
- Antagonize the anti-inflammatory effects induced by formyl peptides in monocytes/PMN from TB patients.

- Mycobacteria butyricum activate FPR1 on neutrophils, resulting in tonic secretion of opioid peptides from neutrophils and in a decrease in inflammatory pain.

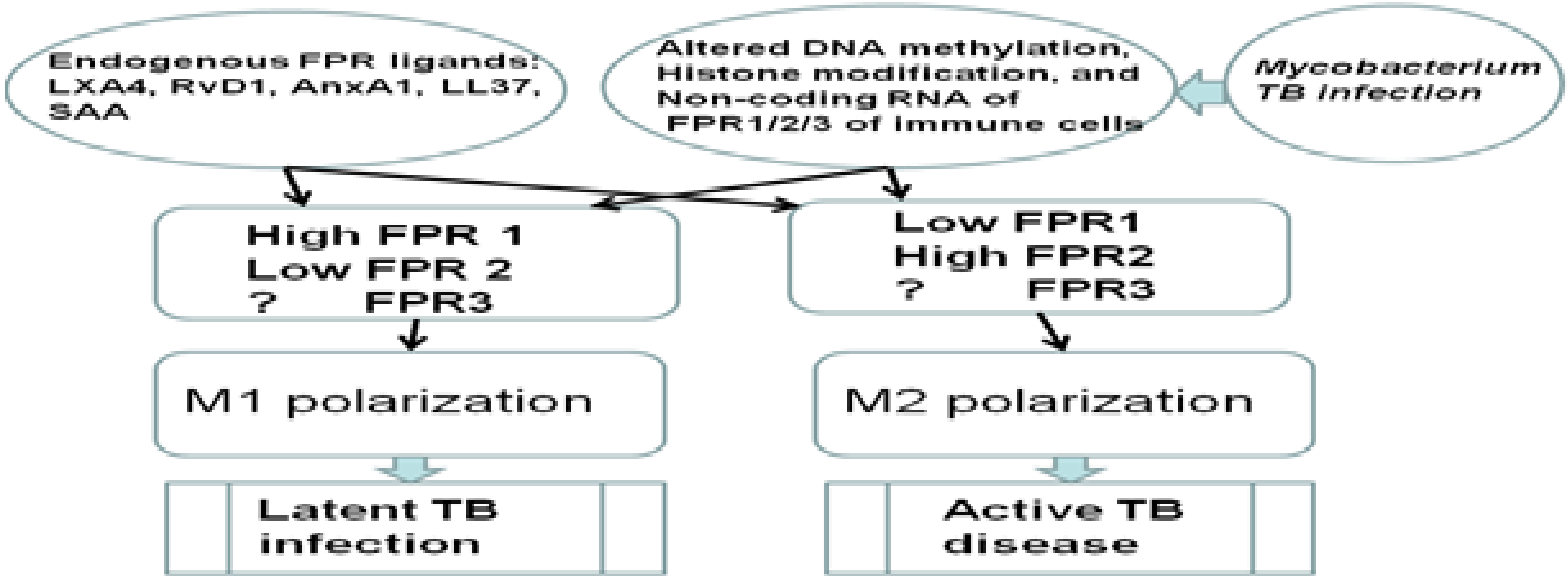
Clin Exp Immunol. 2003 Aug;133(2):267-74.

J Mol Med (Berl). 2007 Jun;85(6):613-21.

PLoS Pathog. 2009 Apr;5(4):e1000362.

