Innate Response to Infection

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Abstract

In response to an infection, the innate part of the immune system is the first line of host defense. This response is non-specific but rapid, and a source of information for next step acquired immune response. Findings that some cellular elements of the innate immune system reveal properties placing them as an interface between "innate" and "adaptive" systems, and others use newly discovered killing strategies, might change the understanding of how the innate immune system functions.

Keywords: Infection; Innate immune system; PRRs; Immune response

Introduction

The innate immune response to an infection is a first line rapid reaction consisting of two elements: recognition of invading microorganisms and complex biochemical and cellular consequences of this fact [1]. The innate immune response has been regarded as relatively non-specific but sensitive. The cellular elements of innate immunity are represented by phagocytic cells and antigen-presenting cells-granulocytes, macrophages and dendritic cells (DCs) respectively, cytotoxic NK cells, γδ T lymphocytes [2]. The innate immune system recognizes conserved microbial structures called pathogen-associated molecular patterns (PAMPs) that have been implicated in activating the host innate response. These structures are sensed by germ line-encoded pattern recognition receptors (PRRs) represented by Toll-like receptors (TLRs) expressed at the cell surface or intracellularly and Nod-like receptors (NLRs) [3]. The early immune reaction includes complement activation, phagocytosis and immune activation by different families of PRRs.

Toll-like Receptors (TLRs)

The TLRs have been identified in most cell types and are expressed constitutively or in an inducible way in the course of infection.

TLRs are type 1 transmembrane glycoproteins characterized by extracellular ligand-binding leucin-rich repeat (LRR) domain and cytoplasmic signaling Toll/interleukin-1 (IL-1) receptor (TIR) domain [4]. They are primarily responsible for PAMPs detecting in the extracellular environment. TLRs located on plasma membrane detect hydrophobic lipids and proteins. Nucleic acids are detected by TLRs located in endosomes [5]. Up to now, 10 TLRs have been identified in humans. TLR1,2,4,5,6 are primarily expressed on the cell surface and recognize PAMPs derived from bacteria, fungi, and protozoa, whereas TLR3,7,8,9 are exclusively expressed within endocytic compartments and recognize nucleic acid PAMPs (single/double stranded RNA or DNA) derived from various viruses and bacteria [6]. Recognition of PAMPs by TLR1,2,4,5,6 primarily induces the production of cytokines, and TLR7,TLR9 induce type I interferon. TLR3,7,9 are specific for viral detection. TLR3,7,9 are endosomal-localized TLRs and are transported to the endosomal compartment via endoplasmic reticulum-localized protein UNC93B1 [6].

UNC93B1 is required for intracellular TLR response and determines how efficiently each TLR is able to move from ER to the endolysosomes [7].

These receptors differentially recruit the adaptor proteins Mal (MyD88 adaptor-like) and MyD88 (myeloid differentiation primary response gene 88) and/or TRIF (TIR-domain-containing adaptor inducing IFNB) and TRAM (TRIF-related adaptor molecule) [5]. The result of triggering by adaptors different signaling pathways is the activation of NF-κB (nuclear factor kappa B), MAPK (mitogen-activated protein kinase) and IRF 1,3,5,7 (interferon regulatory factors-3,-5,-7) [8]. All these transcription factors cause expression of interferons, cytokines, chemokines, and influence cellular maturation as well as survival [5].

The TLR-induced signaling pathways can be divided into dependent on or independent of the adaptor MyD88 or TIR domain-containing adaptor inducing IFN B (TRIF). All TLRs except TLR3 induce the MyD88-dependent signaling responsible for cytokines expression.

TLR3 was shown to recognize self-messenger RNA. TLR3 recruits TRIF and activates TRIF dependent signaling. The TIR receptor domain-containing adaptor protein inducing interferon β (TRIF) is an adaptor that is critical for the production of type I interferons (interferon β or α)-through the activation of a family of transcription factors-interferon regulatory factors (IRFs). TRIF stimulates IRF3 and IRF7 as well as a NF-κB pathway with delayed activation [9]. cDCs stimulated with TLR3 PAMPs activate the TRIF-dependent signaling pathway through recruitment of TRIF to induce transcription of inflammatory cytokines and type I interferon through IKK complex and TBK1/IKKi respectively, via the activation of NF-κB and IRF3/IRF7 [6]. TBK1 is a pleiotropic kinase involved in both innate immunity and tumor genesis.

