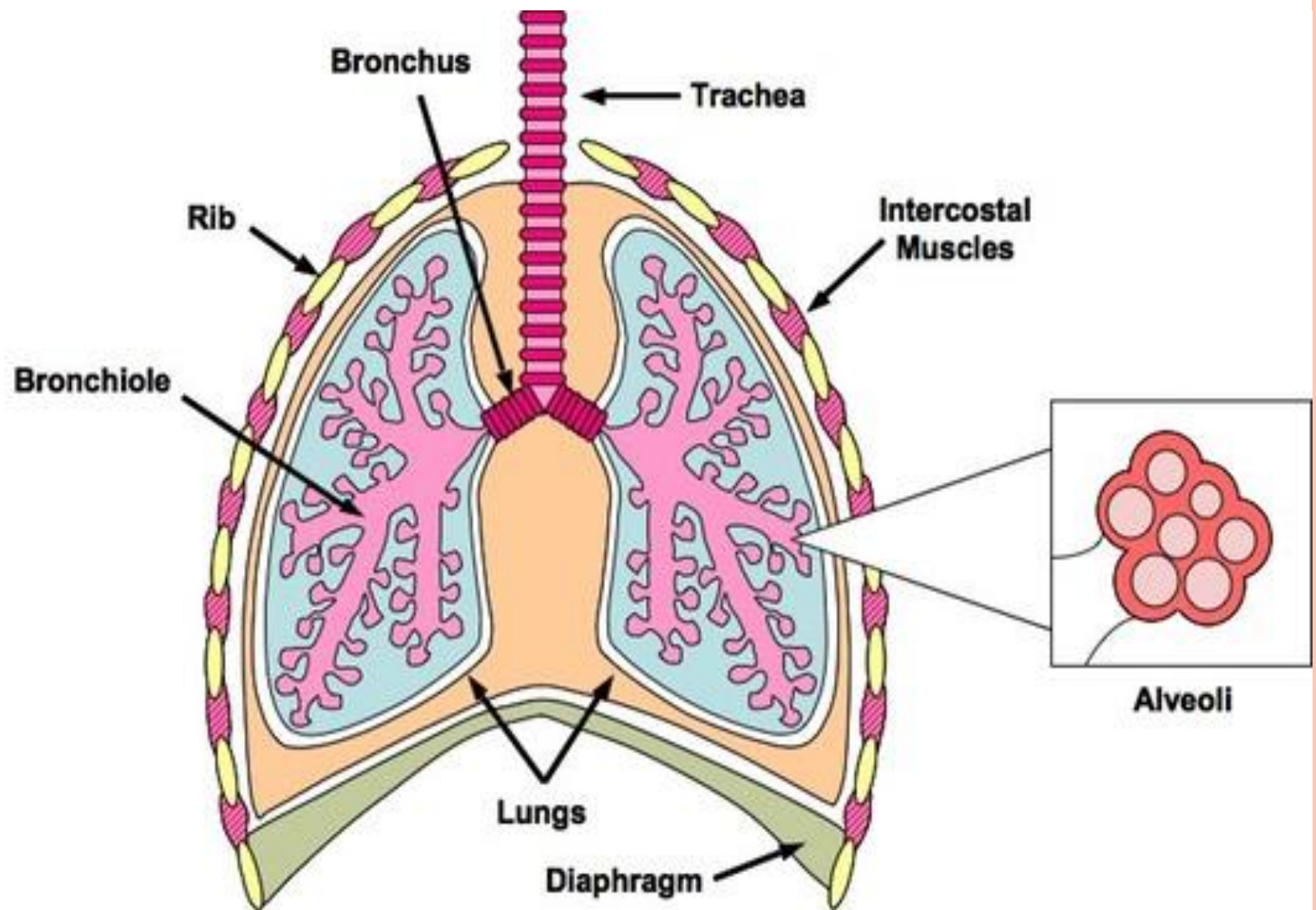





# IMMUNOPHYSIOLOGY OF LUNG



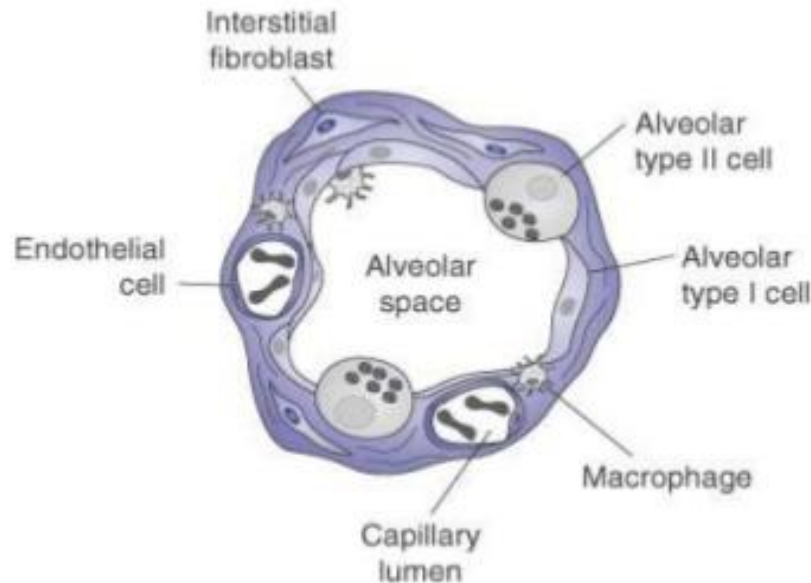


## Structures and Functions of the Alveoli

Feature	Function
Bronchioles split to form an abundance of alveoli	Large surface area for gas exchange
Alveolar surface is moist	Allows gases to dissolve in water for diffusion
The wall of the alveoli and capillary is one cell thick	Allows efficient diffusion of gases
Network of blood capillaries surrounded by alveoli	To allow the carbon dioxide to be brought to the alveoli and for the oxygen to be taken away



