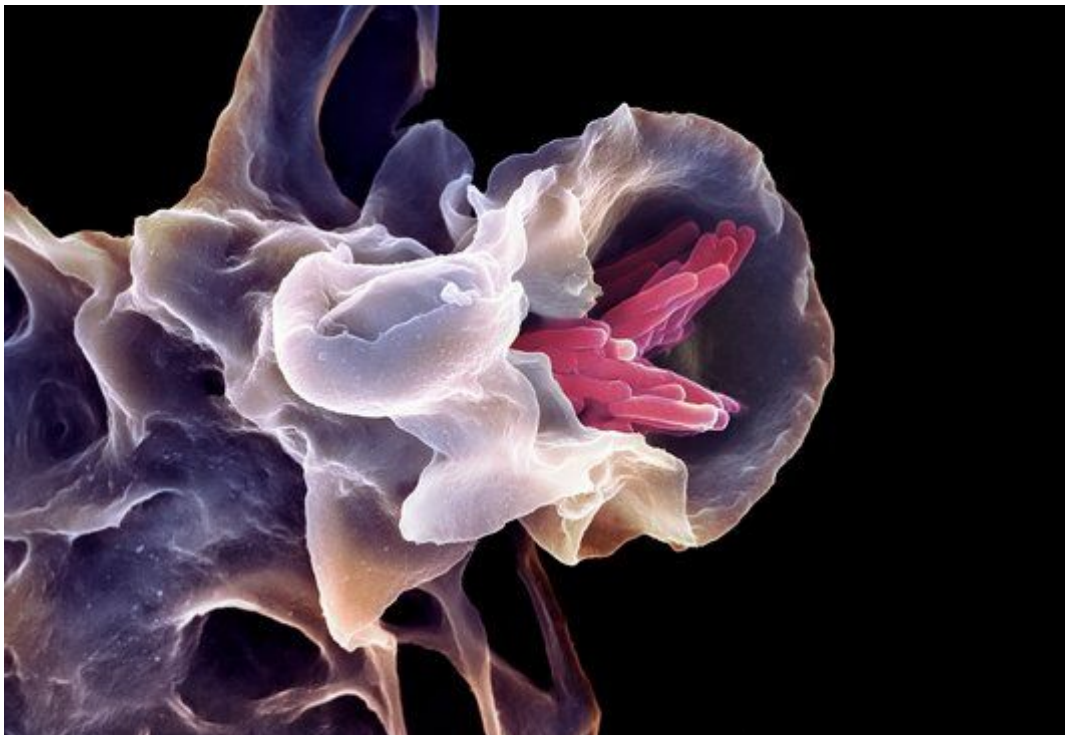
UBC – Experimental Medicine
MEDI 501

Macrophage Differentiation and Function

Zakaria Hmama, PhD
IIRC/Infectious Diseases

hmama@mail.ubc.ca

http://www.id.med.ubc.ca/Faculty/Faculty_Hmama.htm



October 15 2013

Macrophage discovery

In 1882 Mechnikov identified motile cells in the larvae of starfishes…concluding they were important to the animals' immune defenses. He called them macrophages.

Mechnikov extended his observations to human white blood cells and discovered that macrophages are able to engulf and kill bacteria, a process that he called phagocytosis.

Ilya Ilyich Mechnikov (1845-1916)

1908 Nobel Prize (Physiology of Medicine)

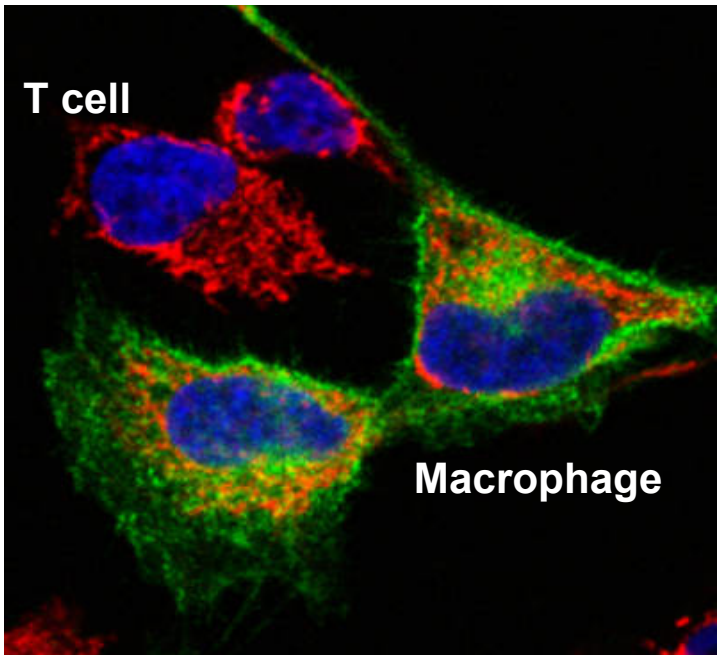
Macrophage function

The macrophage intervenes a the crossroad of innate and adaptive immunity,

Macrophage's function starts with "search and destroy" and finishes with stimulation of T cells.

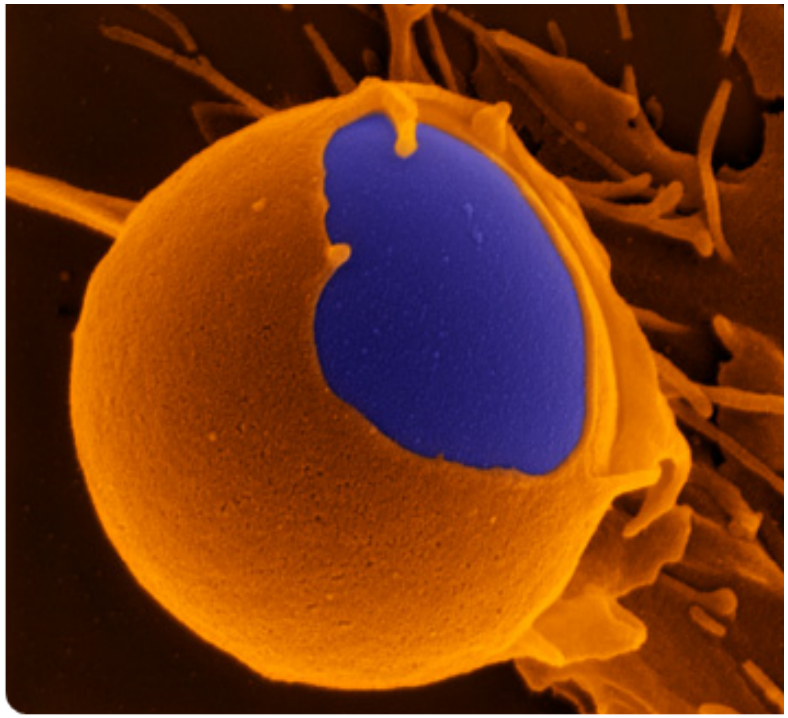
Phagocytosis

Ag presentation



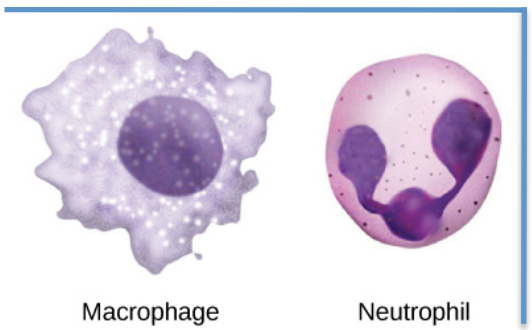
Lecture Topics

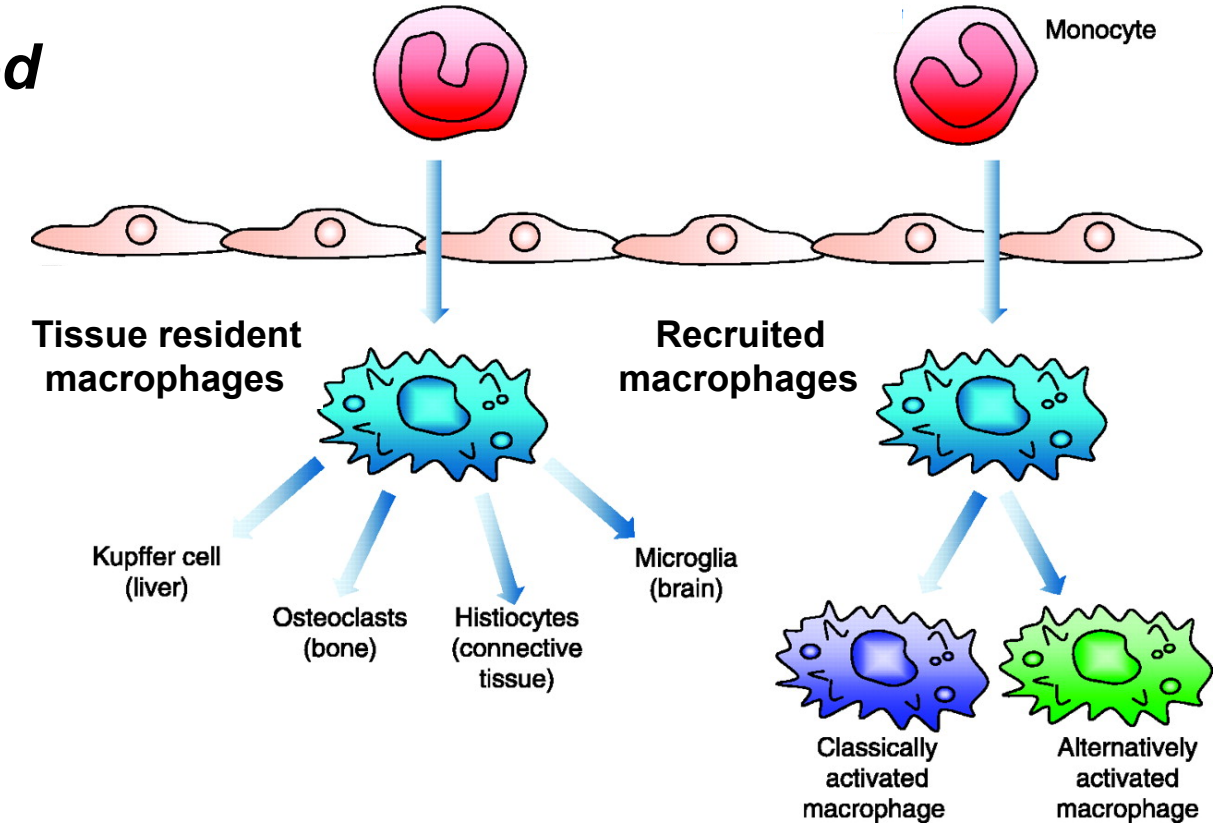
- Monocyte/ Macrophage differentiation
- Monocyte/ Macrophage cell lines
- Phagocytosis
- Efferocytosis
- Mechanisms of intracellular killing



Monocyte/ Macrophage differentiation

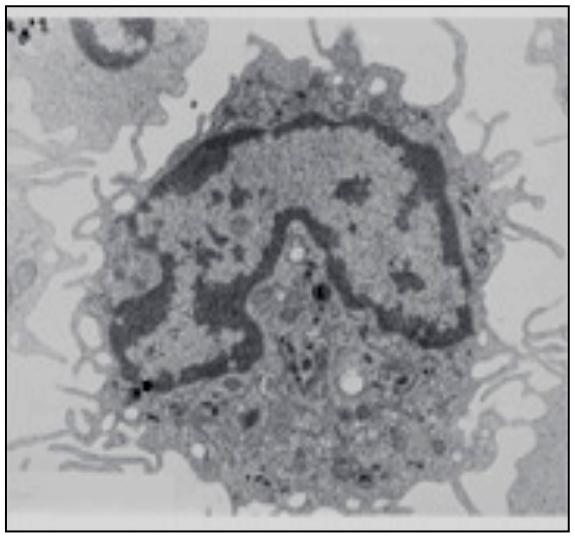
Differentiated tissue macrophages arise from monocytes recruited from the blood.
Unlike short-lived PMNs, macrophages survive in the body for several months.