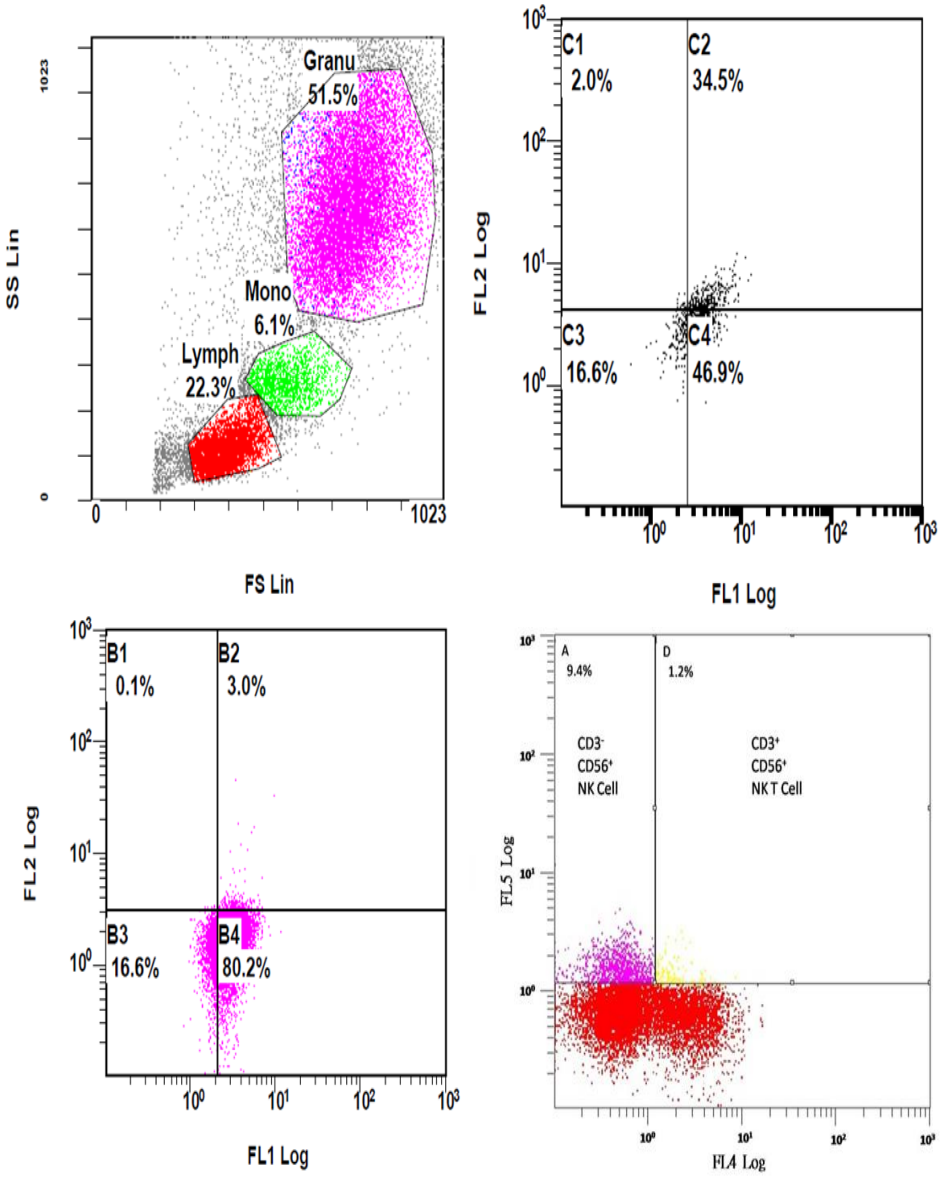
Hypothesis of the current study

- Monocyte M1/M2 polarization and the FPR1/2/3 expressions of peripheral blood immune cells may be different between
  - ➢active pulmonary TB patients, latent TB infection (LTBI) patients, and non-infected healthy subjects (NIHS)
  - ➢between TB patients with and without specific clinical phenotypes, such as high bacterial load, advanced lesions on chest radiograph, and systemic symptoms



Study subjects and Method

- Blood $CD14^{+}CD209^{-}$M1 / $CD14^{+}CD209^{+}$M2a monocyte percentage by flowcytometry
- FPR1/FPR2/FPR3 protein expressions of blood M1 monocyte, M2 monocyte, neutrophil, natural killer (NK) cells, T helper (Th) cell, and cytotoxic T (Tc) cell measured by flowcytometry.
- Participants
  - 43 patients with sputum culture (+) active pulmonary TB disease
  - 11 subjects with LTBI (IGRA+, contact Hx+)
  - 23 non-infected healthy subjects (NIHS; IGRA-, contact Hx+)