# What is the Pulmonary Interstitium?

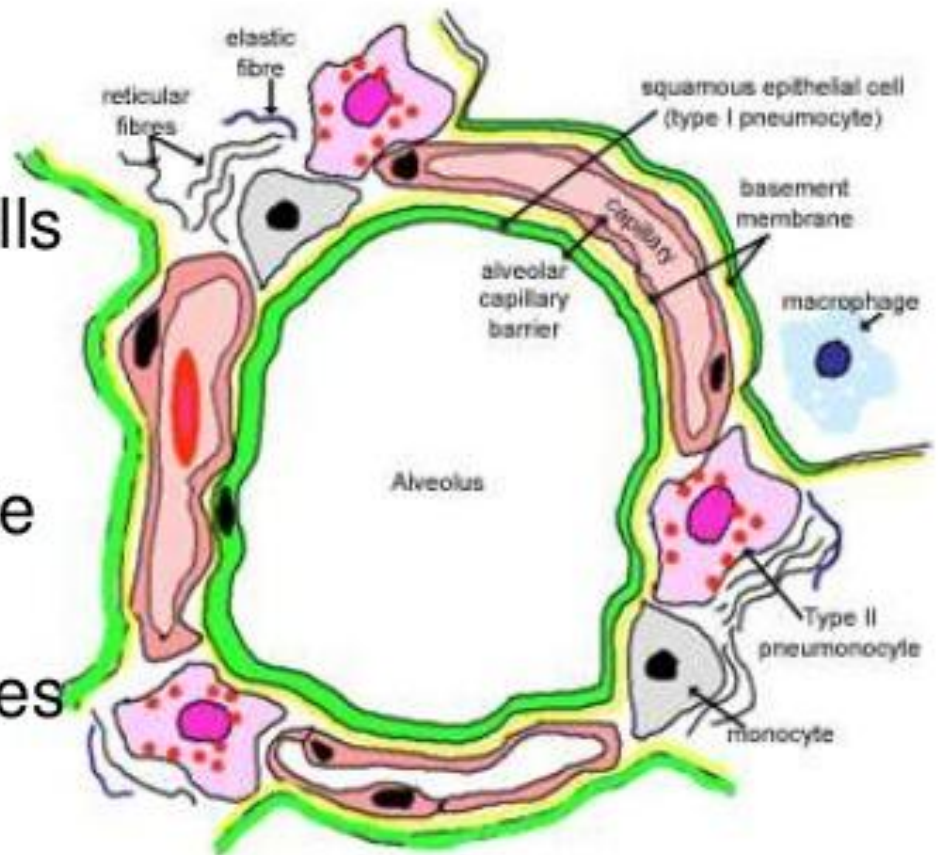


Interstitial compartment is the area of the lung between the alveolar epithelial and capillary endothelial basement membranes



# Cells in Alveolus

- ✓ Type I cells :  
simple squamous cells  
forming lining
- ✓ Type II cells :  
or septal cells secrete  
**surfactant**
- ✓ Alveolar macrophages



For more than 70 years, surfactant was perceived to be a soap-like substance that reduced surface tension in the lung and made breathing easier. With the advent of molecular techniques, it was discovered that one of the surfactant proteins, SP-A, was structurally **homologous to an immune protein of the complement cascade, C1q**. Since then, an entire family of proteins, known as collectins, has been identified, and the role of the innate immune system has garnered increasing attention. In vivo and in vitro studies provide compelling support for the surfactant proteins SP-A and SP-D as mediators of various immune-cell functions. More recent studies have shown novel roles for these proteins in the clearance of apoptotic cells, direct killing of microorganisms and initiation of **parturition**.



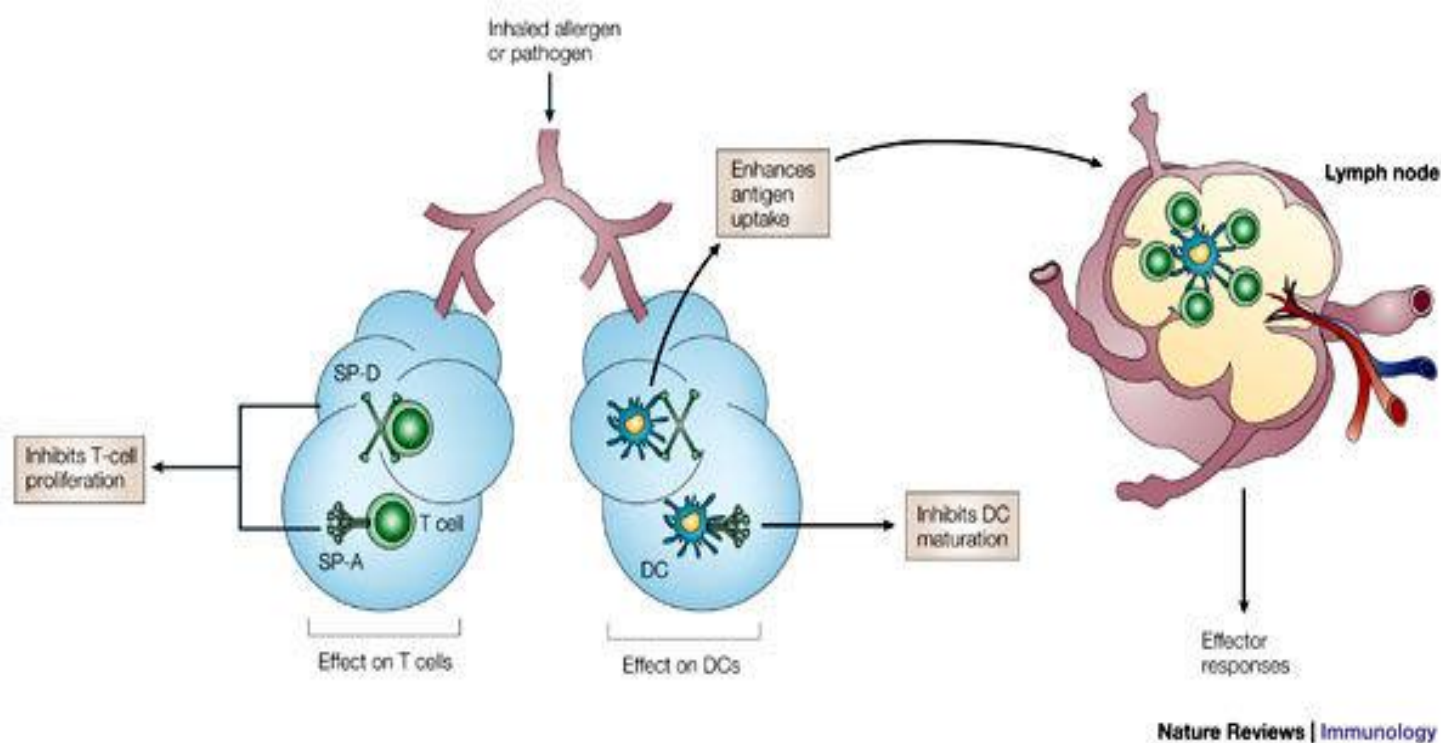
Augmented production of SP-A by the maturing fetal lung at term provides a key hormonal stimulus for the cascade of inflammatory signaling pathways within the maternal uterus that culminate in the enhanced myometrial contractility leading to parturition. This hormonal signal, transmitted to the uterus **by fetal AF macrophages** reveals that the fetal lungs are sufficiently developed to withstand the critical transition from an aqueous to an aerobic environment.