TLR signaling can be controlled by membrane-associated regulators, such as CD14, CD11b, MHC II, TNFR, and CD36. The next line of defense against TLR-mediated overresponses is through several intracellular regulators. Ubiquitin-modifying enzymes Nrdp-1, CHIP, A20, TRIM5, and DTX (recruited by NLRP4) are reported to be responsible for negative or positive regulation of molecules associated with TLR signaling. MHC II and TAG in endosomes,

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TANK (catalyzed by MARCH5) and ECSIT, as well as NLRX1, in mitochondria, UNC93B1 and viperin in ER, and Rab 10 in the Golgi apparatus participate in TLR-triggered innate immune response [10]. MiRNA-21, miRNA-148/152, miRNA-466l, miRNA-29 and miRNA-146a have been described as controlling the gene expression of TLR signaling at the post-transcriptional level [10].

Expression of certain signaling molecules is differentiated between innate cell types, and thus the response to identical PAMPs may differ between cells both in nature of effector molecules and the kinetics of the response (5). TLRs have the capacity to recognize endogenous and harmful self-antigens, so their function may not be restricted to the recognition of only extrinsic pathogens. A recent observation is that TLRs are involved in recognition of endogenous ligands [11], and TLR4 is involved in the recognition of extra-domain A-containing fibronectin [12], fibrinogen [13], several heat shock proteins (HSPs) [14].

TLRs and Sepsis

Sepsis is one of the most challenging health problems worldwide. Tsujimoto et al. consider that TLRs may play a key role in the development of organ dysfunction and mortality occurring in sepsis [15]. Punnet et al. stated that phagocytes from patients with sepsis had an upregulation of TLR4 and TLR2, however shock-inducing inflammatory responses mediated by these TLRs were inhibited by ES-62, an immunomodulator secreted by the filarial nematode *Acanthocheilonema viteae* [16]. ES-62 subverted TLR4 signaling to block TLR2- and TLR4-driven inflammatory responses via autophagosome-mediated downregulation of TLR adaptor-transducer MyD88. *In vivo*, ES-62 protected mice against endotoxic and polymicrobial septic shock by TLR4-mediated induction of autophagy and was protective even when administered after the induction of sepsis. He concluded that administration of ES-62 or synthetic small-molecule derivates, alone or in combination with antibiotics, after initiation of sepsis might offer a suitable new therapeutic tool for treatment septic shock as well as other microbe-mediated diseases in humans, in whom out-of-control inflammation can lead to a fatal outcome [16].

It was shown that *Streptococcus pneumonia* and *Hemophilus influenza* use TLR2 and TLR4, respectively, to downregulate cell-cell interactions and facilitate translocation across the epithelium [17].

NLRs

In contrast to TLRs, the NLRs and RLRs are intracellular cytosolic sensors. RLRs are helicases that primarily recognize viruses, and NLRs are involved in bacterial recognition. NLRs-NOD-like receptors are a family of molecules sensing a wide range of ligands within the cytoplasm of cells and there are 23 members [6] of this family in humans. NLRs are expressed in different cell types including immune and epithelial cells. Some NLR family members are expressed primarily in phagocytes including macrophages and neutrophils. NLRs are multi-domain proteins composed of a variable N-terminal effector region consisting of the caspase recruitment domain (CARD), pyrin domain (PYD), acidic domain, or baculovirus inhibitor repeats (BIRs), a centrally located NOD that is critical for activation, and C-terminal leucine rich repeats (LRR) that senses PAMPs. The CARD and PYD domains are members of the death domain-fold superfamily, and are involved in cellular processes including inflammation and apoptosis. CARD and PYD domains mediate interactions with other CARD and PYD-containing proteins. BIR-containing proteins are regulators of apoptosis in the so-called inhibitor of apoptosis proteins (IAPs). A