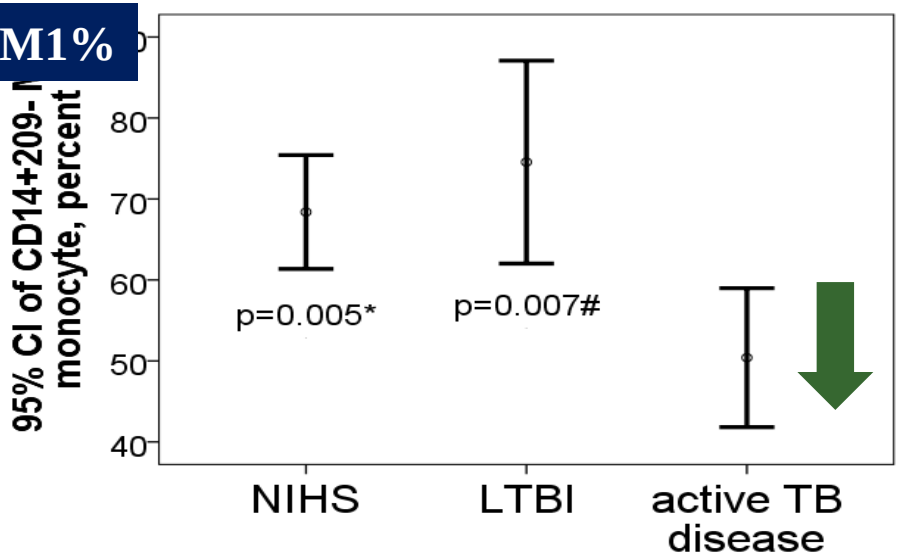
Demographic, co-morbidity, and clinical data of all the 70 study participants

	Non-infected healthy subjects N = 23	Subjects with latent TB infection N = 11	Patients with active pulmonary TB disease N = 43	P value
Age, years	51.4±17.2	53.2±14	56.3±13.6	**0.512**
Male sex, n (%)	10 (43.3)	6 (61.1)	43 (57.5)	**0.542**
Co-morbidity, n (%)				
Hypertension	7 (26.9)	3 (27.3)	13 (28.9)	**0.982**
Diabetes mellitus	4 (15.4)	2 (18.2)	13 (28.9)	**0.393**
COPD/Asthma	2 (7.7)	0 (0)	6 (13.3)	**0.374**
Chronic hepatitis	3 (11.5)	2 (18.2)	4 (8.9)	**0.672**
Chronic kidney disease	0 (0)	1 (5.6)	0 (0)	**0.231**
Heart failure	0 (0)	0 (0)	1 (2.2)	**0.66**
Alcoholism, n (%)	0 (0)	0 (0)	3 (6.7)	**0.468**
Current Smoking, n (%)	3 (15.8)	1 (10)	9 (30)	**0.304**
IGRA (+), n (%)	0 (0)	11 (100)	NA	
Acid fast bacilli 1-4, n (%)			25 (55)	
Drug-resistant TB, n (%)			10 (25)	
Systemic symptoms, n (%)			**15 (34.8)**	

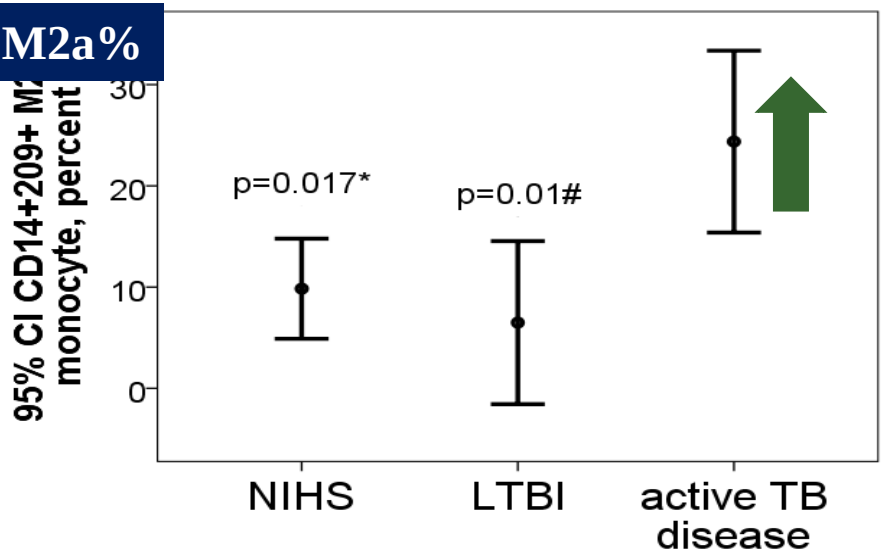
CD14^{+}CD209^{-} M1 monocyte percentage was significantly decreased in active TB group as compared with either NIHS or LTBI group, and negatively correlated with FPR3 expression of M1 monocyte.

