**Phase III or Chemotaxis:**

When leukocytes move towards the site of infection along a chemical gradient by a process known as chemotaxis. When leukocytes reach to site of infection they remain there by the expression of adhesion molecules i-e integrins that attach leukocytes within the extracellular matrix. Integrins or CD44 attract leukocytes.

**Types of chemo attractants:**

There are two types of chemo attractants:-

1. Exogenous chemo attractants
2. Endogenous chemo attractants

Exogenous chemo attractants include bacterial products and arachidonic acid metabolites e.g LTB4 and endogenous chemo attractants include components of complement system i-e C5a. These are also called chemokines.

These chemo attractants bind with the receptor on surface of leukocytes. These are G-protein coupled receptors. As a result there is induction of secondary messenger pathway and increase the activity of GTPase and cyclase enzymes. After binding with leukocytes these chemo attractants trigger the assembly of contractile enzymes necessary for the contractile assembly changes.

Leukocytes move by extending pseudopods in the direction of movement. Leukocytes move towards the site of infection. After entering into the tissue the neutrophills replaced by monocytes and macrophages after 24 hours. This is the chemo taxis process. These macrophages engulf foreign particles.

**Leukocytes activation:**

Once the leukocytes have been recruited to site of infection, they must be activated to perform cellular function. The stimuli for leukocytes activation are:-

* Microbial products
* Necrotic substances
* Inflammatory mediators

The activated leukocytes perform various functions that are:

1. phagocytosis
2. Intracellular destruction of phagocytic particle by substance produced by phagocytes i-e ROS, lysosomal enzyme and reactive nitrogen species.
3. Extracellular destruction of microbes by liberation of substance into extracellular matrix by extracellular traps.
4. Release of inflammatory mediators that amplify the recruitment of various leukocytes and trigger the inflammatory response.

 **Phagocytosis:**

The process of engulfment of solid material by phagocyte is called phagocytosis. The phagocytes are polymorphonuclear neutrophils and macrophages.

**Steps of phagocytosis:**

There are 3 steps of phagocytosis:-

1. Recognition and attachment of phagocytes with the particle to be digested
2. Engulfment
3. Killing and degradation of ingested material

**Recognition and attachment :**

For the purpose, there are three receptors on the surface of phagocytes which are:-

* Mannose receptors
* Scavengers receptors
* Receptors for opsonins

**Mannose receptors:** Mannose receptors recognize mannose and fructose residue in the glycoproteins and glycopeptides of microbial cell membranes. In mammalian cell membrane, mannose and fructose residue are not present. The glycoproteins and glycopeptides of mammalian cell membrane contains N-acetylgalactosamine.

Due to absence of mannose and fructose residue in mammalian cell membrane, phagocytes recognize only foreign particles.

**Receptors for opsonins:** The receptors for opsonins enhance the attachment of phagocytes to microbial cell. Opsonins are the proteins coated on surface of microbial cell. These opsonins are complement component C3b, IgG antibodies and collectins.

The receptors against C3b on surface of phagocytes are complement receptor 1 and 3 (CR1 and CR3)

Receptors for IgG antibodies are Fc.

Receptors for collectins are C1q on the surface of phagocytes.

**Engulfment:**

The attachment of microbial product within the phagocyte lead to the engulfment of microbial product as a result a lot of signals will be integrates and that lead to cytoskeletal changes and membrane remodeling.

After that pseudopods surrounding the objects and the vacuole form is phagosome that contain foreign particle. Membrane of phagosomes fuse with lysosomes result in formation of phagolysosome. This is the engulfment of foreign particle.

**Killing or degradation:**

Killing or degradation involve:-

1. Intracellular killing
2. Extracellular killing

**Intracellular killing:**

It consists of oxygen-dependent mechanism and lysosomal based degradation.

**Oxygen dependent killing:** The phagocytes contain NADPH-oxidase in the cell membrane. It is also called phagocytic oxidase and contains at least 7 proteins that are non-functional. During phagocytosis, these proteins translocate into membrane of phagolysosome and become a functional enzyme.

This NADPH cause oxidation of reduced nicotinamide adenine dinucleotide phosphate. As a by-product oxygen is reduced to superoxide anion.

This superoxide anion in presence of superoxide dismutase is converted into hydrogen peroxide.

H2O2 acts as weak bactericidal agent. The lysosomal granules contains myeloperoxidase that convert hydrogen peroxide into hypochlorous acid that is a powerful bactericidal agent.

Similarly inducible nitric oxide synthase convert arginine into nitric peroxynitrile which is also a free radical. These cause degradation of foreign particle by lipid peroxidation or by halogenation.

In mature phagocytes myeloperoxidase enzyme is not present so hydrogen peroxide is converted to hydroxyl free radical.

It is also a powerful bactericidal agent which is myeloperoxidase independent pathway. The degraded particles are then removed by lysosomal enzyme hydrolase. The most important enzyme for this purpose is elastase enzyme.

In addition to ROS some other constituents of leukocytes are capable of killing microorganisms. These constituents include:-

* Major basic proteins → toxic to parasites
* Phospholipase enzyme → destruction of membrane phospholipids
* Defensins → peptides that cause destruction by creating holes in cell membrane.

**Extracellular killing:**

In case of extracellular degradation when phagocyte is unable to engulf the foreign particle e.g in case of immune complex formation that triggers leukocytes more activation. These are more activated and such phagocytes are called frustrated phagocytes.

In this case phagocytic vacuoles remain open that release substance outside that cause destruction by extracellular traps method. These traps are network that contains anti-microbial proteins or nuclear material that destroys the microbial substance. These traps prevent the spreading of microorganisms by trapping them into their network.