Increased amounts of surfactant lipids and proteins are secreted by the fetal lung into the amniotic fluid during the final third of gestation. Recent studies show that surfactant protein A (**SP-A**) **acts on amniotic-fluid macrophages to induce their migration to the pregnant uterus and the secretion of inflammatory mediators. This results to initiation of parturition.**





# Surfactant proteins regulate dendritic-cell and T-cell functions, thereby providing a link between innate and adaptive immunity.



Surfactant protein A (**SP-A**) has been shown to **inhibit the maturation of dendritic cells (DCs)**, whereas **SP-D** has been shown to **enhance the uptake and presentation of antigen**. Both SP-A and SP-D inhibit T-cell proliferation. As DCs migrate to the lymph nodes and undergo a maturation process, they acquire the ability to present antigen and activate T cells in the lymph nodes.



A consequence of apoptotic-body uptake by a phagocyte is induction of an anti-inflammatory response by the phagocyte. For example, macrophage uptake of apoptotic cells results in release of antiinflammatory mediators, such as transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-10 and prostaglandin E2. This response is in contrast to the release of proinflammatory cytokines that occurs when phagocytes ingest a microorganism. In addition to enhancing the uptake of apoptotic cells, SP-A also enhanced the release of TGF- $\beta$  by macrophages, indicating that SP-A can promote resolution of inflammation at several levels of the apoptotic-cell clearance process.



The roles of macrophages in clearing apoptotic cells and cellular debris in health and disease are equally as important as the participation of these cells in immunological responses. However, each function requires **plasticity** within the resident macrophage population so that **pro-inflammatory responses to tissue debris or to innocuous antigens are inhibited**, **but effective immune responses to pathogenic microorganisms are not compromised**. The ability of tissue macrophages to adapt and to carry out such disparate functions led to their broad classification as either classically activated M1 macrophages or alternatively activated M2 macrophages. Since their initial description, the functional and phenotypical characteristics of macrophages within the M1 phenotype have remained mostly unaltered, but the M2 macrophage category has been expanded to accommodate a broad range of macrophage functions in wound healing and in immune regulation. A transcriptional analysis of human alveolar macrophages that were polarized *ex vivo* using *interferon- $\gamma$  (IFN $\gamma$ )*, or with *interleukin-4 (IL-4)* and *IL-13*, highlighted **41 and 33 genes** that were associated with M1 macrophages and M2 macrophages, respectively.

The alveolar membrane is the largest surface of the body in contact with the outside environment. Like the skin and the gastrointestinal mucosa, the lungs are continuously exposed to a diverse array of microbes and organic and inorganic particulate materials. Innate immune mechanisms defend the air spaces from the array of microbial products that enter the lungs on a daily basis and are evident from the nasopharynx to the alveolar membrane. Particles **1  $\mu$ m** in size and smaller, the size of bacteria and viral particles, are carried to the alveolar surface where they interact with soluble components in alveolar fluids (e.g., IgG, complement, surfactant, and surfactant-associated proteins) and alveolar macrophages. Normally, alveolar macrophages account for approximately 95% of airspace leukocytes, with 1 to 4% lymphocytes and only about 1% neutrophils, so that the alveolar macrophage is the sentinel phagocytic cell of the innate immune system in the lungs.

The soluble constituents of airway and alveolar fluids have an important role in innate immunity in the lungs. In the conducting airways, constituents of airway aqueous fluids include lysozyme, which is lytic to many bacterial membranes; lactoferrin, which excludes iron from bacterial metabolism; IgA and IgG; and defensins, which are antimicrobial peptides released from leukocytes and respiratory epithelial cells. IgG is the most abundant immunoglobulin in alveolar fluids, and complement proteins and surfactant-associated proteins serve as additional microbial opsonins.



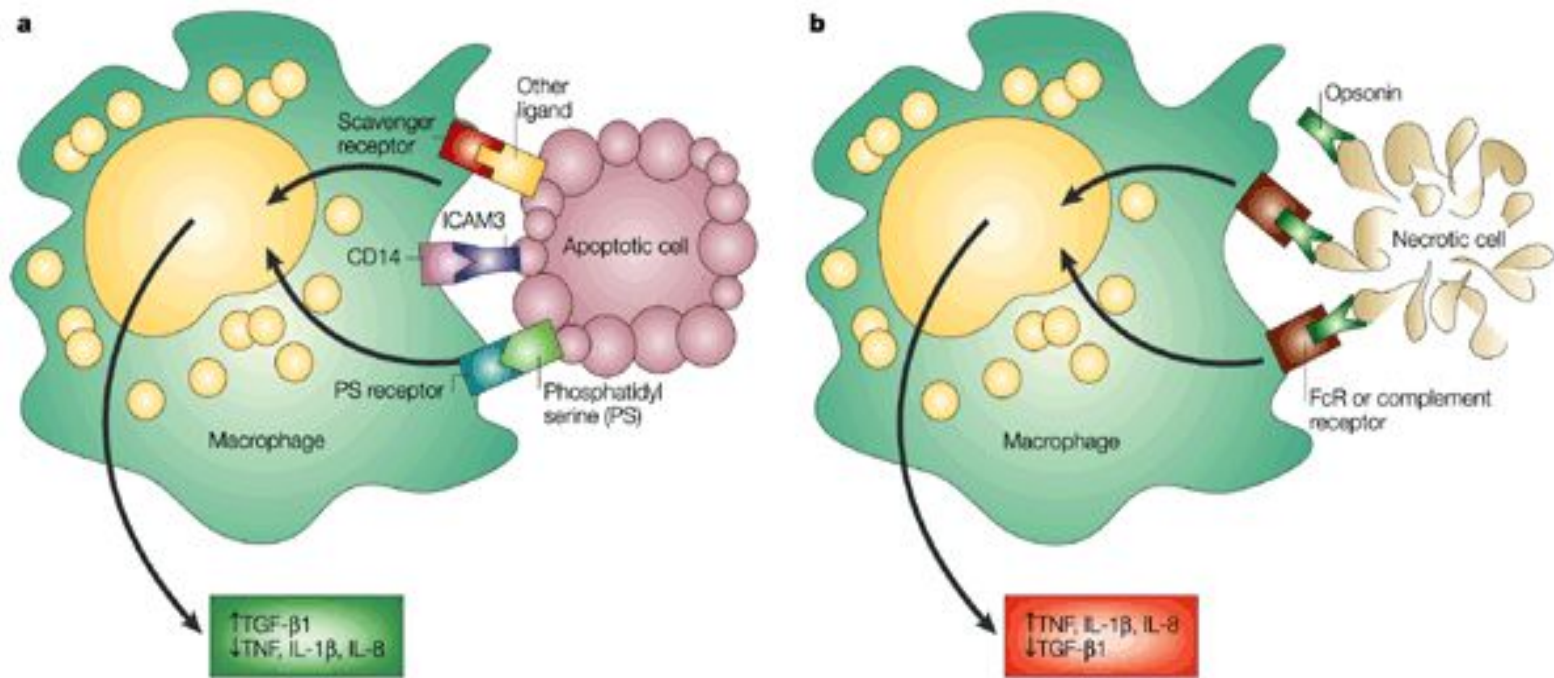
Alveolar macrophages are long-lived, with a turnover rate of only approximately **40% in 1 year**. By contrast, substantial turnover of both lung tissue and peritoneal macrophages occurs within a period of **21 days**.