CD14^{+}CD209^{+} M2a monocyte percentage was increased in active TB group versus either NIHS or LTBI group, and positively correlated with FPR3 expression of M2a monocyte.

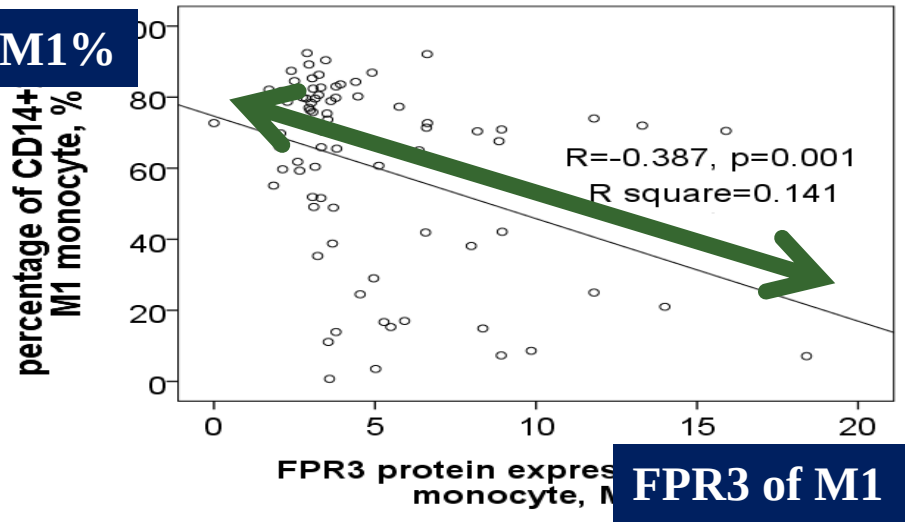
M1%



M2a%

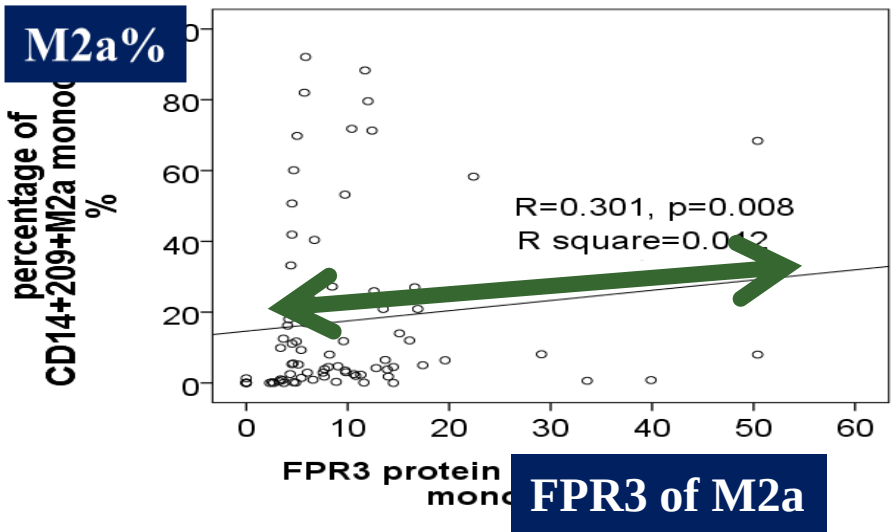


M1%



FPR3 of M1

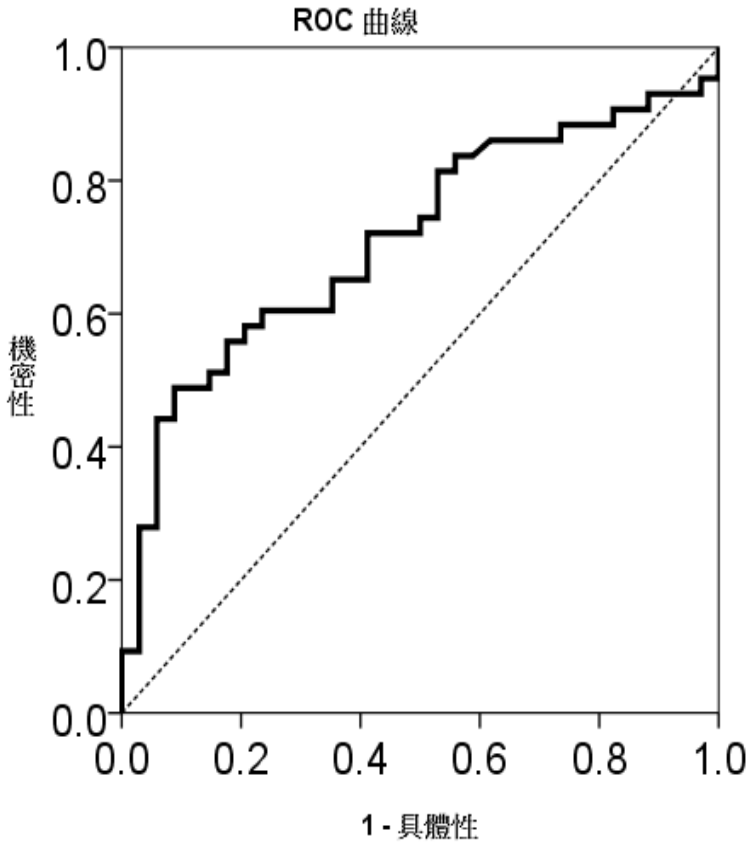
