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Neutrophils in tuberculosis: will code be unlocked ?

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Abstract

Tuberculosis is a devastating disease throughout the world both in humans and animals. Its history is vast, which dates back to era of Robert Koch. There is a huge amount of immunological studies in the aspect of tuberculosis but there remain many unanswered questions. Neutrophils, cells of First line defence are being neglected in tuberculosis. Macrophages are considered as the key player in case of tuberculosis. Researches reveal that neutrophils play some interesting roles; it can be called as a bi-directional weapon. It plays instrumental role in killing mycobacterium, recruiting macrophages and also works hand in hand with macrophages in order halt the spread of the organism. Neutrophils also activates innate immunity, secretes some substances like ectosomes, which favour in trapping the mycobacterial organisms. Whether neutrophils drills the mycobacterium or gets succumbed to it depends on the stage of infection. Neutrophils at times act like a suicidal bomber, by carrying the organisms to different organs and spreading the infections. In chronic cases they are also implicated to granuloma formation, the classic sign of TB.

Keywords: granuloma, *Mycobacterium tuberculosis*, neutrophil, trojan horse

Introduction

Mycobacterium tuberculosis spares no one throughout the world and when it is unleashed it lashes more than 4000 people /day (WHO report 2010 –global tuberculosis control). Days are growing fast since the start of the research in Tuberculosis (TB) but the susceptibility to infection remains unanswered. The consequence of the TB ranges from early, asymptomatic clearance through latent infection to clinical disease [1]. Whenever there is talk about immunity in case of TB, macrophages are the first thing that comes to our mind and not neutrophils, though the later is the first line of defence in the body.

Neglected neutrophils

Neutrophils are the poorly ranked components of the host defence in case of TB. The reason behind this low profile chapter of neutrophils is because of the inherent difficulties in working with these cells. The key hurdles for neutrophils are:

1) they are short lived
2) easily activated
3) cannot be cryopreserved [1].

Hence *in-vitro* study is not feasible.

Newer concepts of neutrophils in TB

* Neutrophils are the commonly affected phagocyte in human TB [2].
* Neutrophils significantly contribute to control of TB in human blood [3].
* Interferon in human whole blood which was neutrophil driven contributes to disease pathogenesis [4].

Facts about neutrophils

Neutrophils kill organism both by oxidative (phagocytic) and non oxidative (degranulation) [5]. There are 3 types of granules seen in neutrophils. They are:

* Azurophilic granules (or "primary granules")-contains proteins like myeloperoxidase, bactericidal/ permeability-increasing protein (BPI), defensins, and the serine proteases neutrophil elastase.
* Specific granules (or "secondary granules") – contains lactoferrin and cathelicidin.
* Tertiary granules - contains cathepsin and gelatinase.

Neutrophils in TB: two way traffic?

Neutrophil contribute to the early defence against mycobacteria. The scientific community is split, regarding the action of neutrophils in TB. LPS used to recruit neutrophils to the lungs of rat during airborne infection with 200 *M.tuberculosis*, decreases downstream colony forming units in lung [6]. Depleting granulocytes in mice before intra tracheal infection with 10^5 organisms, increases the number of colony forming units downstream in lung and spleen [7]. Science is not always constant and consistent. On the other side some works proved negative to the above mentioned action of neutrophils in TB. For example: no effect on bacterial load in mice with 10^6 colony forming unit of *M.tuberculosis, M.bovis*, BCG or *M. fortuitum* in case of granulocyte depletion [8]. Murine experiments with RB6-8C5 monoclonal antibodies to deplete granulocyte receptor positive cells, in addition to the target (Ly6G antigen) on neutrophils, it also target Ly6C on plasmocytoid dendritic cells, mono-

cytes [9]. The stage of infection plays a crucial role: in established TB disease, higher neutrophil counts are associated with poor prognosis [10].

Begin with the basics

The preliminary steps taken by neutrophils after the entry of organism into the body are;

1. Recruitment
2. Recognition
3. Phagocytosis
4. Killing.

Neutrophil recruitment

Neutrophil recruitment occurs at the site mycobacterial infection. Recruitment occurs at the perivascular sites within an hour when intra-venous infection with *M.tuberculosis* [11]. There is infiltration of neutrophils in liver within 2 hours of systemic challenge with *M.avium* [12]. There is skin infiltration of neutrophils within 3 hours of BCG infection in rabbits [13]. In case of mice it takes 4 hours for skin infiltration with the same challenge study as that of rabbit [14].

Mechanism of recruitment: In sensitized animals there is a powerful immune response to mycobacterial challenge [11]. Interleukin 17 (IL 17) and IL 23 produced from T helper 17 (Th 17) cell are the key in recruitment of neutrophils [15]. IL 8 from macrophage also plays a key role [16]. In simpler terms we can say that the initial signal releases the inflammatory cytokine which activates local endothelium, coordinated with increase in adhesion molecules like Intra Cellular Adhesion Molecules (ICAM), E selectin, P selectin. These signals cause influx of neutrophils.