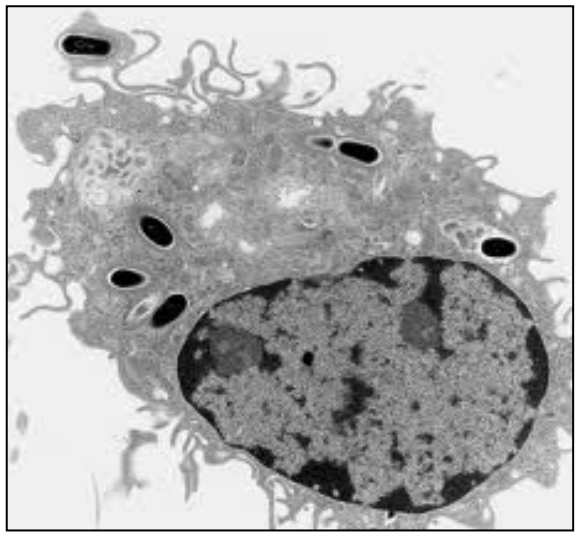


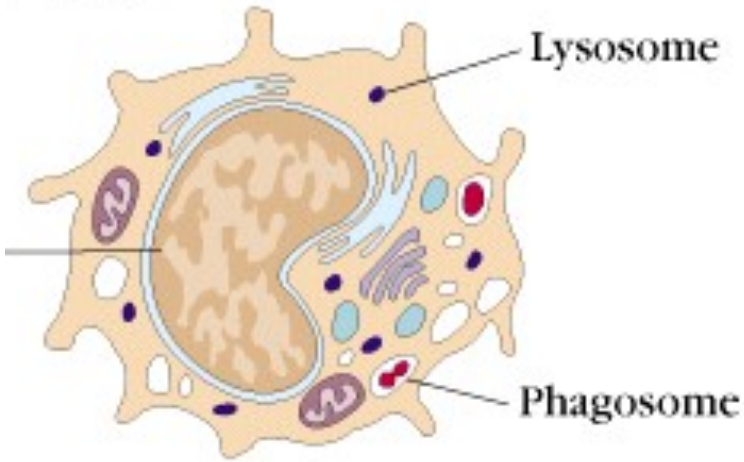
Monocyte/ Macrophage differentiation

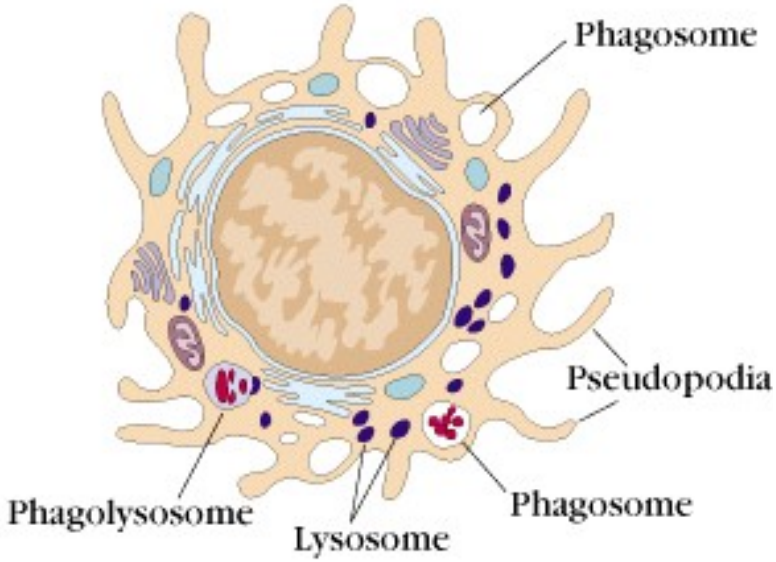
Monocyte



Macrophage





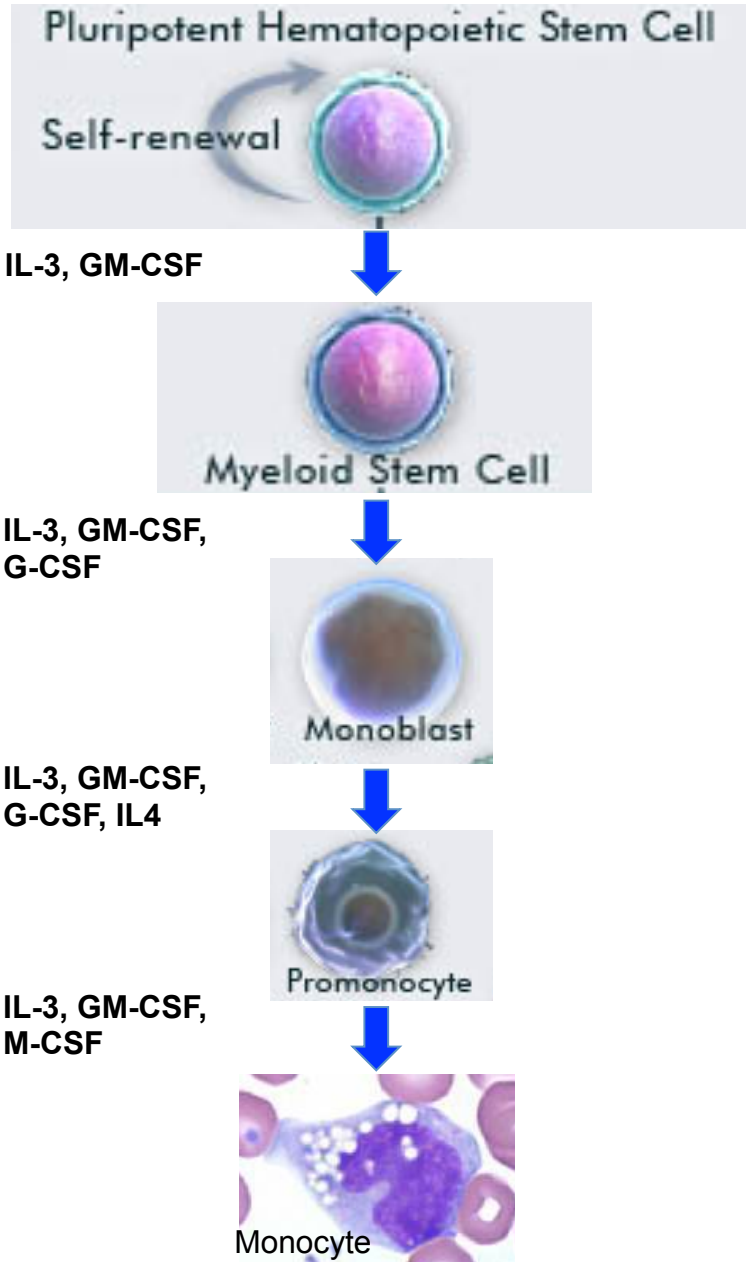


Monocyte/ Macrophage differentiation

Monocytes are produced by the bone marrow from hematopoietic stem cell precursors called monoblasts.

Monocytes constitute between 3 to 8% of the leukocytes in the blood.

They circulate in the bloodstream for about one to three days and then move into tissues throughout the body.

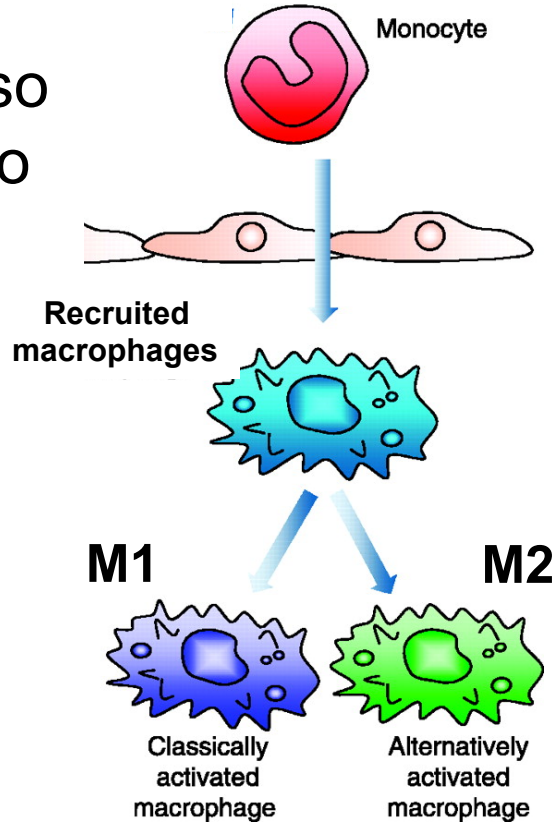


Monocyte/ Macrophage differentiation

M1 *vs* M2 macrophages

Like T Helper cells, recruited macrophages also polarize into distinct phenotypes in response to endogenous and exogenous stimuli.

By analogy to Th1 and Th2, macrophages are currently referred to as: M1- (**CAM**) and M2- macrophages (**AAM**).

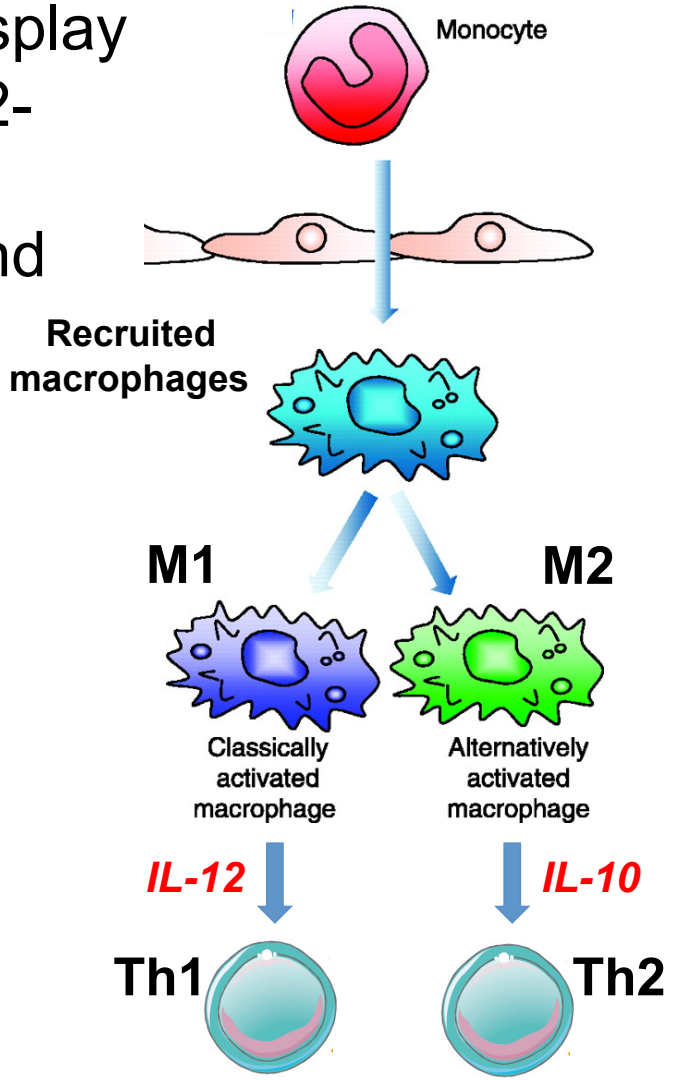


Monocyte/ Macrophage differentiation

M1 *vs* M2 macrophages

Classically **a**ctivated **m**acrophages (M1) display an acute inflammatory phenotype, while M2-polarized or **a**lternatively **a**ctivated **m**acrophages ensures anti-inflammatory and regulatory functions.

M1 phenotype supports proinflammatory Th1 responses driven by cytokines such as IL-12, while M2 phenotype supports Th2 anti-inflammatory processes driven by IL-10.



Monocyte/ Macrophage differentiation

M1 *vs* M2 macrophages

NATURE IMMUNOLOGY VOLUME 12 NUMBER 3 MARCH 2011

PMID: 21240265

IRF5 promotes inflammatory macrophage polarization and T_H1-T_H17 responses

Thomas Krausgruber[1], Katrina Blazek[1], Tim Smallie[1], Saba Alzabin[1], Helen Lockstone[2], Natasha Sahgal[2], Tracy Hussell[3], Marc Feldmann[1] & Irina A Udalova[1]

Here we show that IRF5 expression in macrophages was reversibly induced by inflammatory stimuli and contributed to the plasticity of macrophage polarization. High expression of IRF5 was characteristic of M1 macrophages, in which it directly activated transcription of the genes encoding interleukin 12 subunit p40 (IL-12p40), IL-12p35 and IL-23p19 and repressed the gene encoding IL-10. Consequently, those macrophages set up the environment for a potent T helper type 1 (TH1)-TH17 response. Global gene expression analysis demonstrated that exogenous IRF5 upregulated or downregulated expression of established phenotypic markers of M1 or M2 macrophages, respectively. Our data suggest a critical role for IRF5 in M1 macrophage polarization and define a previously unknown function for IRF5 as a transcriptional repressor.

IRF5 is a member of the interferon regulatory factor (IRF), a group of transcription factors with diverse roles

Monocyte/ Macrophage differentiation

M1 *vs* M2 macrophages

NATURE IMMUNOLOGY VOLUME 11 NUMBER 10 OCTOBER 2010 PMID: 20729857

The Jmjd3-*Irf4* axis regulates M2 macrophage polarization and host responses against helminth infection

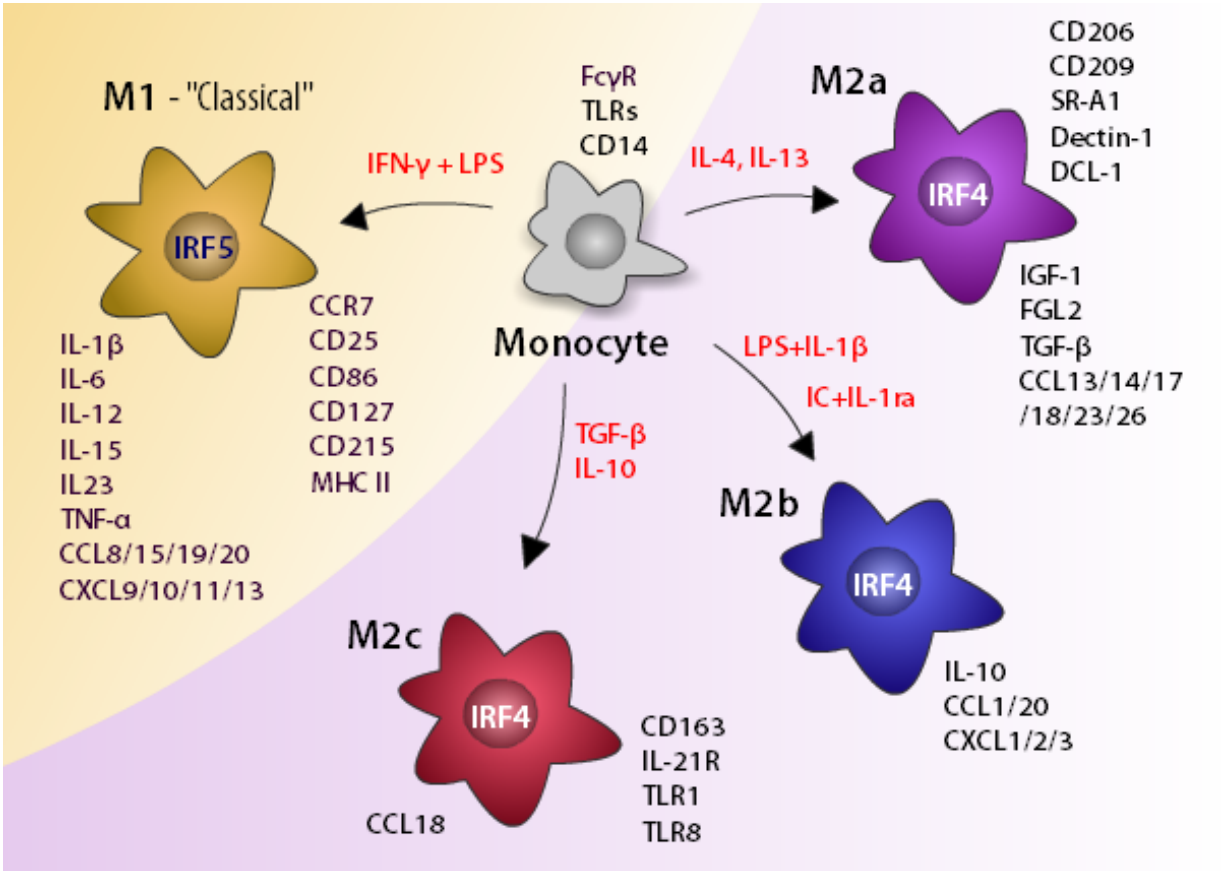
Takashi Satoh[1,2,8], Osamu Takeuchi[1,2,8], Alexis Vandenbon[3], Koubun Yasuda[4], Yoshiaki Tanaka[5], Yutaro Kumagai[1,2], Tohru Miyake[1,2], Kazufumi Matsushita[1,2], Toshihiko Okazaki[1], Tatsuya Saitoh[1,2], Kiri Honma[6], Toshifumi Matsuyama[6], Katsuyuki Yui[6], Tohru Tsujimura[7], Daron M Standley[3], Kenji Nakanishi[4], Kenta Nakai[6] & Shizuo Akira[1,2]

This study revealed a pathway involving the IFN regulatory factor-4 (IRF4) and the histone demethylase jumonji domain containing-3 (JMJD3) that regulates the polarization of marophages into M2 phenotype.