M2a%



FPR3 of M2a

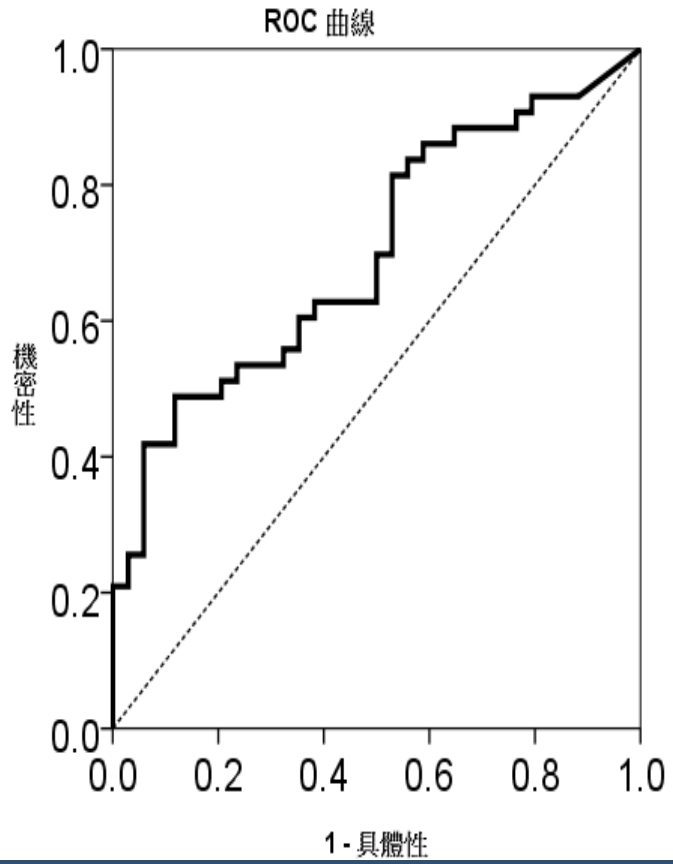
Diagnostic accuracy of the reverse of M1 monocyte percentage and M2a/M1 percentage ratio measured at diagnosis for discrimination between active TB disease and LTBI+NIHS

Reverse of M1%



AUC: o.717, 95%CI 0.597-0.0828, P=0.001
0.0139: Sensitivity 82.1%, Specificity 58.8%