Phagocytosis of mycobacteria by neutrophils

Neutrophils engulf the entered mycobacteria [17]. The mechanisms which cause this interaction are:

1) Direct recognition 2) Opsonisation.

1) Direct recognition: Pattern Recognition Receptors makes this interaction possible [18]. Toll Like Receptor 2 (TLR2) also involved in this interaction. There is impaired control of *M.tuberculosis* and *M. avium* infection in TLR2 deficient mice [12]. Lipoarabinomannan or 19 kDa lipoprotein of mycobateria is the ligand for TLR2. Blocking TLR4 reduces IL8 production, in response to infection [19]. Hence TLR4 may also be involved in this interaction between neutrophils and mycobecteria.

2) Opsonisation: Apart from direct recognition, opsonisation seems to play a key role in the phagocytosis of the mycobacteria by neutrophils. There is reduction in the ability of Ficoll isolated neutrophils to phagocytose mycobacteria after heat inactivation of serum [20].

Do neutrophils dissect mycobacteria?

This remains a controversial stuff. Theoretically speaking the neutrophils kill and stop the infection at an early stage. But in the absence of killing, the neutrophils traffics the infection to different organs of the body. Thus playing the role of "granulocytic Trojan Horse" [21]. Mice treated with anti IL17 during *M.tuberculosis* infection shows 100 fold decrease in number of organism in spleen [22]. Treatment with IL17 causes reduction in neutrophil recruitment.

Mechanism of killing

A member of the α-defensin family, Human Neutrophil peptides (HNP) plays a prime role in killing mycobacteria. They are cationic proteins seen in the azurophil granules which bind to anionic molecules [23]. Exceptions exist here too. *M.avium, M.kansasii, M.smegmatis* and *M.tuberculosis* fail to fuse to azurophil granules [24]. lysX gene of *M.tuberculosis* is same as mprF gene of *Staphylococcus aureus* that increase the lysine content of membrane lipids, hence negative charge is decreased and susceptibility to HNP also reduced [25].

Macrophages seem to take up free HNP and this increases their ability to kill mcobacteria [26]. Phagocytosis of apoptotic neutrophils by macrophages causes restriction of mycobacterial growth [27].

Neutrophil-macrophage co-operation

Clearing of cytotoxic, short lived neutrophils are carried out by macrophages. Macrophages are attracted by the chemokines derived from neutrophil [28]. Macrophage chemotaxis is also stimulated by mycobacterial lipoarabinomannan [29].

Process of apoptosis- pro inflammatory or anti inflammatory?

Controversy surrounds here too. Apoptosis is an anti inflammatory process resulting in induction of Tissue Growth Factor ß (TGF ß), prostaglandin E2 (PGE2) and inhibits IL 6, IL 8 and Tissue Necrosis Factor (TNF) [30]. Sometimes the reverse may happen; ingestion of apoptotic cell with pathogen may result in pro inflammatory effect [30]. This may be due to expression of heat shock proteins [31] or activation of macrophages by neutrophil proteases.

Phagocytosis of apoptotic cell may produce anti/pro inflammatory effect based on the mycobacteria inside the neutrophil is alive or dead. If the organism is killed, there is anti-inflammatory effect. If the organism is living it causes pro-inflammatory effect.

Effect of neutrophils on acquired immunity

Neutrophils produce IL 12, macrophage inflammatory protein (MIP 1α) [32] which attract T lymphocytes and cause its maturity. Neutrophils can also produce IL 10 which might limit acquired immunity [33].

Neutrophil Extra cellular Traps (NETs)

Nuclear chromatin, mitochondrial DNA and granular anti microbial proteins form the NETs [34]. Pro-inflammatory stimuli like IL8, TNFα forms NETs [35]. NETs can trap mycobacteria [36]. NETs formed against mycobacterial infection are unable to kill mycobacteria instead it kills *Listeria monocytogene*

which is found as a co-infection. Hence NETs can localize mycobacteria which may be the basis for granuloma formation.

Ectosomes (ECTs) from *M. tuberculosis* infected neutrophils

Vesicles released in response to pro-inflammatory stimuli from cell membrane are called as ectosomes [37]. ECTs are rich in cholesterol, express CD35 marker [38]. ECTs from neutrophil bind to endothelium selectively and macrophages but not to red blood cells, confirming its role in immune response.

Conclusion

Neutrophils are seen in the early stages of the mycobacterial infection. In chronic cases the same neutrophils may act in the pathology of granuloma formation. Thus neutrophil act as a "Double edged Sword". The role of neutrophils in killing the mycobacteria is still doubtful. It may disseminate the organism to various organs by acting as a granulocytic trojan horse.

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