Monocyte/ Macrophage differentiation

M1 *vs* M2 macrophages

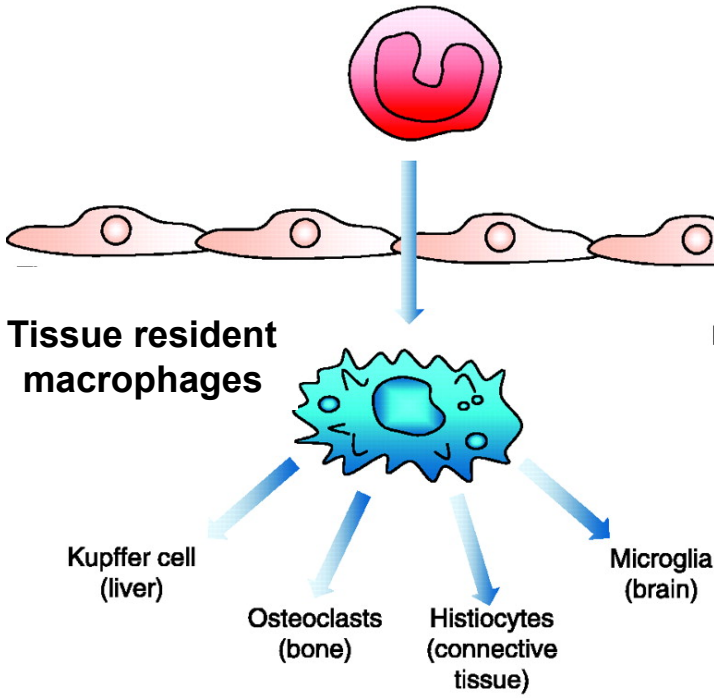
M2 cells can be further classified into 3 different subsets, M2a, M2b, and M2c, based on the type of stimulation and the subsequent expression of surface molecules and cytokines.



Monocyte/ Macrophage differentiation

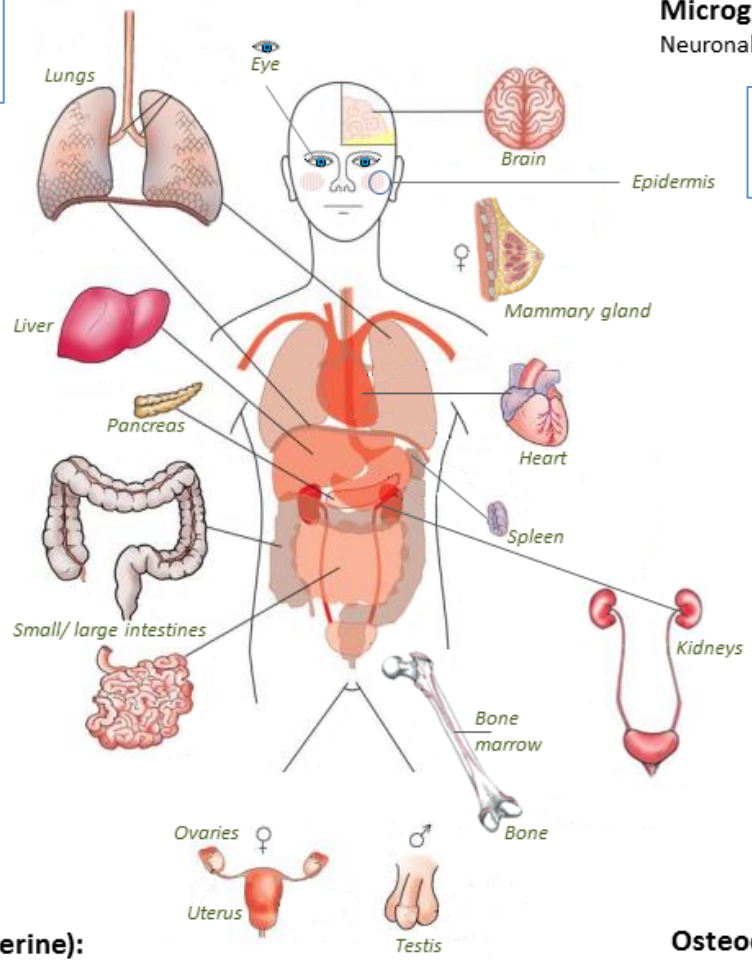
Resident macrophages

We commonly think of macrophages as cells of the immune system, and forget their central function in many other aspects of embryonic development, homeostasis and wound repair.



Monocyte/ Macrophage differentiation

Resident macrophages



Macrophage (eye):
Vascular remodelling

Alveolar Macrophage:
Immune surveillance

Microglia:
Neuronal survival, connectivity, repair

Langerhans cells:
Immune surveillance; control keratinocyte proliferation / differentiation

Kupffer Cells:
Clearance of debris from blood; liver tissue regeneration after damage

Macrophage (mammary):
Branching morphogenesis; ductal development

Macrophage (pancreas):
Islet development

Macrophage (cardiac):
Healing and remodelling of infarcted heart

Crypt Macrophages:
Immune surveillance

Macrophage (splenic marginal zone):
Immune surveillance

Macrophage (kidney):
Ductal development

Macrophage (ovary):
Steroid hormone production and ovulation

Bone marrow macrophage:
Erythropoiesis; stem cell niche

Macrophage (uterine):
Cervical ripening

Osteoclasts:
Bone remodelling

Macrophage (testis):
Steroid hormone production

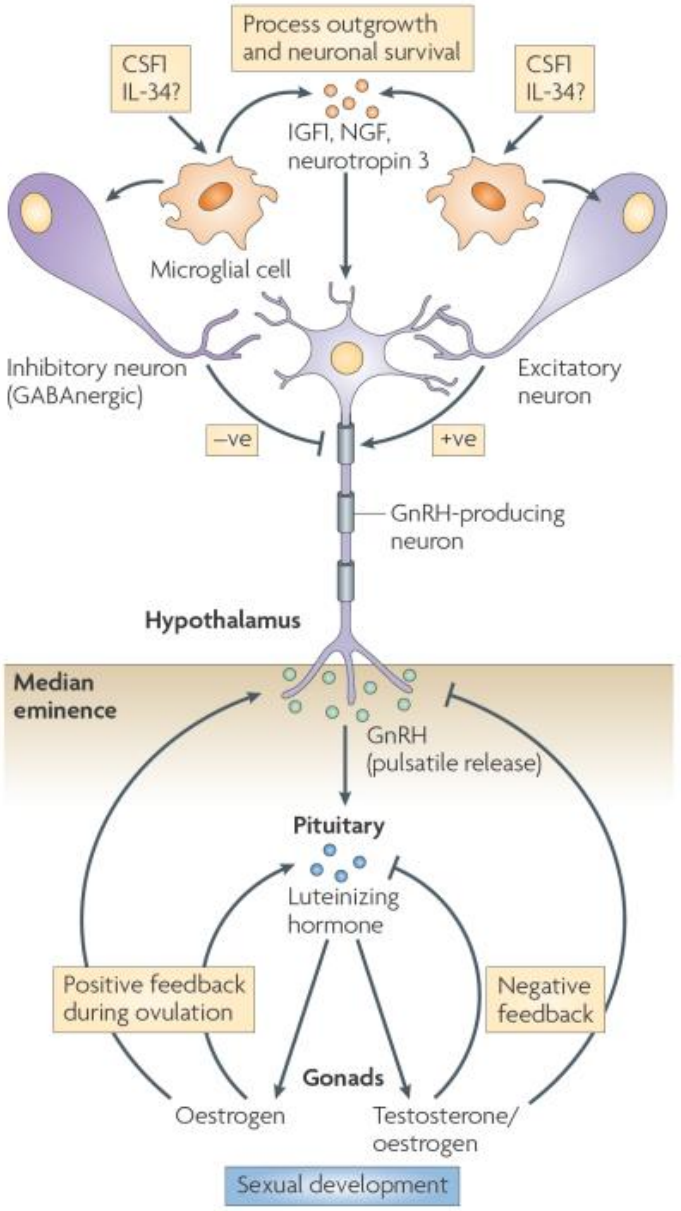
Monocyte/ Macrophage differentiation

Resident macrophages

Ex: Microglial cells

In the brain, microglial cells respond to colony-stimulating factor 1 receptor (CSF1R) signalling to produce factors that are required for the establishment of neuronal connectivity.

Microglial cells also regulate the hypothalamic–pituitary–gonadal axis through negative signalling from GABAnergic neurons and positive signalling from excitatory neurons, which allows gonadotrophin-releasing hormone (GnRH) to be released into the median eminence. This induces the release of luteinizing hormone by the pituitary, which controls testosterone and oestrogen biosynthesis in the gonads.