Alveolar macrophages are avidly phagocytic and ingest all types of inhaled particulates that reach the alveolar spaces.

Remarkably, one of the primary roles of the alveolar macrophage is to keep the **airspace quiet**, and they ingest large numbers of inert particulates like amorphous silicates and carbongraphite particles without triggering inflammatory responses. Normally, the airspace environment is a relatively quiet place despite the array of microbial and other products that enter the airspaces by inhalation or subclinical oropharyngeal aspiration.

Alveolar macrophages are the masters of contradictory function. They are essential for steady-state 'hoovering' of daily cellular debris but are also ideally placed to initiate a strong inflammatory response to something more pathogenic. How do alveolar macrophages so rapidly distinguish between these two functions?





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The non-inflammatory clearance of apoptotic cells (**a**) compared with the pro-inflammatory ingestion of necrotic cells and debris (**b**). **a** | Macrophages recognize apoptotic cells through specific cell-surface receptors; subsequent phagocytosis promotes the release of anti-inflammatory mediators, such as transforming growth factor-1 (TGF-1), and suppresses the production of pro-inflammatory mediators. **b** | However, necrotic-cell debris does not express specific receptors and is phagocytosed through alternative mechanisms, such as Fc receptors (FcR), that promote the release of pro-inflammatory mediators, such as tumour-necrosis factor (TNF). IL, interleukin; ICAM3, intercellular adhesion molecule.

## Apoptotic cells



Release ATP and related nucleotides

"Find me" signals  
Normal clearance



## Necrotic cells



Danger signals  
Over activation  
Inappropriate downregulation

P2Y2R P2Y6R P2Y12R Others

Recruitment of monocytes,  
macrophages and DCs

P2Y2R P2Y6R P2X7R Others

Recruitment of macrophages,  
neutrophils and DCs

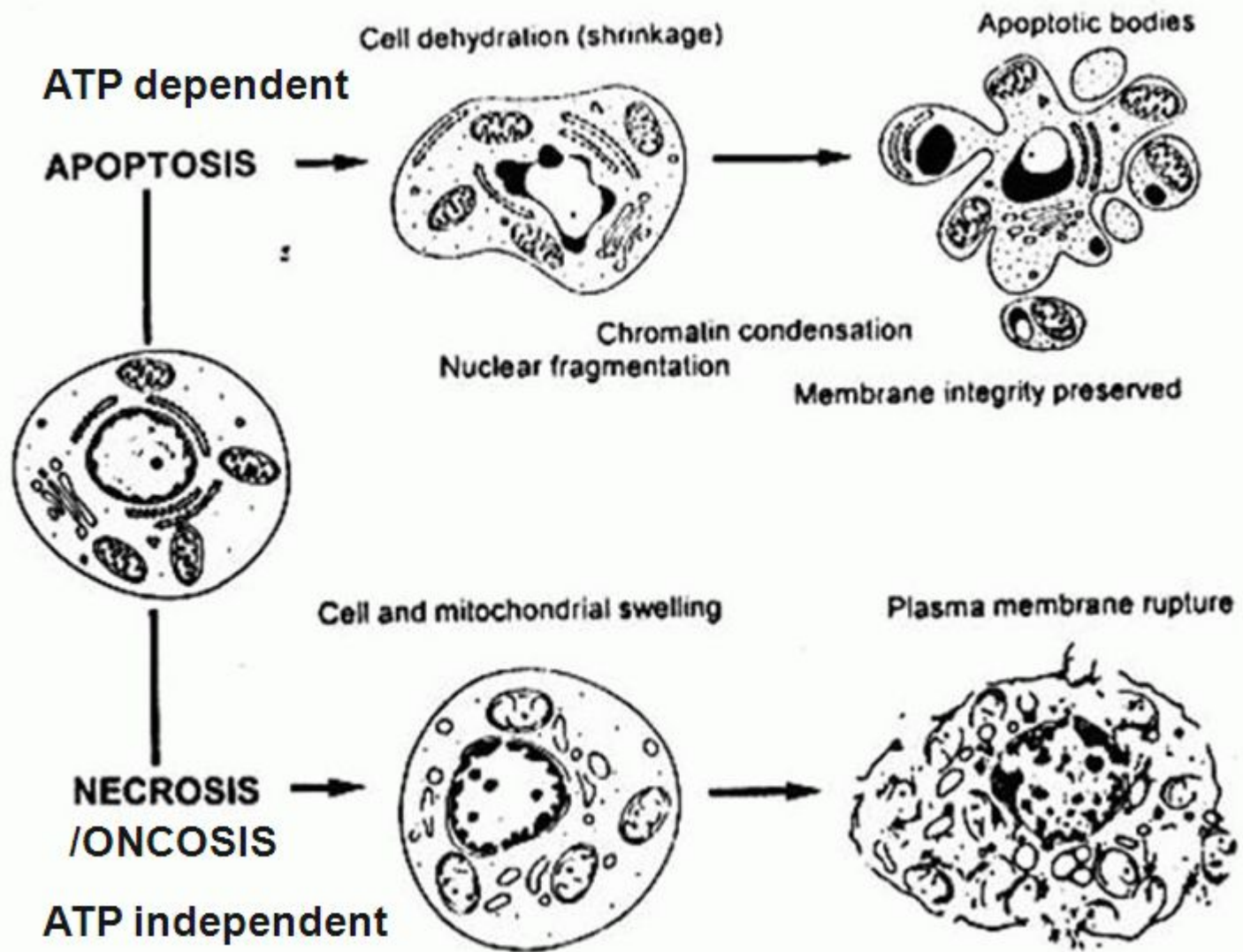
Mediating chemotaxis  
Modulating phagocytic activity  
Promoting phagocytic clearance