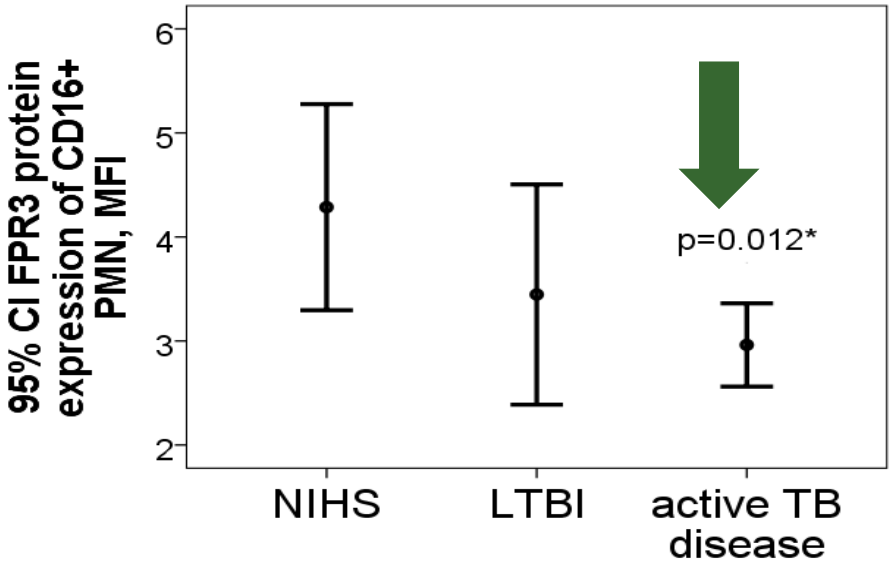
M2a/M1 % ratio



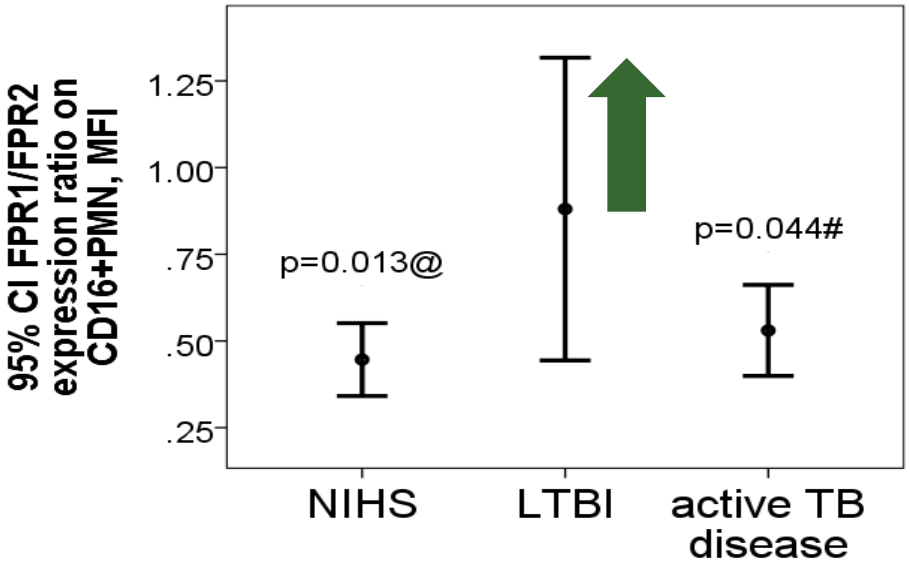
AUC: 0.698, 95%CI 0.582-0.814, p=0.003
0.721: sensitivity 62.8%, specificity 61.8%

FPR3 protein expression of CD16^{+} neutrophil was decreased in active TB group versus NIHS group, while FPR1 over FPR2 expression ratio on CD16^{+}neutrophil was increased in LTBI group as compared with either NIHS or LTBI group.