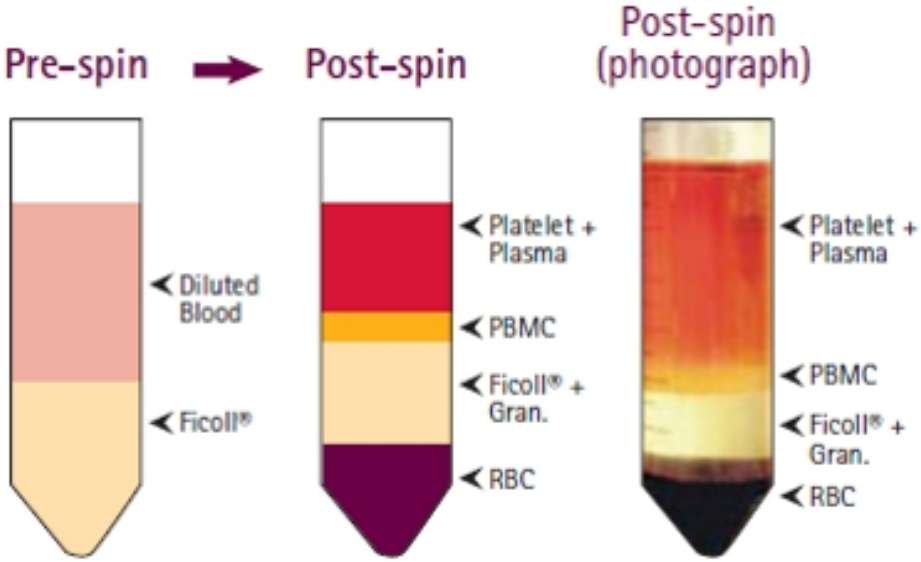


Nat Rev Immunol. 2009 April; 9(4): 259–270. **PMID: 19282852**

Monocyte/ Macrophage Cell lines

In vitro M1/ M2 differentiation

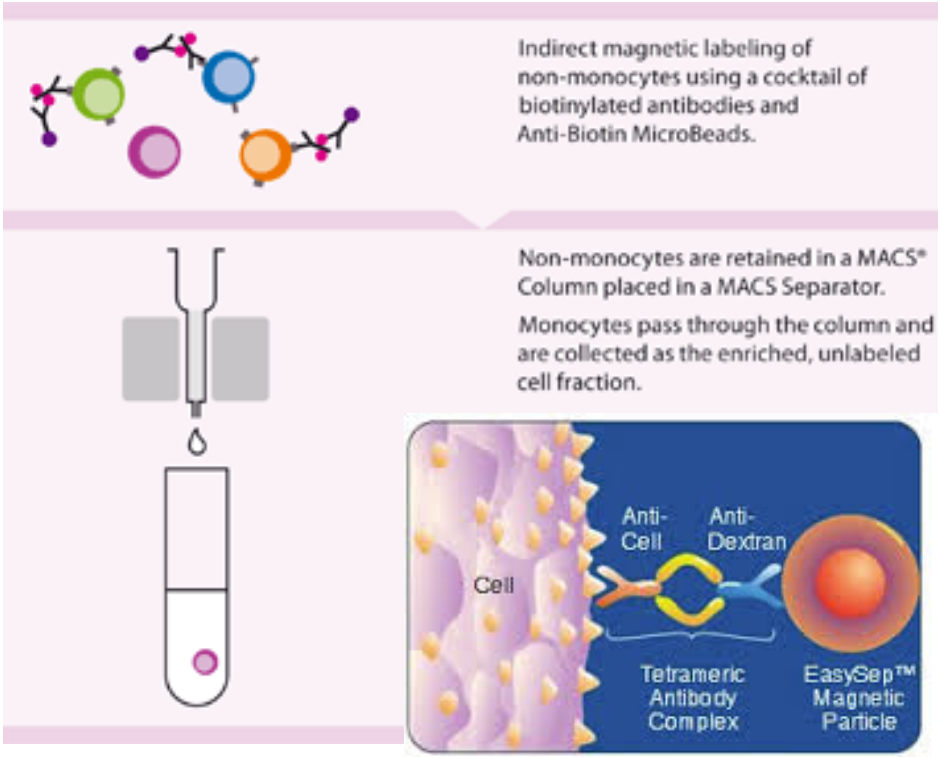
PBMC isolation



PBMC,
Peripheral Blood Mononuclear Cells:
Monocytes + Lymphocytes

100 ml blood led to 5-15 million monocytes

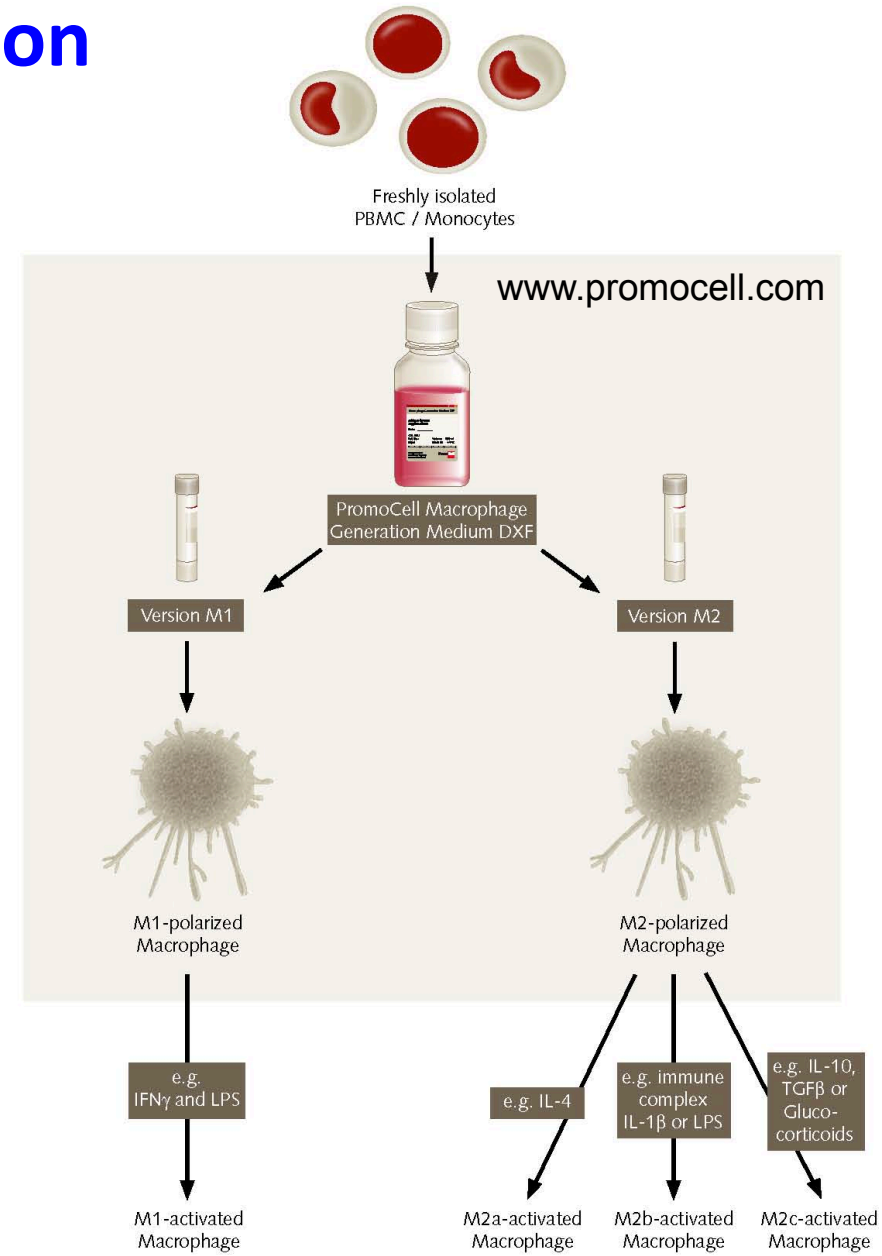
Monocyte negative selection



Monocyte/ Macrophage differentiation

In vitro M1/ M2 differentiation

Highly pure M1- or M2-macrophages can be differentiated from monocytes or PBMC in defined culture media plus a combination of appropriate stimuli



Monocyte/ Macrophage Cell lines

Limited cell numbers represent a barrier to the use of blood monocytes in protocols requiring very large numbers of macrophages.

Research projects based exclusively on macrophage differentiation *in vitro* involves large number of healthy blood donors, is costly and is time consuming.

Monocytic cell lines of varying degrees of differentiation can be readily expanded in vitro and thus are frequently used to model macrophage function.

Most common monocytic cell line are: U-937, HL-60 and THP-1.

Monocyte/ Macrophage Cell lines

Int J Cancer. 1976 May 15;17(5):565-77. **PMID: 178611**

Establishment and characterization of a human histiocytic lymphoma cell line (U-937).

Sundström C, Nilsson K.

U-937 cell line was isolated from a 37 year old male patient and are used to study the behavior and differentiation of monocytes.

U937 cells mature and differentiate in response to a number of soluble stimuli, adopting the morphology and characteristics of mature macrophages.

Monocyte/ Macrophage Cell lines

blood

1979 54: 713-733 **PMID: 288488**

Characterization of the continuous, differentiating myeloid cell line (HL-60) from a patient with acute promyelocytic leukemia

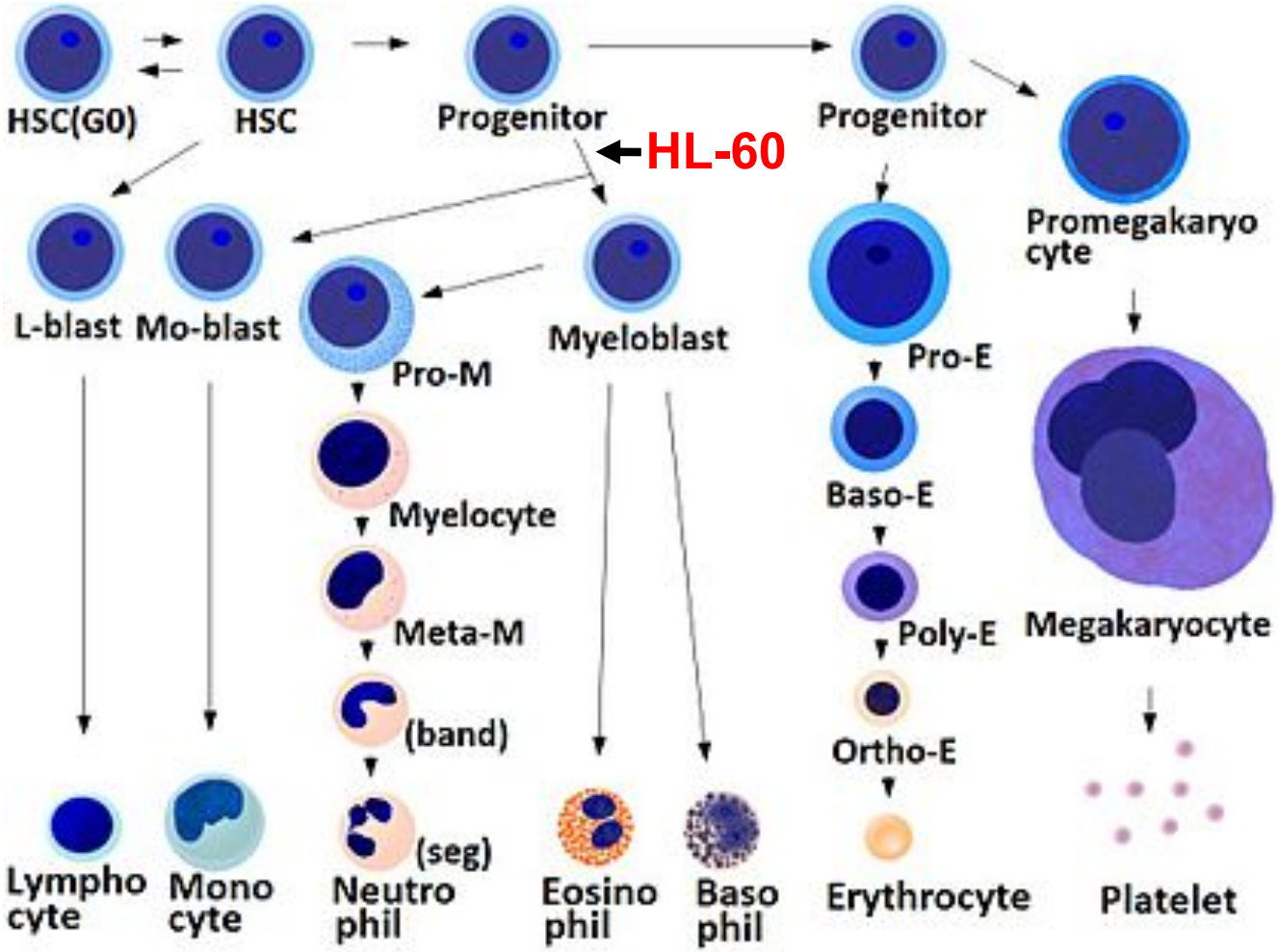
R Gallagher, S Collins, J Trujillo, K McCredie, M Ahearn, S Tsai, R Metzgar, G Aulakh, R Ting, F Ruscetti and R Gallo

The HL-60 cells lack specific markers for lymphoid cells, but express surface receptors for Fc fragment and complement (C3), which have been associated with differentiated granulocytes. They exhibit phagocytic activity and responsiveness to a chemotactic stimulus commensurate with the proportion of mature cells…

The HL-60 cultured cell line provides a continuous source of human cells for studying the molecular events of myeloid differentiation.

Monocyte/ Macrophage Cell lines

HL-60 is committed to both the monocyte and granulocyte branches of the myeloid lineage



Monocyte/ Macrophage Cell lines

HL-60 can be differentiated *in vitro* into monocyte- or PMN-like cells

Proc. Natl. Acad. Sci. USA
Vol. 75, No. 5, pp. 2458–2462, May 1978
Medical Sciences PMID: 276884

Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds

(myeloid differentiation/liquid suspension culture/granulocyte/hemopoiesis)

STEVEN J. COLLINS, FRANCIS W. RUSCETTI, ROBERT E. GALLAGHER, AND ROBERT C. GALLO

AACR
Cancer Research

Control of Macrophage Cell Differentiation in Human Promyelocytic HL-60 Leukemia Cells by 1,25-Dihydroxyvitamin D_3 and Phorbol-12-myristate-13-acetate

Shin-ichi Murao, M. Anne Gemmell, Michael F. Callaham, et al.

Cancer Res 1983;43:4989-4996. **PMID: 6576856**

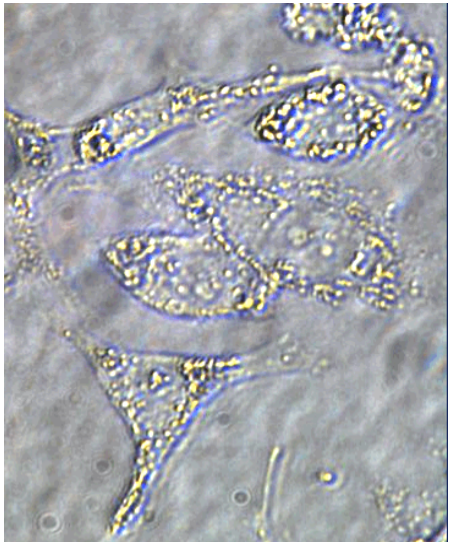
Monocyte/ Macrophage Cell lines

Int J Cancer. 1980 Aug;26(2):171-6. **PMID: 6970727**

Establishment and characterization of a human acute monocytic leukemia cell line (THP-1).

Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K.

The monocytic nature of the cell line was characterized by: (1) the presence of alpha-naphthyl butyrate esterase activities which could be inhibited by NaF; (2) lysozyme production; (3) the phagocytosis of latex particles and sensitized sheep erythrocytes; and (4) the ability to restore T-lymphocyte response to Con A. The cells did not possess Epstein-Barr virus-associated nuclear antigen. These results indicate that THP-1 is a leukemia cell line with distinct monocytic markers. During culture, THP-1 maintained these monocytic characteristics for over 14 months.



Monocyte/ Macrophage Cell lines

OPEN ACCESS Freely available online

PLoS one

The Identification of Markers of Macrophage Differentiation in PMA-Stimulated THP-1 Cells and Monocyte-Derived Macrophages

Marc Daigneault, Julie A. Preston, Helen M. Marriott, Moira K. B. Whyte, David H. Dockrell*

Department of Infection and Immunity, Medical School, University of Sheffield, Sheffield, United Kingdom

PLoS ONE 5(1): e8668. doi:10.1371/journal.pone.0008668
PMID: 20084270

This study compared the phenotype of the promonocytic THP-1 cell line after various protocols of differentiation with VD3 and PMA in comparison to primary human monocytes or monocyte-derived macrophages.

The findings suggest a modified PMA differentiation protocol that enhances THP-1 differentiation into macrophage.

Monocyte/ Macrophage Cell lines

Limitation of monocytic cell lines

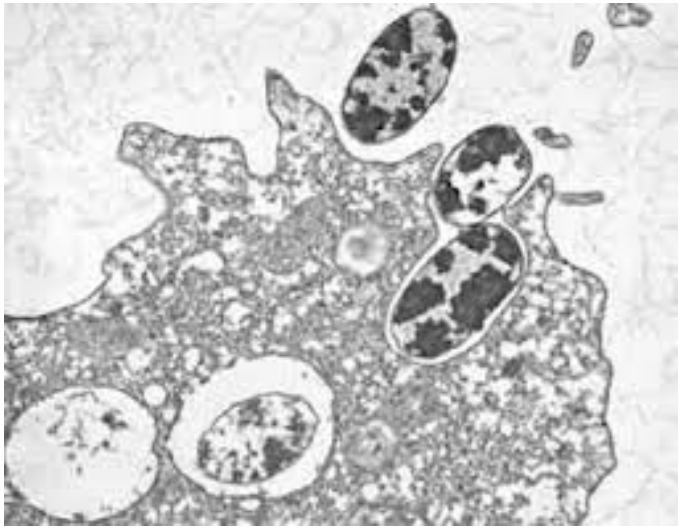
Monocytic cell lines have obvious advantages in terms of ease of growth and maintenance. However, cell lines responses do not always accurately reflect the behavior of differentiated tissue macrophages.

Confirmation of pertinent data with monocytes-derived macrophages is strongly recommended.