Injury resolution

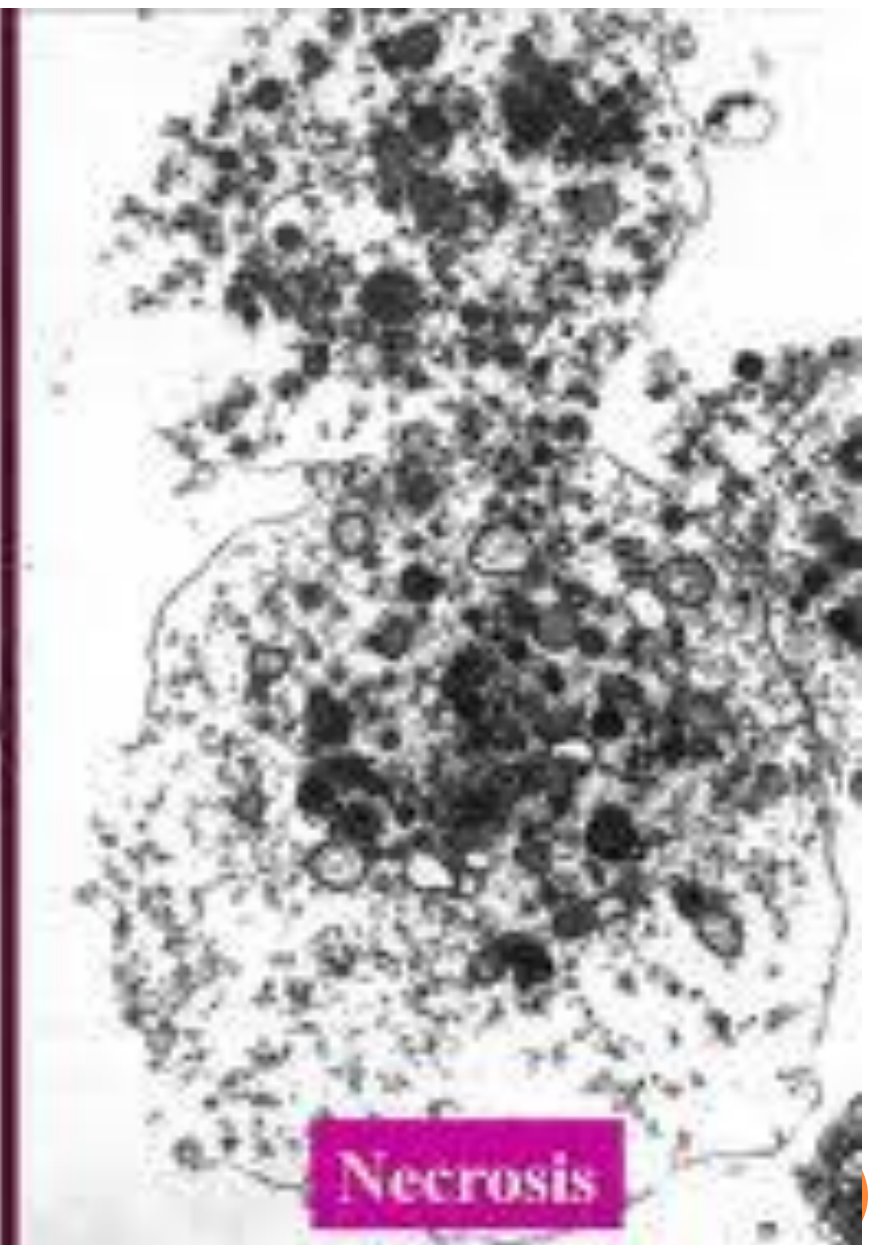
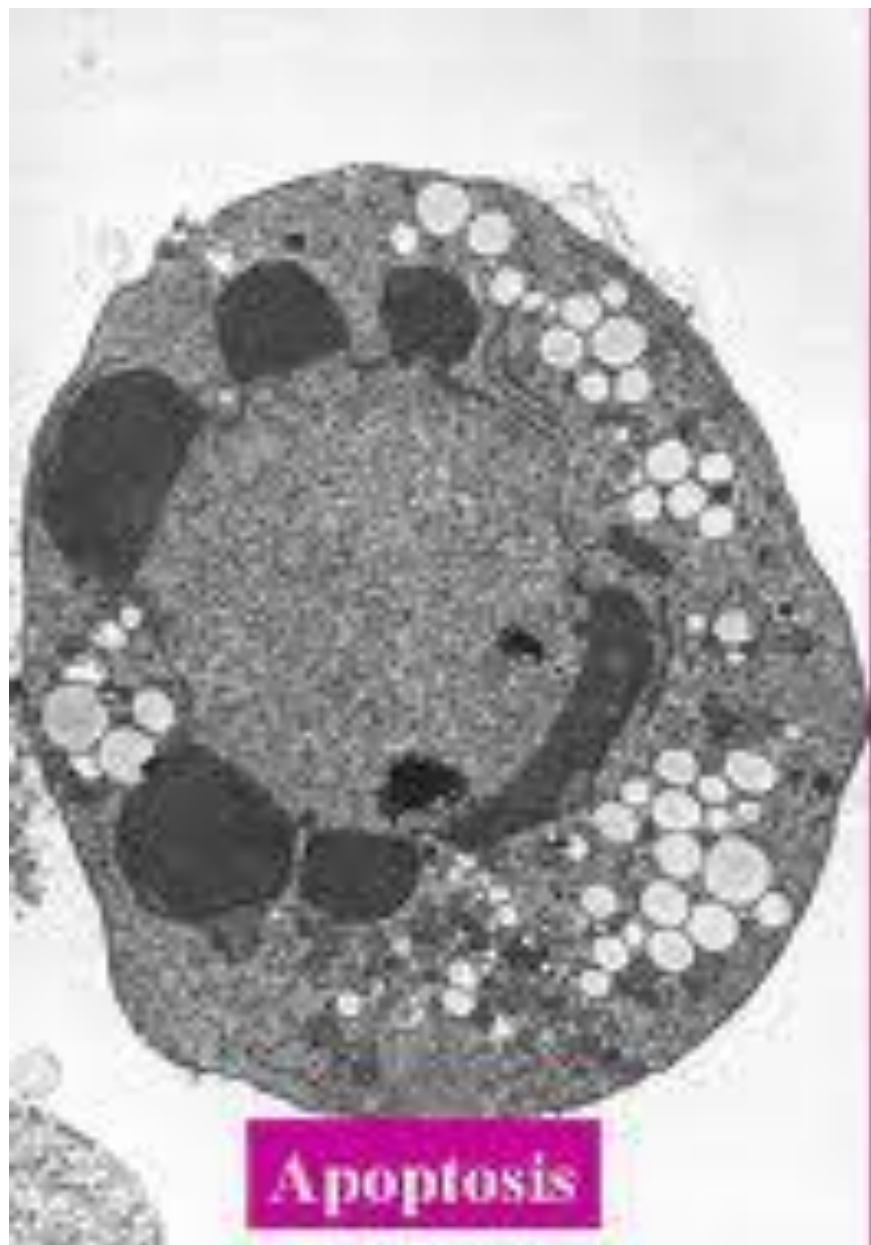
Production of proinflammatory cytokines  
Neutrophils accumulation and tissue  
damage