class of IAPs called neuronal apoptosis inhibitor proteins (NAIPs) are NLR family members [18]. NLRs harboring a pyrin-domain, or a baculovirus inhibitor apoptosis protein repeat (BIR) domain in their N terminus are not involved in the transcriptional activation of inflammatory mediators and are components of the inflammasome that regulates caspase-1 activation [19]. NLRs act as proteins that create signaling platforms that trigger NF-κB and MAPK signaling pathways to induce the inflammatory cytokines production and activate the inflammasome initiating the proteolytic cleavage. The inflammasome is a multiprotein-complex required for the maturation or activation of pro-IL-1 family cytokines to its active IL-1 family cytokines. Activation of the inflammasome requires two steps. First, NF-κB dependent up-regulation of the pro-forms of the cytokines, and next, convertion of the inactive form of the cytokine to a bioactive form by inflammasome. This process results in maturation and production of cytokines such as IL-1β and IL-18 [6].

NOD1 and NOD2

NOD1 and NOD2 which harbor CARDs in addition to NOD and LRR domains, activate NF-κB via an adaptor RIP2/RICK. NOD1 and NOD2 induce transcriptional upregulation of pro-inflammatory cytokine genes. NOD1 and NOD2 recognize the structure of bacterial peptydoglicans, g-D-glutamyl-mesodiaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP). Sabbah stated that the expression of NOD2 is involved in 5'-triphosphate RNA-induced type I IFN-production and host defense against respiratory syncytial virus infection [20]. Cells that are constantly exposed to microbial stimuli *in vivo* and are characterized by reduced TLR signaling (e.g intestinal tissues), can become re-sensitized if intracellular NOD1 and NOD2 signaling is triggered by the presence of invasive bacteria. The results from several studies suggest that the intracellular NOD1 and NOD2 play a critical role in host defense when TLR signaling is reduced such as in intestinal cells or inhibited via tolerization [21].

RLR

RLR family consists of three elements: RIG-I, MDA5, and LGP2. These receptors recognize the RNA from RNA viruses in the cytoplasm of infected cells and induce inflammatory cytokines and type I interferons.

RIG-I and MDA5 recognize viral RNA through their helicase domain and signal through their caspase recruitment domains (CARD). RLR use a common adaptor molecule mitochondria antiviral signaling protein (MAVS). Type I interferons together with interferon-stimulated genes, induce an antiviral state in all infected cells. This inhibits viral replication, induces apoptosis in infected cells, increases the lytic capacity of NK and up-regulates the expression of MHC class I molecules [22].

γδ T cells

Among innate immune cells, γδ T cells bearing the Vγ9Vδ2T-cell receptor (TCR) are able to link innate and acquired immune system. In humans, different subtypes (Vδ1-5) of γδ T cells were found within the γδ T cells pool. Antigen-specific CD3^{+} T-cells using a Vγ9Vδ2 TCR represent less than 5% within the peripheral blood lymphocytes. Vγ9Vδ2 T cells respond to non-protein stress-related molecules, without the need for antigen-presenting cell presentation [23]. Activated Vγ9Vδ2 T cells by secreting TNF-α and IFN-γ induce monocyte-derived dendritic cell maturation and activation [24]. They may recruit and activate neutrophil phagocytes by releasing monocyte

chemotactic protein-2 (MCP-2) and by releasing different cytokines/chemokines they drive other immune cells activation [25]. They mediate NK-like cytotoxicity functions against infected or transformed autologous cells.