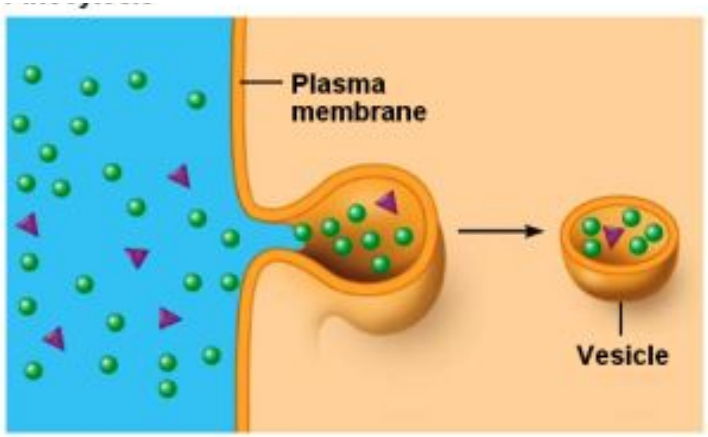
Phagocytosis

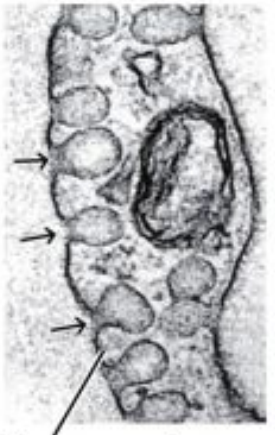
Macrophage endocytosis, 'ingestion by the cell'

In phagocytosis, or "cell eating," the macrophage engulfs debris, bacteria, or other sizable objects. Invagination produces a vesicle called a phagosome.



In pinocytosis, or "cell drinking," the cell engulfs extracellular fluid, including molecules such as sugars and proteins. These materials enter the cell inside a vesicle.

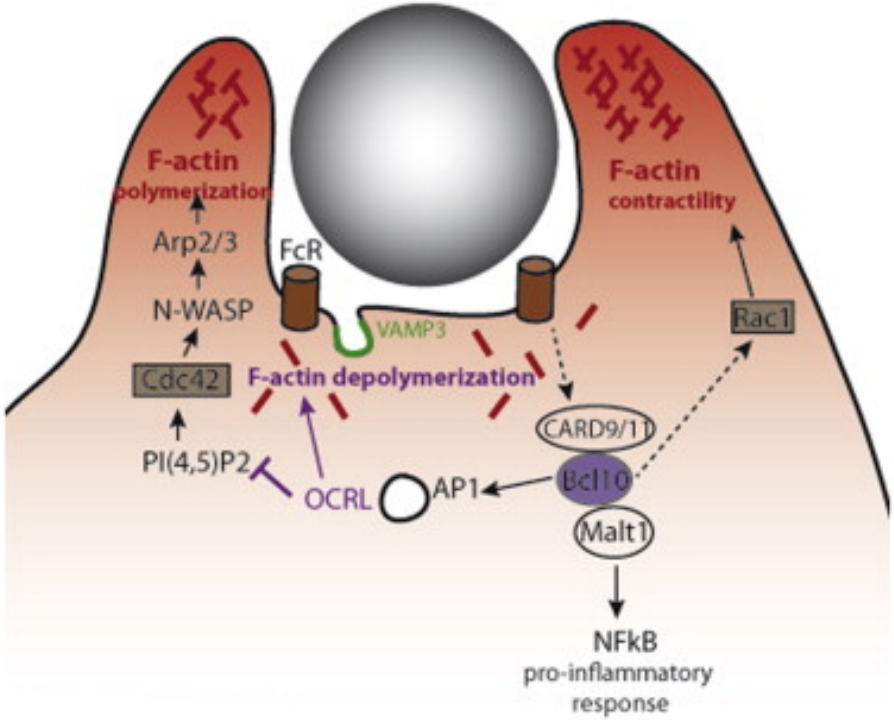




Phagocytosis

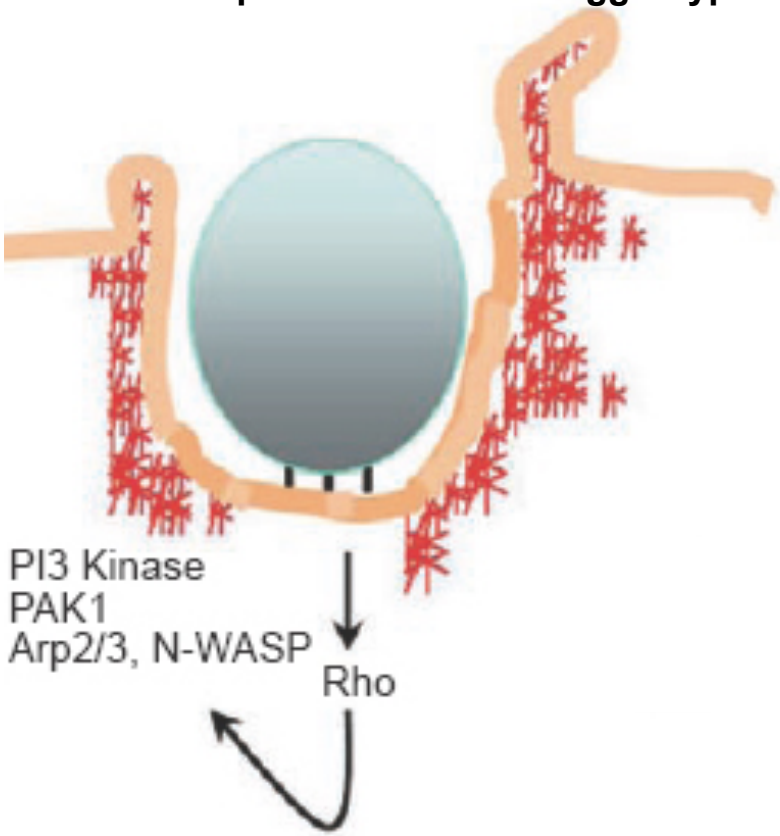
Monocyte/macrophage can perform phagocytosis using intermediary (opsonin) proteins such as antibodies or complement that coat the pathogen.

Fc Receptor mediated or 'Zipper type'



Cdc42- and Rac1-dependent

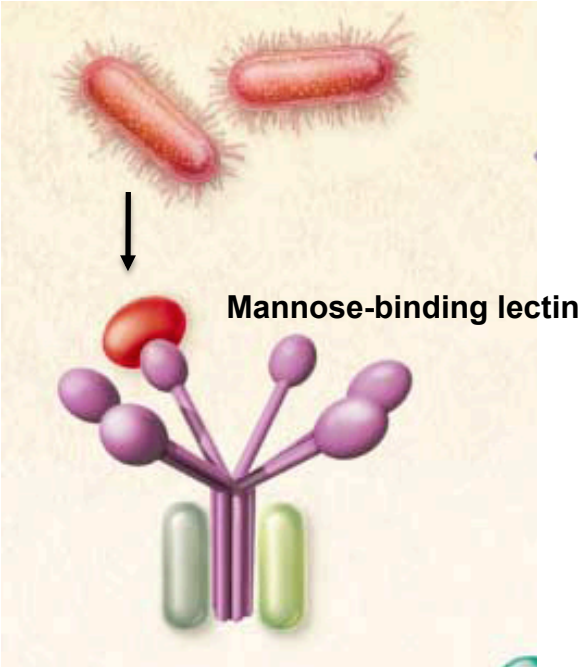
CR3 Receptor mediated or 'trigger type'

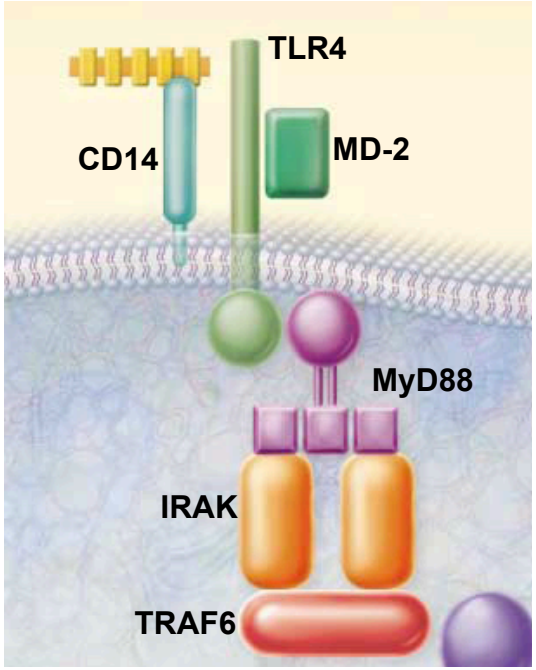


RhoA-dependent

Phagocytosis

Phagocytosis occurs also by binding to the microbe directly via pattern-recognition receptors that recognize pathogens.





Phagocytosis

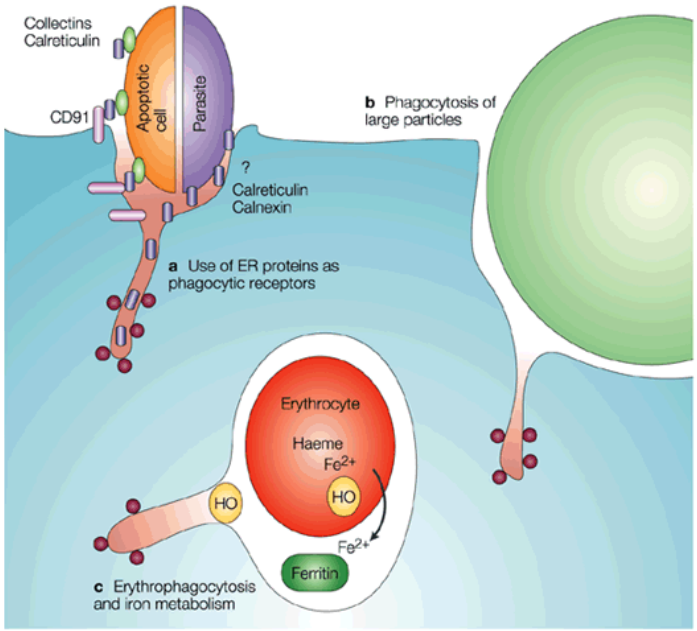
ER-mediated phagocytosis, *novel and controversial mode of phagocytosis*

Cell, Vol. 110, 119–131, July 12, 2002, Copyright ©2002 by Cell Press **PMID: 12151002**

Endoplasmic Reticulum-Mediated Phagocytosis Is a Mechanism of Entry into Macrophages

Etienne Gagnon,[1] Sophie Duclos,[1]
Christiane Rondeau,[1] Eric Chevet,[2]
Pamela H. Cameron,[3] Olivia Steele-Mortimer,[4]
Jacques Paiement,[1] John J. M. Bergeron,[3,5]
and Michel Desjardins[1,5,6]
[1]Département de Pathologie et Biologie Cellulaire
Université de Montréal
Montréal. Québec

Fusion of the ER with the macrophage plasmalemma, underneath phagocytic cups, is a source of membrane for phagosome formation in macrophages. Successive waves of ER become associated with maturing phagosomes during phagolysosome biogenesis.



Phagocytosis

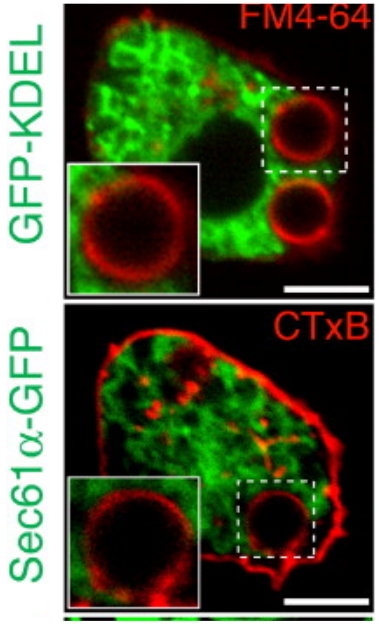
ER-mediated phagocytosis, *novel and controversial mode of phagocytosis*

Cell. 2005 Oct 7;123(1):157-70. **PMID: 16213220**

Quantitative and dynamic assessment of the contribution of the ER to phagosome formation.

Touret N, Paroutis P, Terebiznik M, Harrison RE, Trombetta S, Pypaert M, Chow A, Jiang A, Shaw J, Yip C, Moore HP, van der Wel N, Houben D, Peters PJ, de Chastellier C, Mellman I, Grinstein S.
Programme in Cell Biology, University of Toronto, Ontario M5G 1X8, Canada.

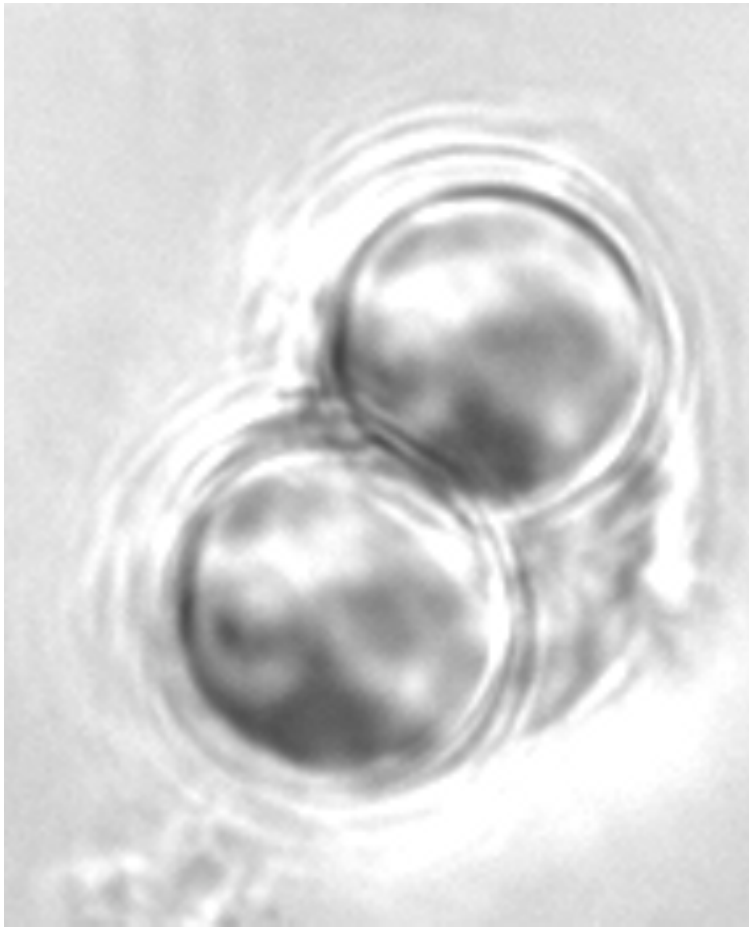
We used a combination of biochemical, fluorescence imaging, and electron microscopy techniques to quantitatively and dynamically assess the contribution of the plasmalemma and of the ER to phagosome formation and maturation. We could not verify even a transient physical continuity between the ER and the plasma membrane, nor were we able to detect a significant contribution of the ER to forming or maturing phagosomes.