Chronic inflammation

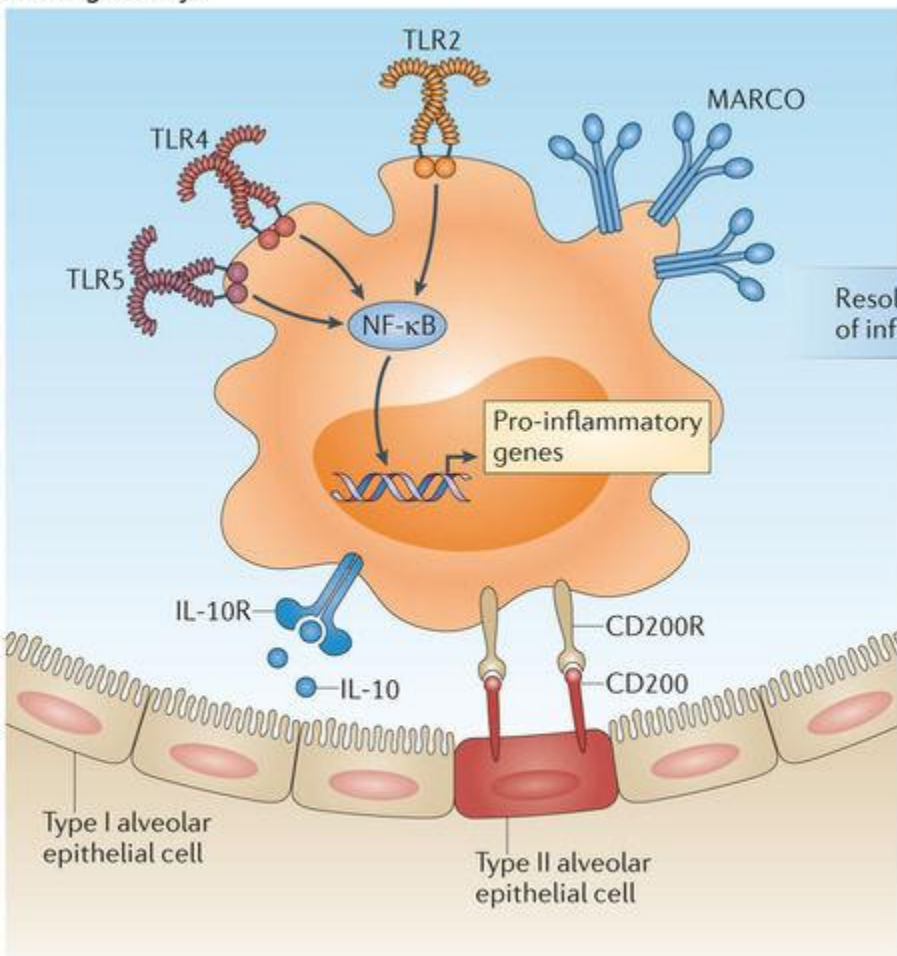




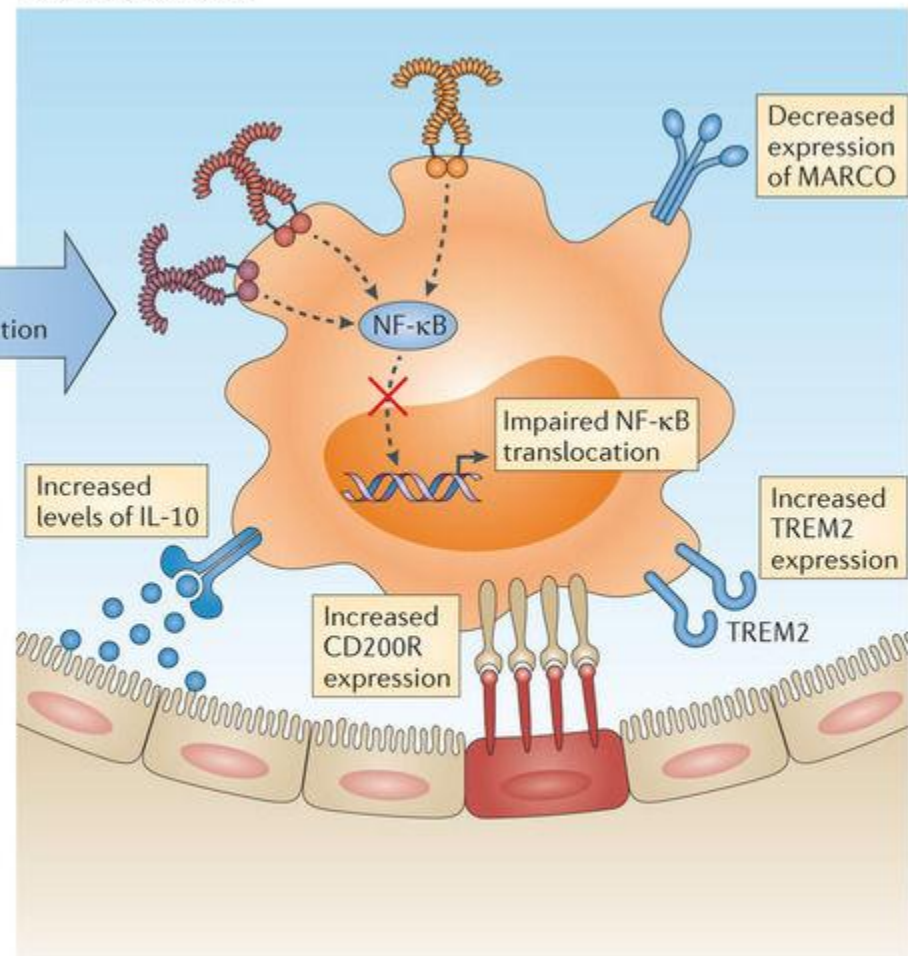




## Resting airways



## Post-inflammation



In healthy individuals, the airspaces are replete with mechanisms that prevent an inflammatory response from occurring. This in turn affects the function and the phenotype of the alveolar macrophages, which are one of the few cell populations to reside in the healthy airspaces, in addition to a small number of lymphocytes. **Alveolar macrophages from mice have been shown to be poor at presenting antigens to T cells, although they are capable of transporting antigens to the lung-draining lymph nodes.** Human alveolar macrophages also induce T cell antigen-specific unresponsiveness as a result of poor antigen presentation and **a lack of expression of co-stimulatory molecules, such as CD86**; this promotes **tolerance** to innocuous antigens. In addition, alveolar macrophages show decreased phagocytic activity compared with lung interstitial macrophages and also have a reduced respiratory burst. Furthermore, they produce immunosuppressive prostaglandins and transforming growth