NK cells

NK cells are innate immune lymphocytes that develop from a common lymphoid progenitor in the bone marrow [26]. NK cells have a biological program that is distinct from the adaptive T and B lymphocytes which develop from the same progenitor [26]. NK cells complete differentiation and maturation in peripheral lymphoid tissues under the direction of cytokines and transcription factors, with IL-15 playing a central role. NK cells during maturation undergo a process that results in tolerance to normal "self" cells and prevents NK based autoimmunity. NK cells constitutively express a number of cytokine receptors, so NK responsiveness is also regulated by cytokines produced by accessory immune cells sensing pathogens [27]. Human NK cells in the periphery may be divided into subsets by their expression of CD56 and CD16 [28]. Mature peripheral NK cells defend the host from pathogens and mediate anti-tumor responses [29].

NK cells present in many tissues contribute to inflammatory processes, particularly through production of IFN-γ [30]. NK cells mediate several effector functions, including production of cytokines and chemokines, and cytotoxicity against appropriately recognized virus-infected and tumor target cells. NK cell recognition of virus-infected and tumor targets depends on complex interplay between signals from activating and inhibitory NK cell receptors [31].

One way that NK cells protect the host is by detecting abnormal surface receptor on target cells. Typical inhibitory NK cell receptor ligands include MHC class I molecules, which are often down-regulated on target infected cells and "stress" ligands such as retinoic acid early-inducible-I (Rae-I) and UL16 binding protein 1 (ULBP-1), which can activate NK cells and are often up-regulated on these target cells. Once triggered NK cells mediate cytotoxicity against cells releasing cytotoxic granules, and producing various cytokines and chemokines, that influence the developing immune response [32].

A number of transcription factors have been identified that contribute to NK cells development and function. Recently, the role of post-transcriptional control has been raised, especially microRNAs (miRNAs) that influence the NK cell molecular program [33]. The function of miRNAs in NK cell biology is complex, with an important role in NK cell development, survival and/or homeostasis, while reducing peripheral NK cell activation [34].

MicroRNAs

MicroRNAs are small, non-coding RNAs regulating numerous cellular functions. MiRNA repression is mediated by targeting sites in the 3'UTR of mRNAs leading to translation suppression or causing mRNA degradation. The classical pathway of miRNA processing consists of transcription from genomic sequences as long primary (pri-miRNA) transcripts that are processed by Drosha |Dgcr8 complex into the pre-miRNA. The pre-miRNA is exported from nucleus through exportin 5, then processed by Dicer complex into a mature 19-26 nucleotide miRNA. The mature miRNA is loaded into RNA-induced silencing complex (RISC) including the Argonaute proteins, and directs down-regulation of proteins levels [35]. NK cells activated with IFN-γ respond by down-regulating a number of miRNAs, so it is possible that miRNA changes are dependent on the mode of stimulation [36].The

knowledge about regulation of NK cells biology by miRNAs is limited, but it is known that they are critical to NK gene regulation [31].

The expression and importance of miRNAs in T and B lymphocytes have been established, little is known about miRNAs in NK cells. Sullivan et al. described studies that have provided the first insights into the expressed mouse and human NK cell miRNA transcriptome using next-generation sequencing that provides novel miRNA identification [37]. Fehniger et al. identified the resting and 24 h IL-15 activated profile of murine splenic NK cells. The miRNA expression profile was supported by two independent next-generation sequencing platforms with validation by qPCR and microarrays [38].

In sepsis, T cell function must be controlled to keep the balance between pro-inflammatory activity and damaging over-activation. The role of micro-RNA 146a in human T cells and its relevance in sepsis is poorly defined. According to the own study results Möhnle et al. identified micro-RNA 146a as a potent inhibitor of Th1 differentiation in human T-cells and concluded that dysregulation of micro-RNA-146a contributes to the pathogenesis of sepsis [39].