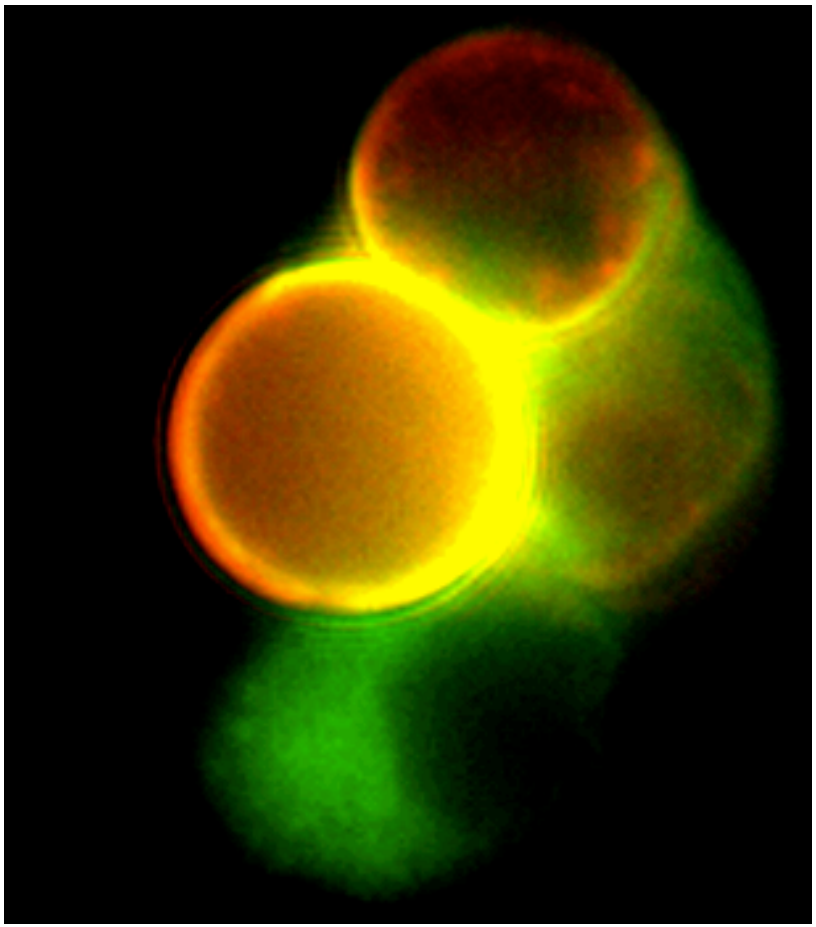


Phagocytosis

ER phagocytosis: Yes for large particle…

11 um latex bead phagosome (Red) fused to the ER (Green)





Hmama lab, unpublished

Phagocytosis

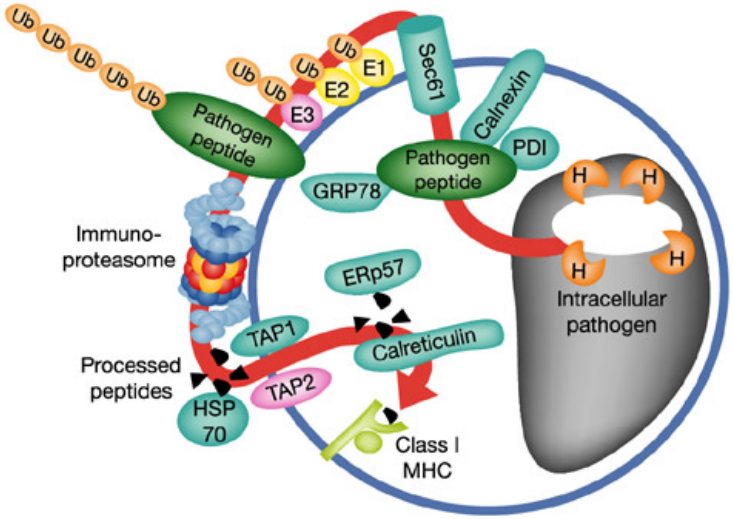
ER-mediated phagocytosis, *novel and controversial mode of phagocytosis*

Nature. 2003 Sep 25;425(6956):402-6. **PMID: 14508490**

Phagosomes are competent organelles for antigen cross-presentation.

Houde M, Bertholet S, Gagnon E, Brunet S,
Goyette G, Laplante A, Princiotta MF, Thibault P,
Sacks D, Desjardins M.
Département de pathologie et biologie cellulaire,
Université de Montréal, C.P.6128, Succ centre-ville,
Montréal, Québec, H3C 3J7, Canada.

It is generally accepted that antigens in the cytoplasm are loaded in the ER and presented at the cell surface on MHC class I molecules, whereas peptides present in phagocytic compartments are presented on MHC class II molecules.

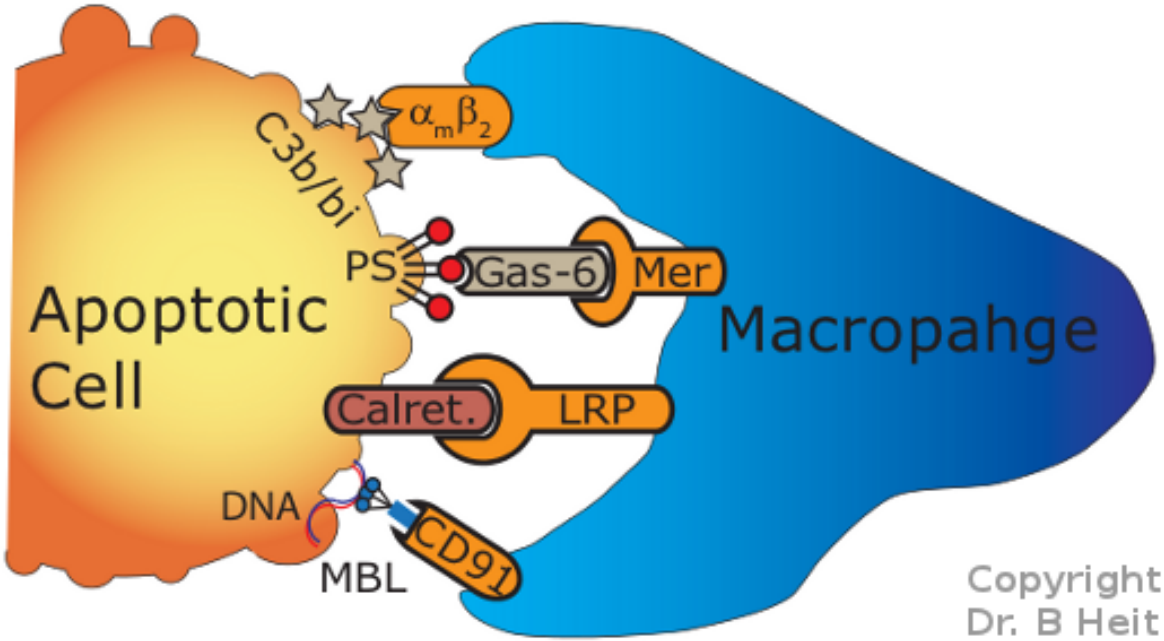


Desjardins et al show that phagosomes display the elements and properties needed to be self-sufficient for the cross-presentation of exogenous antigens, a newly ascribed function linked to phagocytosis mediated by the endoplasmic reticulum.

Efferocytosis

Efferocytosis (from **efferre:** to take to the grave, to bury) is the process of dead cell clearance by phagocytic cells.

Macrophages recognize "eat me" signals displayed by apoptotic cells. These signals include complement fragments (C3b/bi), phosphatidylserine (PS), intracellular proteins (calret) and DNA. Recognition of these signals induces the macrophage to consume (efferocytose) and destroy the apoptotic cell.



Efferocytosis

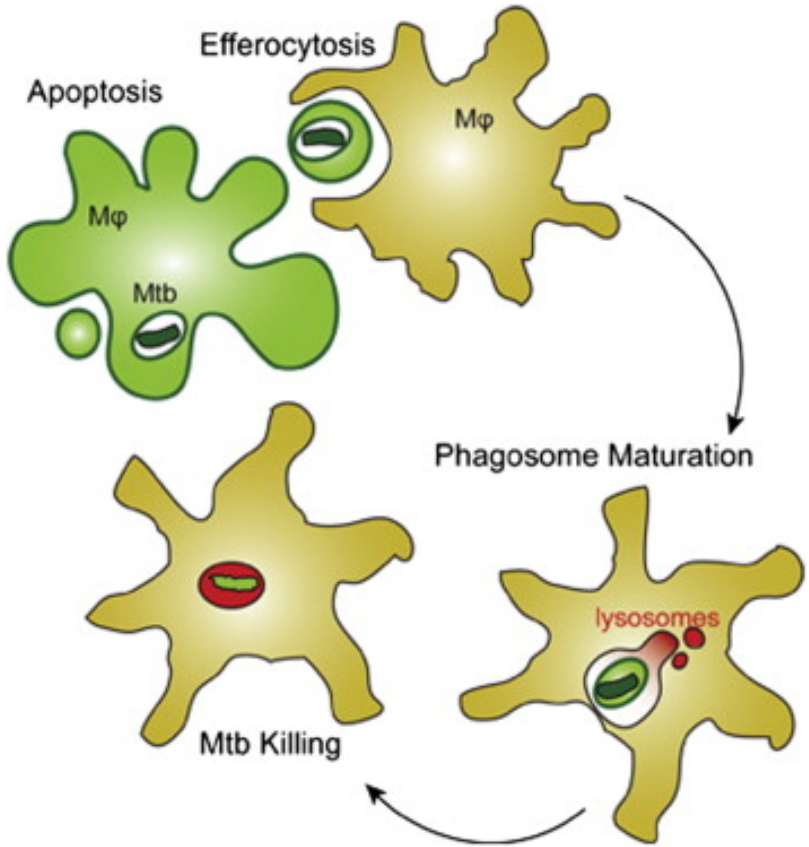
Cell Host Microbe. 2012 Sep 13;12(3):289-300. **PMID: 22980326**

Efferocytosis is an innate antibacterial mechanism.

Martin CJ, Booty MG, Rosebrock TR, Nunes-Alves C, Desjardins DM, Keren I, Fortune SM, Remold HG, Behar SM.SourceDepartment of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115,

The study revealed that after apoptosis, M. tuberculosis-infected macrophages are rapidly taken up by uninfected macrophages through efferocytosis, a dedicated apoptotic cell engulfment process.

Efferocytosis of Mtb sequestered within an apoptotic macrophage further compartmentalizes the bacterium and delivers it along with the apoptotic cell debris to the lysosomal compartment.