factor- $\beta$  (TGF $\beta$ ), which suppress T cell activation. Alveolar macrophages may drive the development of forkhead box P3 (FOXP3)+ regulatory T cells by secreting TGF $\beta$  and retinoic acid, although recent evidence suggests that tissue-resident macrophages in the lungs can also secrete these molecules.

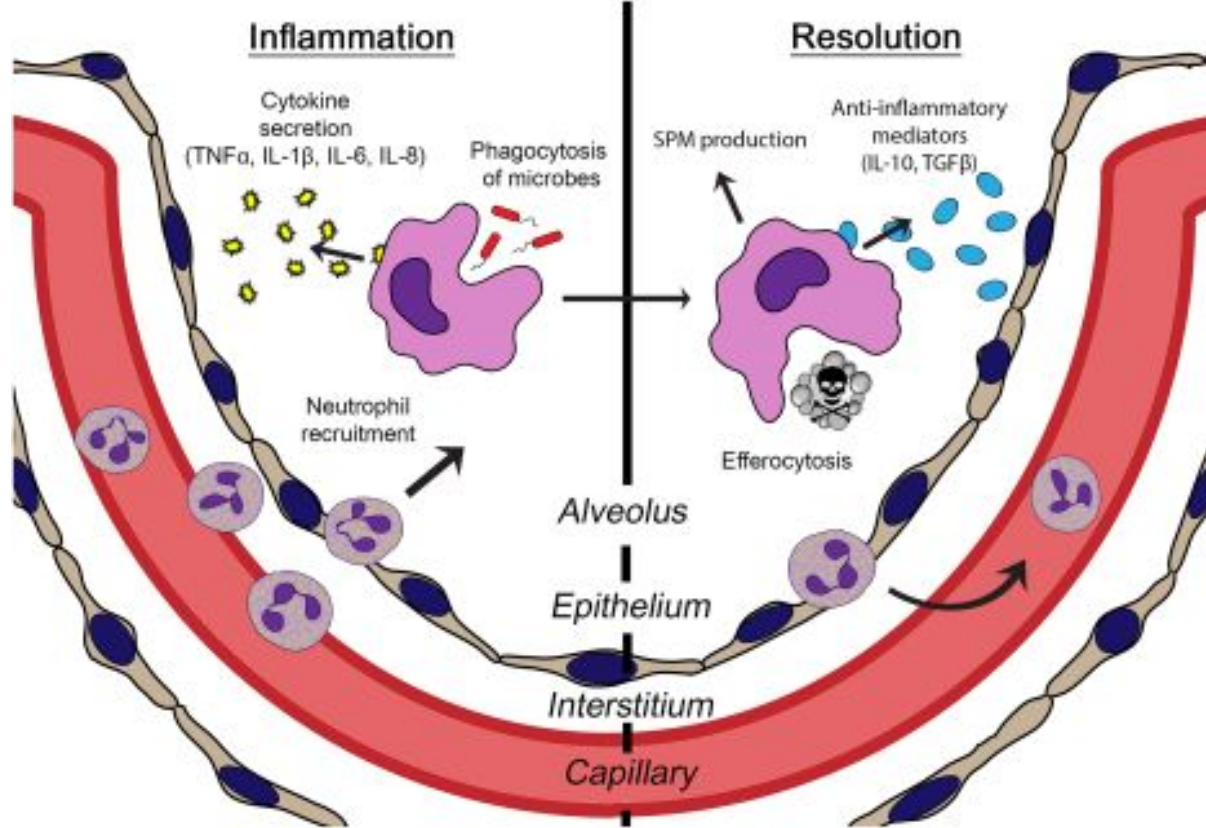


**Table 1 | The specific phenotype of mouse macrophages from different sites**  
**Surface**

<b>Marker</b>	<b>Peritoneal macrophage</b>	<b>Interstitial macrophage</b>	<b>Alveolar macrophage</b>
<i>CD11b</i>	Intermediate expression	Intermediate expression	Not expressed
CD11c	Not expressed	Not expressed	High expression
CD14	Intermediate expression	Intermediate expression	Low expression
CD200R	Low expression*	Intermediate expression	High expression
DEC205	Not expressed expression	Expression unknown	Intermediate
F4/80	Intermediate expression	Low expression	Low expression
Mannose receptor (also known as CD206)	Low expression	Intermediate expression	High expression
MHC class II	Intermediate expression	Intermediate expression	Low expression
SIGLEC-F	Not expressed	Not expressed	High expression