Dendritic Cells

Dendritic cells are antigen-presenting cells (APCs) and are involved in the activation of T cell immune response against invading pathogen. DC derived from bone marrow progenitor cells and comprises a complex lineage or subsets of cells. Both myeloid and lymphoid precursors can give rise to DCs. Precursor DCs differentiate into immature DCs having a potent capacity to internalize and process exogenous antigen. DCs are relatively rare cells and are mainly localized in tissues exposed to external environment, where they reside in an immature form [40]. Immature DCs express a number of PRRs including TLRs, c-type lectin receptors, and nucleotide-binding oligomerization domain-like receptors. DCs participate in cell mediated immunity. The activation of DCs by PAMPs causes a change in chemokine receptor expression, induces the maturation of DCs, increases the number of co-stimulatory molecules (CD80 and CD86), and in turn induces migration to draining lymph nodes [41]. The up-regulation of co-stimulatory (CD80, CD86, CD40) and MHC molecules together with cytokine (TNF-α, IL-1, IL-6, IL-10, IL-12, INF type I and II) and chemokines secretion facilitates antigen presentation by DCs to naïve T cells with an antigen-specific receptor. Recognition of the co-stimulatory molecules (CD80, CD86) by CD28 on T cell membrane is required to fully activate T cells [9]. Geissmann et al. examined blood monocytes in humans and identified two functional subsets as defined by the level of expression of CX_3CR1. Resident CX_3CR1^{high} monocytes were found in the blood and non-inflamed peripheral organs where they homed in a CX_3CR1-dependent way. CX_3CR1^{low} monocytes were short-lived, were actively recruited to inflamed tissues independently of their CX_3CR1genotype, and differentiated into functional DCs that had the ability to stimulate naïve T cells. They were named inflammatory DCs [42]. Only in case of inflammation/infection $Ly6C^{high}$ monocytes emigrate from bone marrow by CCR-2 dependent mechanism, travel through the blood and reach inflamed/infected tissues where they differentiate into inflammatory DCs [43]. Hespel et al. stated that inflammatory DCs may be recruited to reinforce the function of conventional DCs in case of uncontrolled infection. The capacity of conventional DCs to induce immunity or tolerance has not been described for inflammatory DCs, so the two subsets may drive different response. It seems likely that conventional and inflammatory DCs play complementary roles and synergize in the case of inflammation/infection [43].

B Cells

The B cell is an element of the adaptive immune system, secreting a specific antibody that protects against viral and bacterial infection. Recent reports have revealed additional critical function of B cells, as regulators of innate immunity to viral infection. Some B cell subsets (B_1 cells) show characteristics of innate cells-they do not utilize antibody or antigen receptor genes [44]. There are evidences validating view of B cells, as an innate effector cell initiating the earliest response against viral pathogens, independently of antibody. Schneider et al. established the B cell dependence of the IFN β response to infection with cytomegalovirus (CMV) [44]. This innate IFN defense mechanism was independent of TLR pathways and required the signaling of the lymphotoxin (LT)-β receptor, part of the larger super family of cytokines related to TNF. Conditional deletion of the LTβ gene in B cells, but not T cells, confirmed the involvement of LTβ in B cells in the initial response to CMV. The LT-IFN response occurs rapidly, initiating within a couple of hours after infection, before adaptive immunity could contribute [44]. Moseman et al. demonstrated the critical role of the B cell dependent LT-IFN defense pathway in response to the vesicular stomatitis virus (VSV). In the absence of LTB or IFN signaling, VSV infected the lymphatic neurons and spread into central nervous system. These results reveal the innate action of B cells through the LT-IFN pathway [45]. Kelly-Scumpia et al. demonstrated (experimental animal model) that repletion of Rag1$^{-/-}$ mice with B cells improves survival, demonstrating that B cell function in the absence of T cell-dependent antibodies is important for sepsis outcome. Mice deficient in B cells produced decreased levels of IFN-I-dependent cytokines. This study identified a novel role for IFN-regulated B cells in modulating early innate immune responses during bacterial sepsis and identified B cells as participants in a protective IFN-I-dependent circuit during sepsis [46].

CTLs

Protection from intracellular pathogens is dependent on cytotoxic lymphocytes (CLs) which comprise NK cells and cytotoxic T lymphocytes (CTLs). NK cells and CTLs use a common mechanism of cytotoxicity involving the exocytosis of toxic effector molecules and their deliver to the desired target cells.