Mechanisms of Intracellular Killing

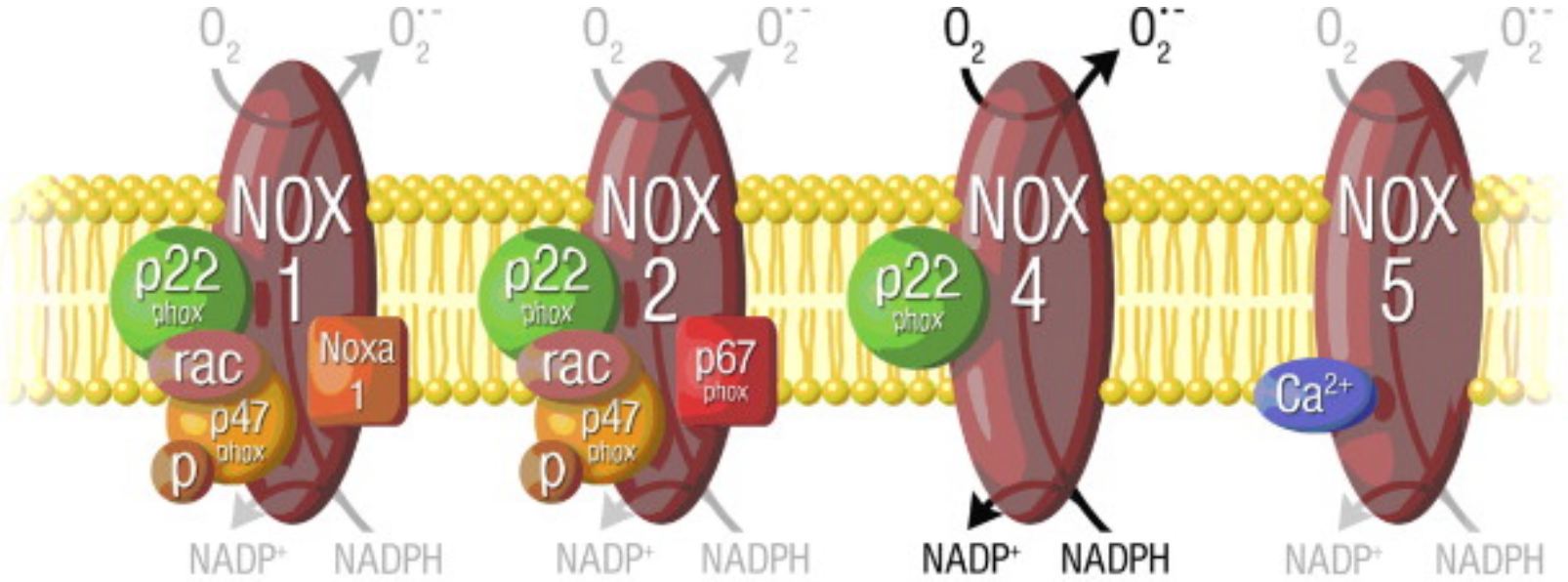
1- NADPH Oxidase: NOX2

2- Inducible Nitric Oxide Synthase: iNOS

3- Phagosome-Lysosome fusion: P-L fusion

Mechanisms of Intracellular Killing

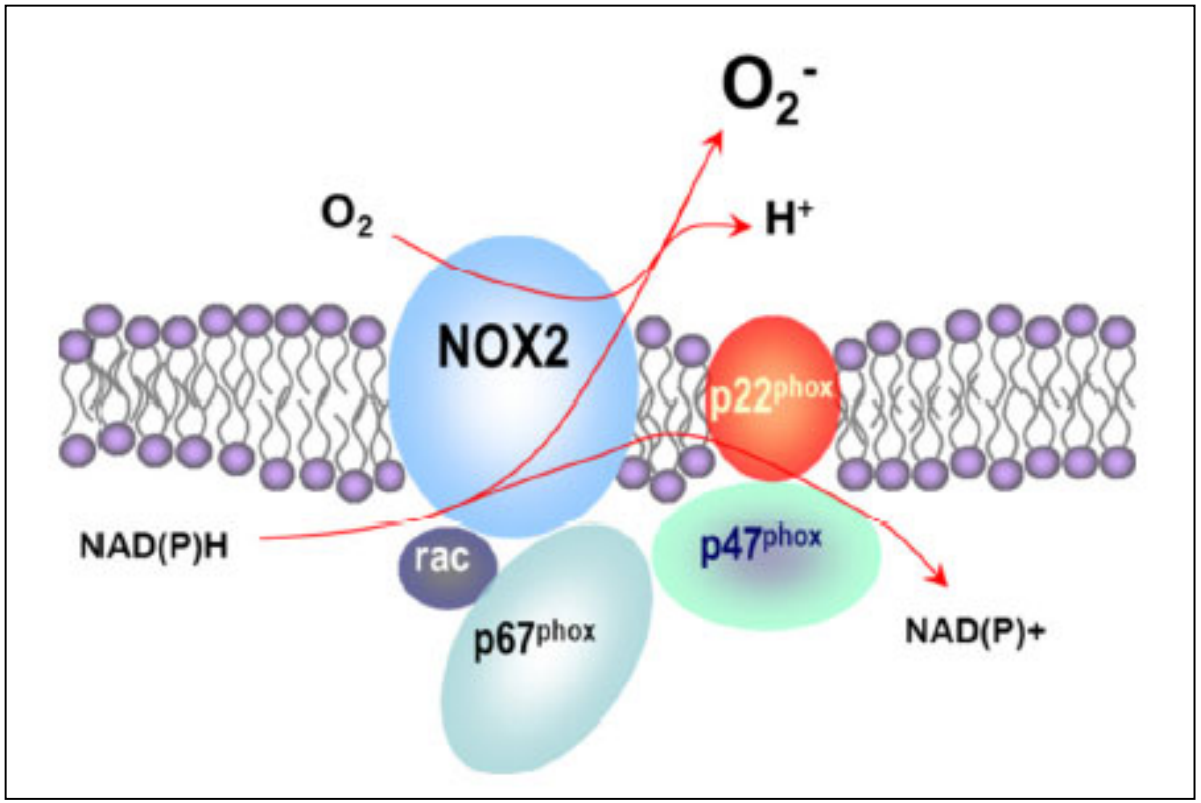
NOX family



	NOX 1	NOX 2	NOX 4	NOX 5
Requires agonist-stimulated activation	+	+	Constitutively active	+
Vascular distribution	VSMC	Endothelium, adventitia, leukocytes	Endothelium, VSMC, adventitia	Endothelium, plaque-associated VSMC
Cellular distribution	Plasma membrane	Plasma membrane	ER	???
Pathophysiology / Function	Hypertension, hypertrophy	VEGF signaling, decreases NO	ROS signaling, proliferation	Atherosclerosis ?

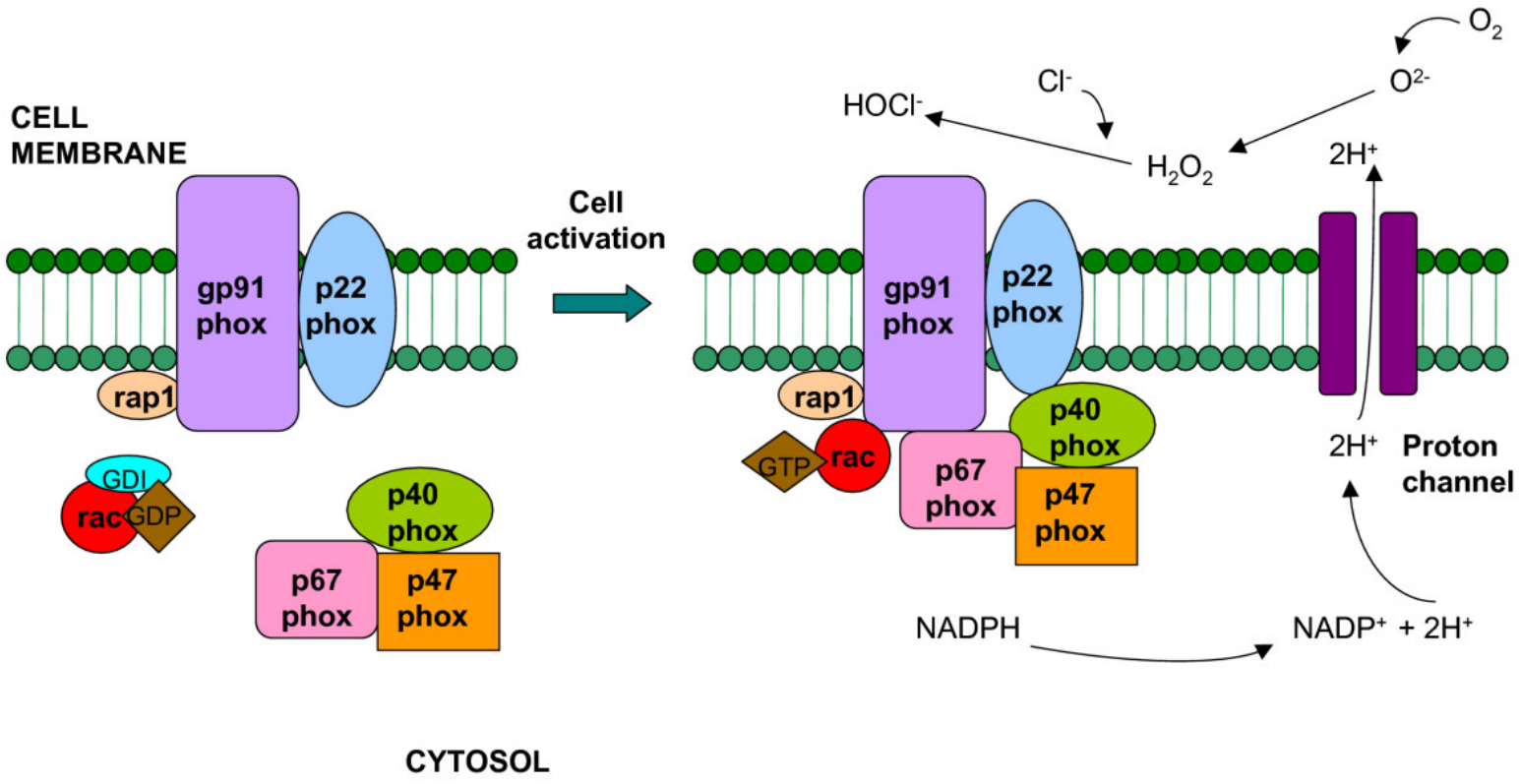
Mechanisms of Intracellular Killing

NOX-2 is a multi-subunit complex that expressed in the macrophage



Mechanisms of Intracellular Killing

Pathogen interaction with surface receptors induces NOX-2 assembly and activation, which culminates in the production of bactericidal Reactive Oxygen Species (ROS): superoxide anion, hydrogen peroxide and hypochlorous acid.



Mechanisms of Intracellular Killing

Chronic Granulomatous Disease (CGD) is disease caused by mutations in any one of the five components of the NADPH oxidase in phagocytes.

Patients with CGD suffer from recurrent, life-threatening bacterial and fungal infections of the skin, the airways, the lymph nodes, the liver, the brain and the bones.

Mechanisms of Intracellular Killing

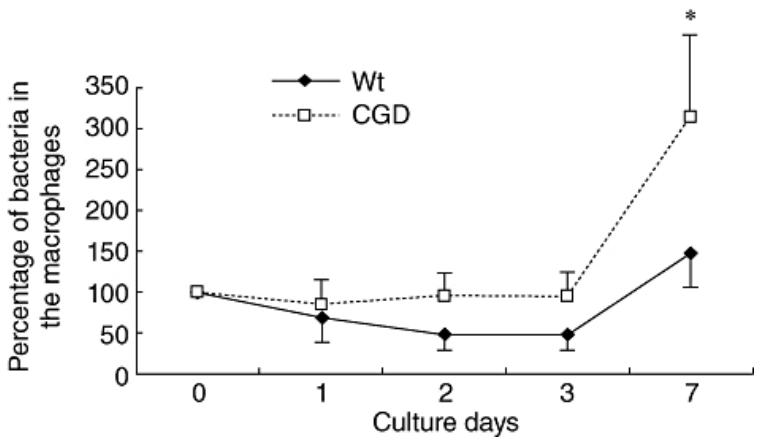
Experimental TB in CGD mice

Clin Exp Immunol. 2010 Jun;160(3):457-60.

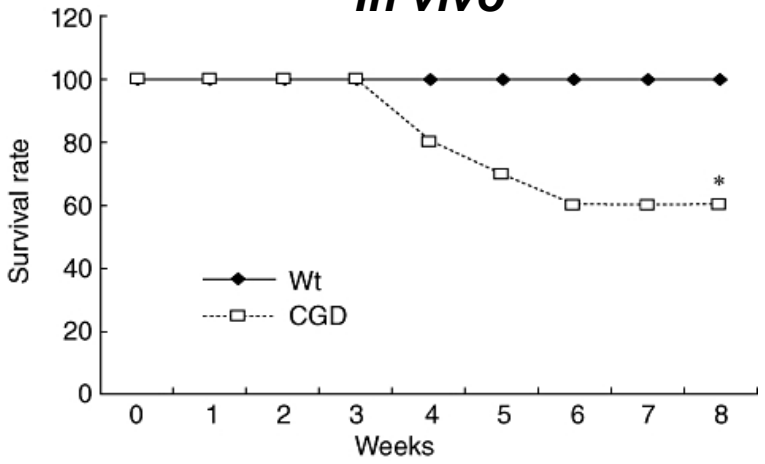
Impaired host defence against Mycobacterium avium in mice with chronic granulomatous disease.

Fujita M, Harada E, Matsumoto T, Mizuta Y, Ikegame S, Ouchi H, Inoshima I, Yoshida S, Watanabe K, Nakanishi Y.SourceResearch Institute for Diseases of the Chest, Kyushu University, Fukuoka, Japan. mfujita@fukuoka-u.ac.jp

In vitro



In vivo



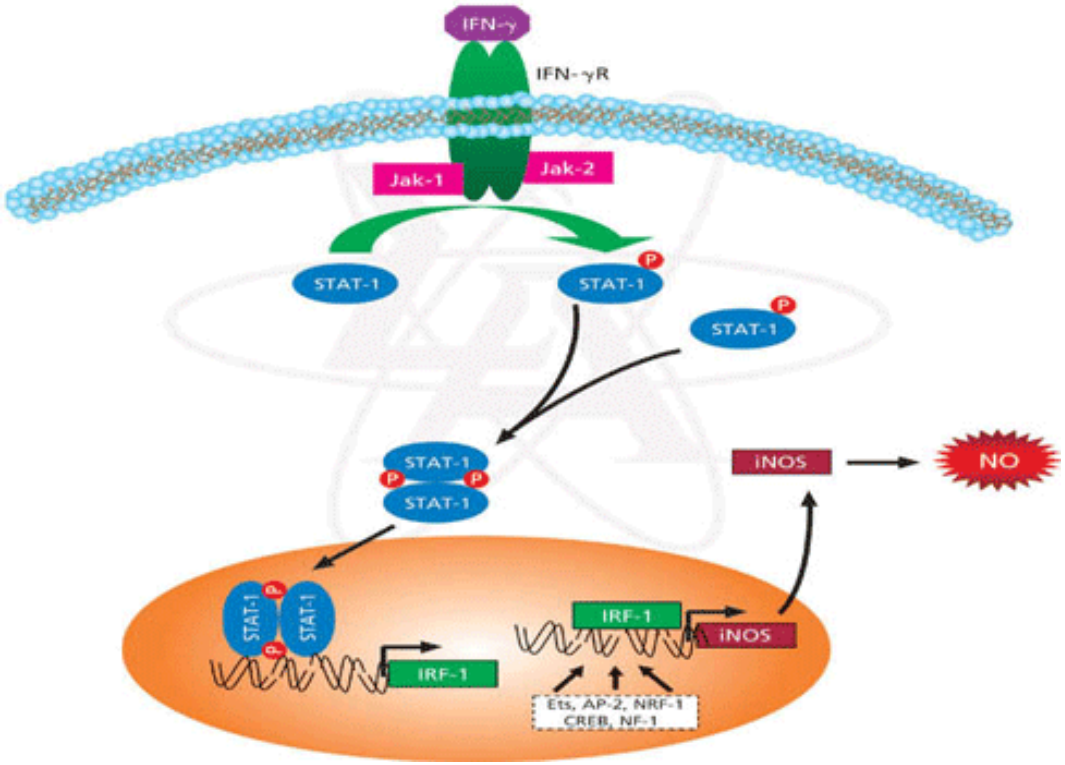
Mechanisms of Intracellular Killing

iNOS

Animal cells make three similar types of nitric oxide synthase (NOS), to produce NO for these different functions. Neuronal and endothelial NOS continually produce low levels of NO used for signaling.

Inducible NOS, on the other hand, makes large toxic bursts of NO to fight pathogens.