FPR3 of PMN

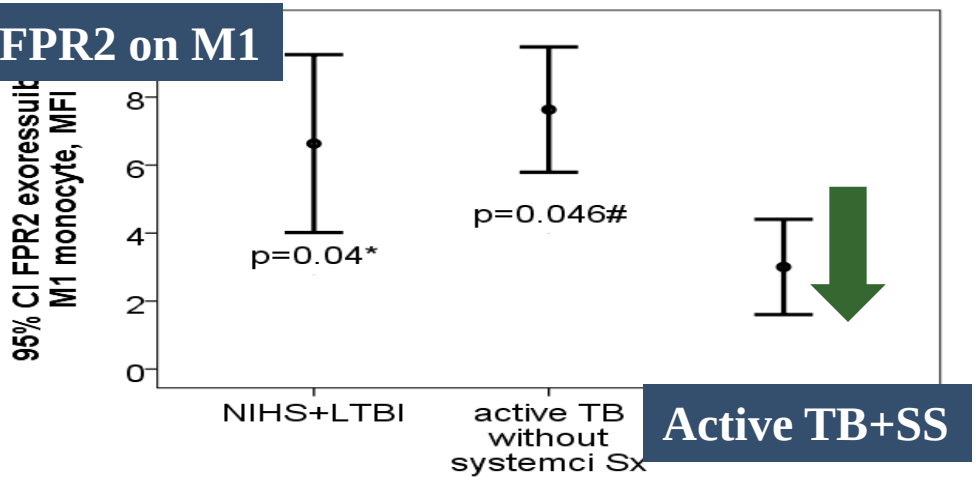


FPR1/FPR2 expression ratio on PMN

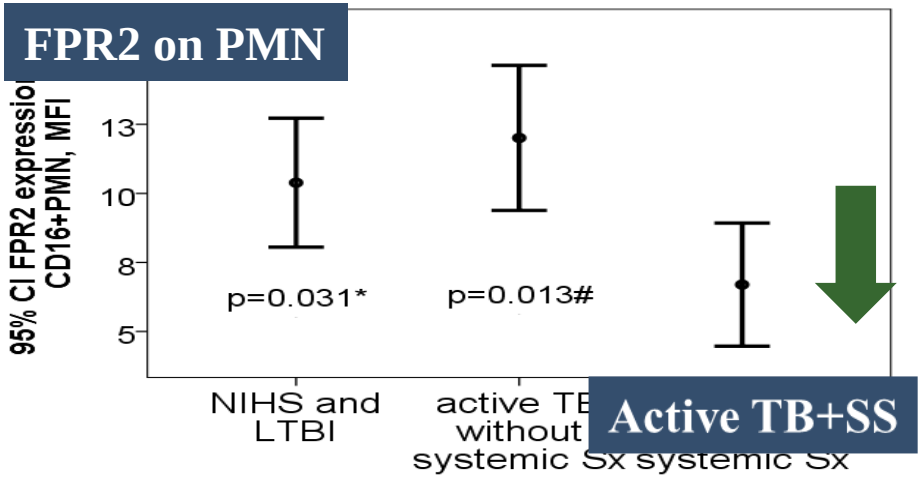


FPR2 expressions on M1 monocyte, CD16+neutrophil, and CD3-CD56+ natural killer cells were all decreased in active TB patients with systemic symptoms (defined as either fever or body weight loss at diagnosis; N=15) as compared with that in those without systemic symptoms (N=28) or subjects without active TB disease (NIHS+LTBI groups; N=34).