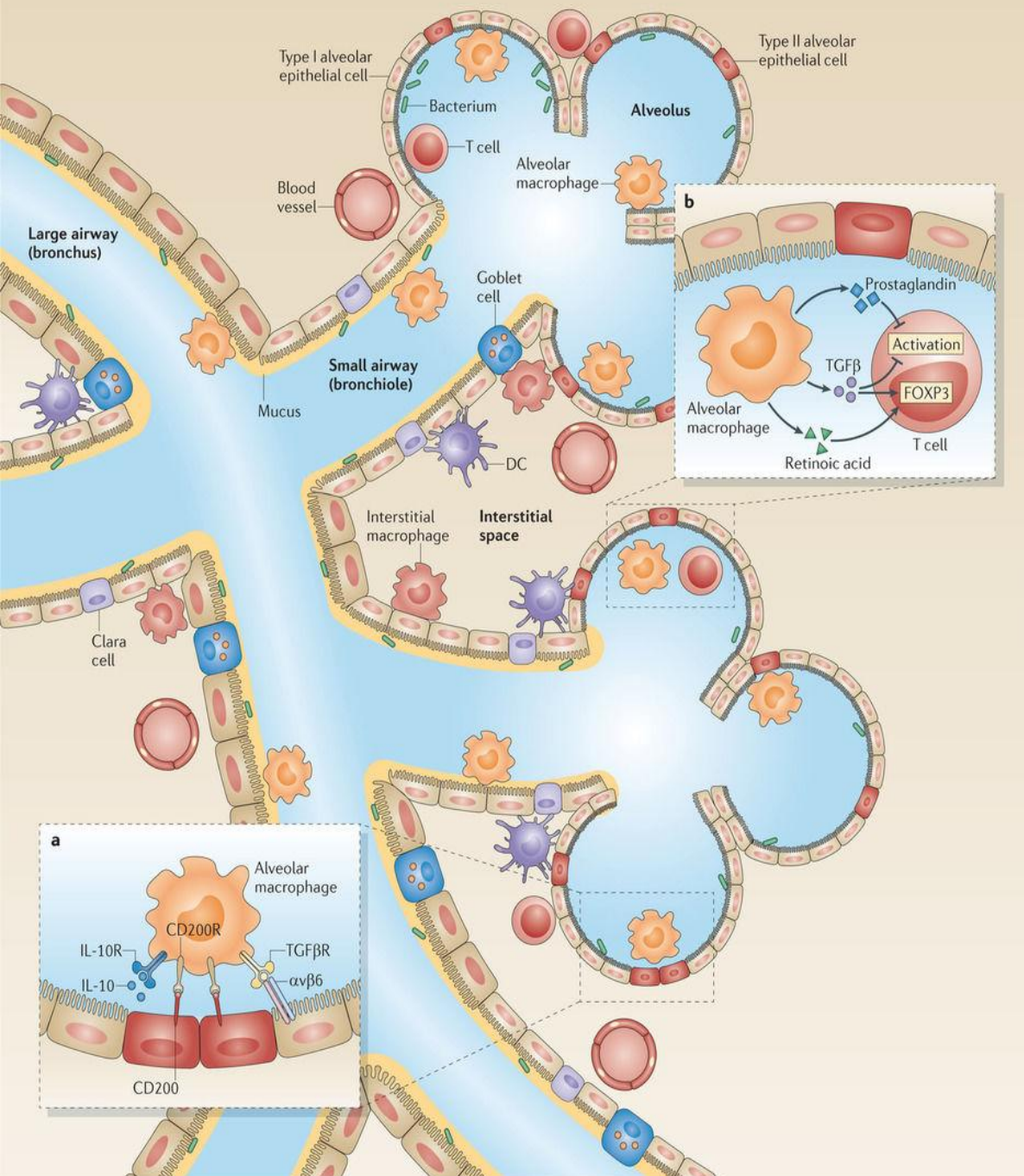






Innate immune functions of alveolar macrophages. As the resident innate immune cell of the pulmonary airspace, alveolar macrophages stand at the forefront of host defence against microbial invaders in the lung. Along with their role in effecting and propagating the inflammatory response by phagocytosing microbes and secreting proinflammatory mediators, alveolar macrophages also facilitate resolution by clearing away dead cells (efferocytosis) and producing anti-inflammatory mediators.





Alveolar macrophages reside in the airspaces juxtaposed with type I alveolar epithelial cells or with type II alveolar epithelial cells. Macrophages found in the larger airways reside within the mucous layer.

Macrophages are also found in the interstitial space between the alveoli and the blood vessels where T cells, dendritic cells (DCs) and a sparse population of B cells also reside. Alveolar macrophages are regulated by the airway epithelium through their interactions with **CD200, which is expressed by type II alveolar cells**, with transforming growth factor- $\beta$  (TGF $\beta$ ), which is tethered to the epithelial cell surface by  $\alpha\beta 6$  integrin, and with secreted interleukin-10 (IL-10). These interactions can also take place in the larger airways, where CD200 and  $\alpha\beta 6$  integrin are also expressed by the bronchial epithelium. The secretion of TGF $\beta$  and retinoic acid by alveolar macrophages can induce forkhead box P3 (FOXP3) expression in both naive and activated CD4<sup>+</sup> T cells that are present in the lumen of the airways. In addition, TGF $\beta$  and prostaglandins suppress T cell activation.

As such, Kupffer cells in the liver, osteoclasts in the bone and alveolar macrophages have very different roles and can be phenotypically differentiated from one another. Tissue macrophage diversity has led researchers to question the origins of these cells and the factors that promote their maintenance. Bone marrow haematopoietic stem cells (HSCs) give rise to circulating monocytes, which can differentiate in tissues into macrophages. However, a recent study showed that the mouse embryo yolk sac is a sufficient source of specific macrophage subtypes in the liver, skin and central nervous system (CNS) in the **absence of HSCs**. Furthermore, initial colonization of the airways with alveolar macrophages occurs in the first few days after birth — a process that is wholly dependent on fetal monocytes. In addition, models of *transplantation, radiation chimaeras, parabiosis and strontium-mediated depletion of blood monocytes* have shown that alveolar macrophages have a **marked capacity for self-renewal and that this is the main means by which these cells are replenished throughout life**. The alveolar macrophage pool is at least partially depleted during influenza infection; however, *in situ proliferation of the remaining alveolar macrophages seems to be capable of replenishing the population. Only in the case of radiation-induced depletion of alveolar macrophages, when any remaining cells have a reduced capacity for proliferation, do HSC-derived circulating monocytes eventually contribute to alveolar macrophage repopulation.*