IFN gamma is a major inducer of iNOS expression

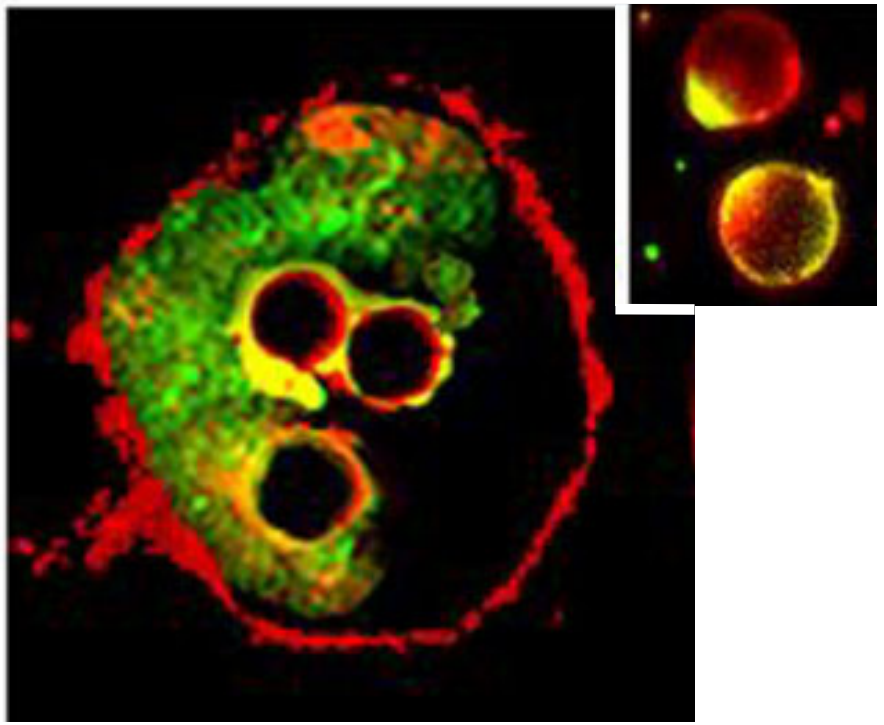


Mechanisms of Intracellular Killing

P-L Fusion

Phagolysosome fusion follow phagocytosis. It is an important immunological function of the macrophage.

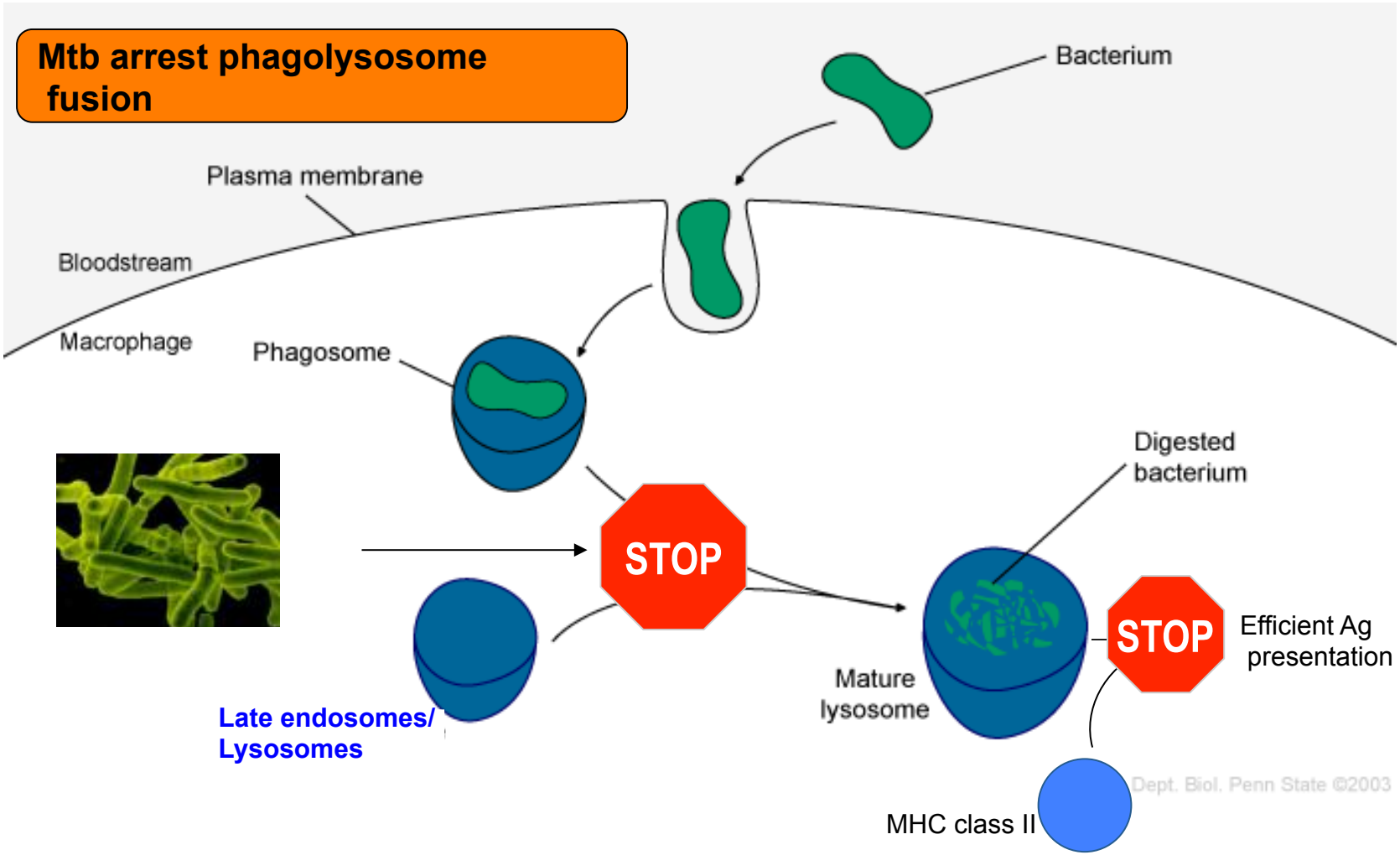
It is fusion between phagosome containing ingested particle or pathogen with a lysosomes containing hydrolytic enzymes.



Hmama Z et al.,J Cell Sci. 2004, 117: 2131-40

Mechanisms of Intracellular Killing

The mycobacterial phagosome, exception to the rule



The Mycobacterial Phagosome

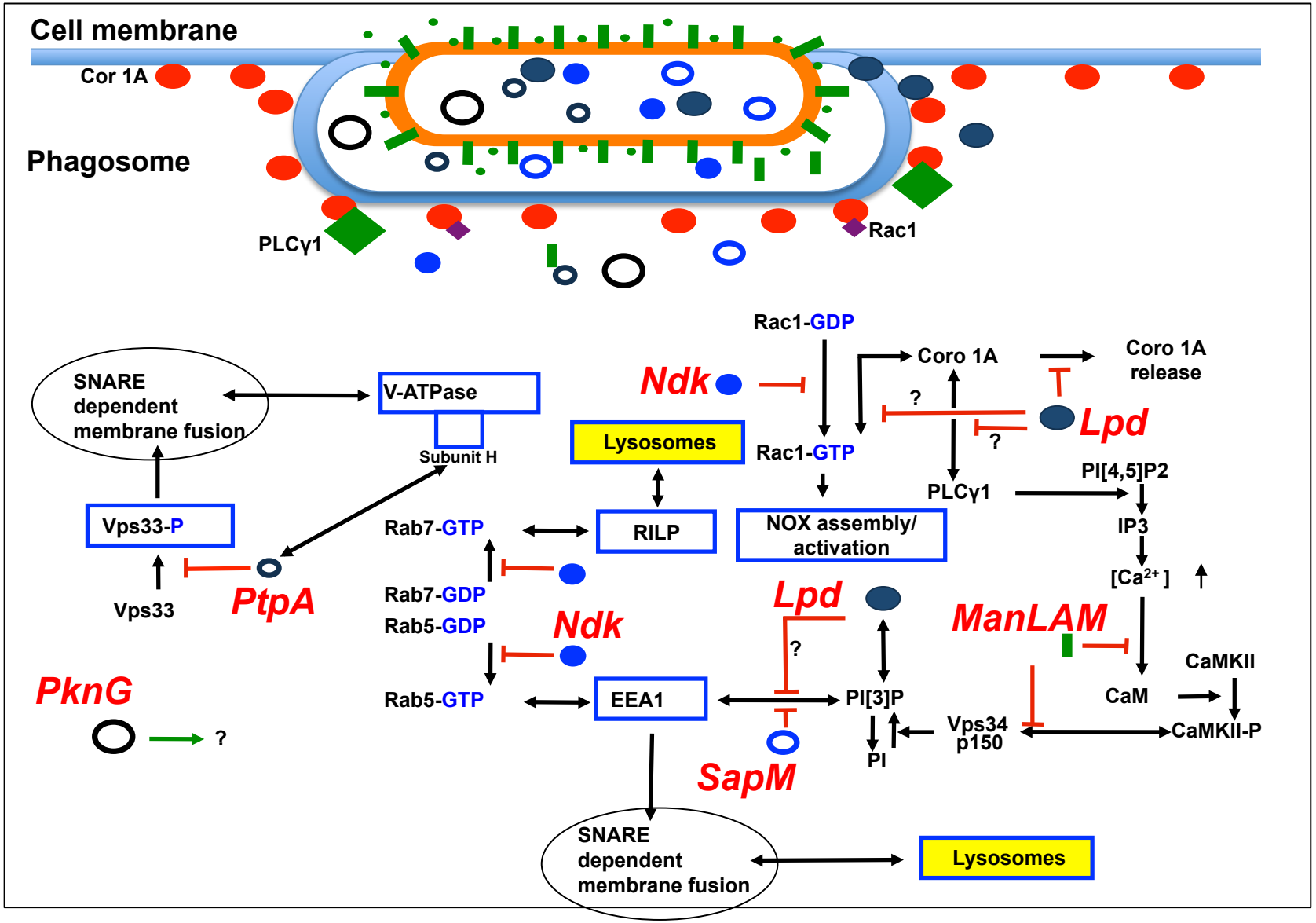
A turning point for cellular mycobacteriology

Today, many investigators are focusing their efforts on the identification of mycobacterial factors that arrest phagosome maturation.

Mycobacterial factors that arrest phagosome maturation represent attractive drug targets.

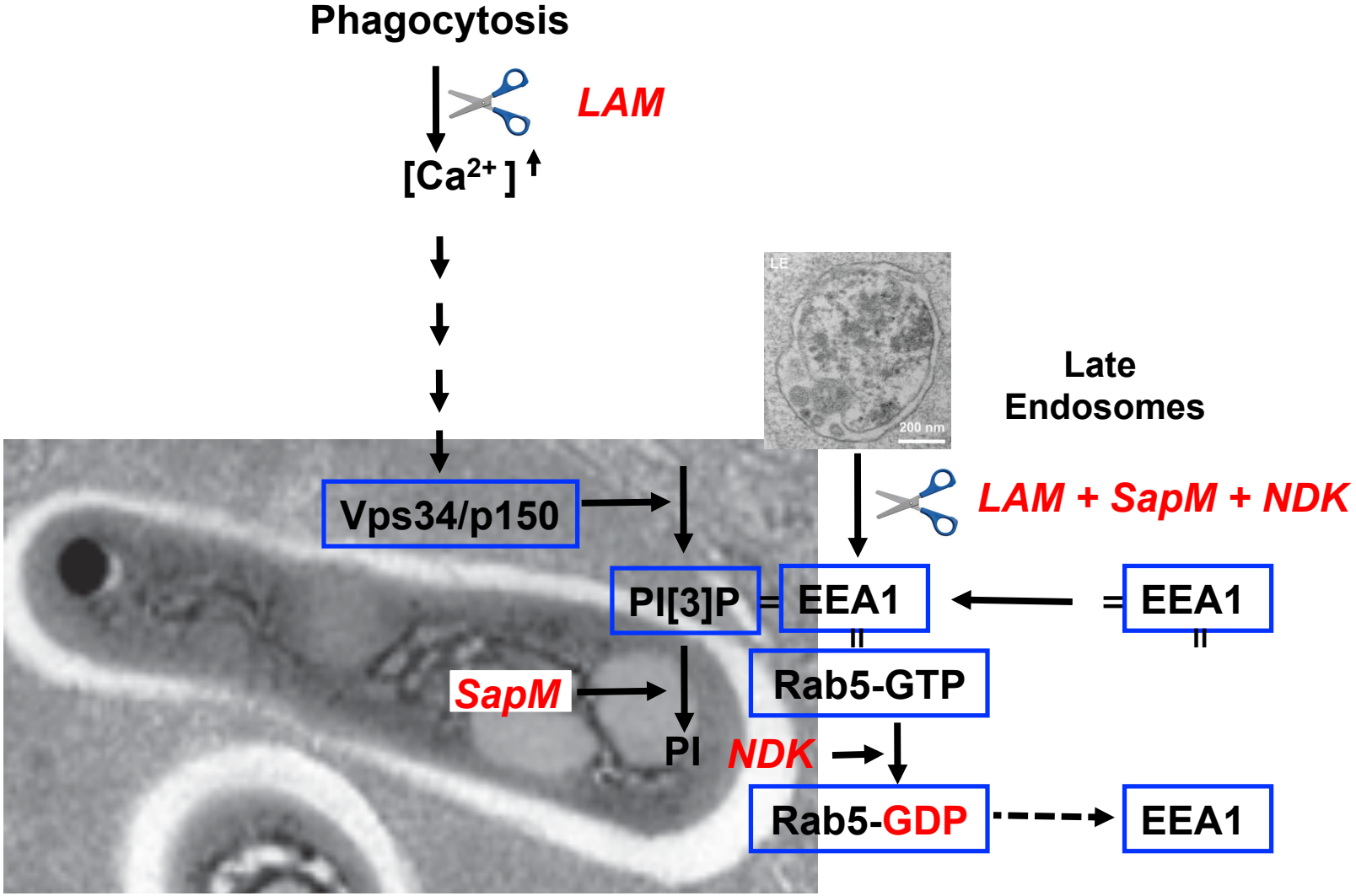
KO of these mycobacterial factors would convert virulent Mtb into protective vaccines.

Mycobacterial phagosome: Current Model



The Mycobacterial Phagosome

Mechanism of phagosomal exclusion of EEA-1 by Mtb



Exam questions

1- Discuss macrophage M1/M2 differentiation and the role of IFR4 and IFR5; PMID: 20729857 & 21240265.

2- Discuss the controversy of ER-mediated phagocytosis; PMID: 12151002 & 16213220.

3- How would you prepare M1-activated macrophages starting from human blood.

4- You want to use cell line-derived macrophages instead of monocyte-derived macrophages. Justify your switch to cell lines. What cell line would you choose? Why? and how would you proceed?