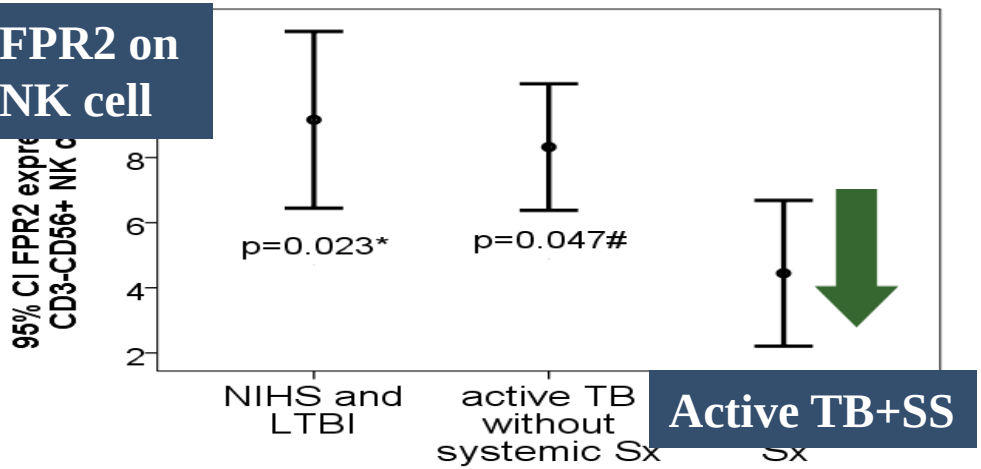
FPR2 on M1



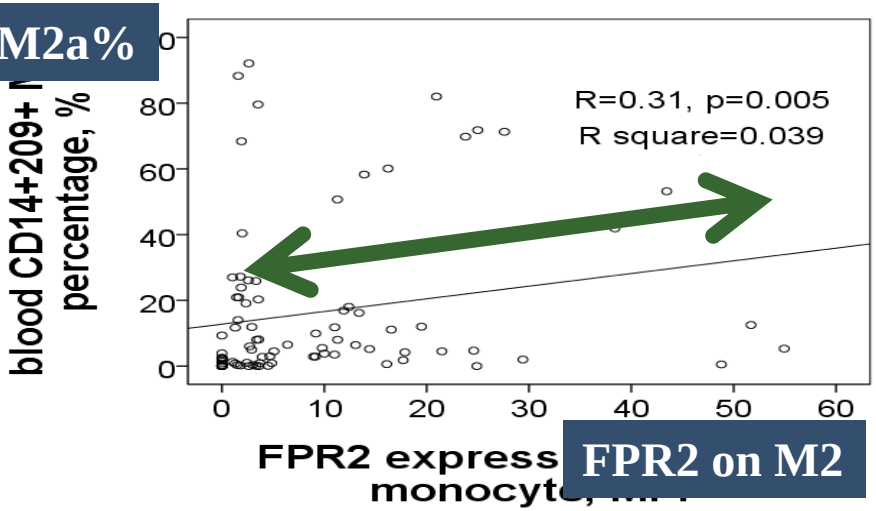
FPR2 on PMN



FPR2 on NK cell



M2a%



Summary and Conclusions

- A potential marker of active TB disease vs. NIHS or LTBI
  - Increased M2a monocyte percentage
  - Decreased M1 phenotypes of blood monocyte
  - Decreased FPR3 expression of blood neutrophil
  - Reverse of M1% showed Good performance of diagnosis for active TB
    - ✓ AUC 0.721, p=0.001, versus LTBI+NIHS
    - ✓ CUT-OFF value of 0.0139: Sensitivity 82.1%, Specificity 58.8
- A potential marker of LTBI
  - Increased FPR1 over FPR2 expression ratio of blood neutrophil
- An association between systemic symptoms in active TB D's
  - Decreased FPR2 expressions on M1 monocyte,
  - Decreased FPR2 expression on neutrophil
  - Decreased FPR2 expression on natural killer cells

Acknowledgement

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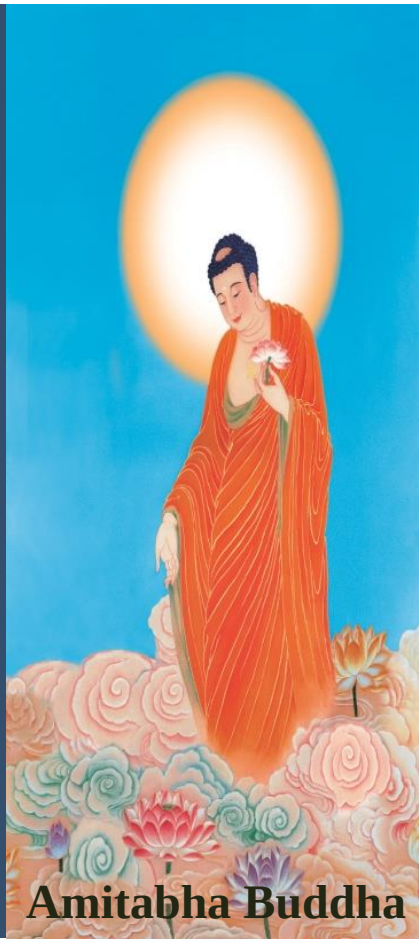

Amitabha Buddha

Thank You for Your Attention