Cell-mediated destruction of unwanted cells is an essential element of the immunity against viruses [47]. Activated CD8$^+$ cytotoxic T cells (CTLs) mediate killing of target cells by secretion of lytic granules or by ligation of death receptors. Cytolytic activity of CTLs is regulated by MHC class I proteins (MHC-I) that limit presentation of antigenic peptides derived from intracellular proteins to CTLs [47]. CTLs have clonotype specific TCR (T-cell receptor) that distinguishes between endogenous and self antigens. The TCR ligands are binary proteins containing an MHC moiety and peptide antigen-peptide MHC or pMHC [47]. After TCR activation the centrosome (microtubule organizing centre-MTOC of T cells) moves to the point of TCR signaling within the immunological synapse (IS). IS is a highly organized interface between CTL and target cell. At the IS, a cascade of activation signals causes a rapid segregation of cell surface receptors into three compartments: central, peripheral and distal supramolecular activation complex (SMAC) [48]. Varma et al. has revealed that TCR signaling occurred in actin-dependent peripheral microclusters at the distal SMAC (dSMAC) which coalesce in the cSMAC, where TCR can be downregulated [49]. After signaling has occurred, the actin and microtubule cytoskeleton polarize towards the synapse [50]. Microtubules having a defined polarity, radiate from

the MTOC (minus end) to the cell periphery (plus end) and granules migrate towards MTOC. Actin, tubulin and cytoskeletal-associated proteins are important to maintain the organization of the IS-T cell. Concomitant with IS-T cell formation, a variety of cytoskeletal proteins are recruited to this region, which is also a site of polymerization of active actin [51]. Some studies have suggested that actin filaments facilitate access of granules to the site of synapse formation [50]. Perforin-containing granules have been shown to colocalise with actin after recruitment to the presynaptic membrane, and a small clearance in polymerized actin enables free movement of granules to the site of secretion [52]. Granule movement toward MTOC is mediated by the kinetics of intracellular Ca^{2+} accumulation [53]. A mature synapse at the contact area of CD8$^+$ T cells is formed rapidly and the dynamics of molecular segregation is CD8-dependent [53]. The formation of the bull's eye structure at the CTL contact surface appears when the CTL contact professional antigen-presenting cells (APC). The APC may possess a mechanism facilitating recruitment of MHC and adhesion molecules to the contact area [54]. Jenkins et al. stated that once the MTOC/centrosome is polarized to the synapse, the granules move to the plasma membrane, dock and release their contents into specialized cleft. The granules release their contents into the secretory domain after they are delivered to the cSMAC point on the plasma membrane. Lytic granules contain cytolytic proteins such as perforin and granzymes, lysosomal hydrolases such as cathepsins B and D, β-hexosaminidase and lysosomal membrane proteins-LAMP-1, LAMP-2, LAMP-3. LAMP-1 is a marker of degranulation [50]. According to Jenkins et al. studies while the centrosome/MTOC polarizes to the synapse with weak signals, lytic granule movement requires a stronger threshold of signaling [55]. Jenkins concluded that MTOC polarization though being essential for the delivery of granules to the synapse, is not a predictive measure of cytotoxicity. The recruitment of lytic granules to the cSMAC is the decisive step in cytotoxicity.

Anikeeva and Sykulev proposed a model in which the kinetics of Ca^{2+} - mediated downstream signaling determines how rapidly granules are recruited to the MTOC [56]. The model outlines that the granule movement to the MTOC is regulated by signaling kinetics, and the initial MTOC reorientation toward the CTL/target cell interface does not depend on Ca^{2+} signaling. According to their suggestions if the signaling kinetics is fast, the granules are recruited to the MTOC prior to its polarization and the concentrated granules are then delivered by subsequent MTOC polarization to the centre of IS-the shortest pathway. They concluded that the difference in signaling kinetics in CTL dictates the choice of the path of granule delivery that is translated into a more rapid release and more efficient destruction of target cells by CD8$^+$ CTL [47].

It has been recognized that memory CD8$^+$ T cells, which play a role in adaptive response, during an infection may be activated non-specifically through a process called bystander activation.

Data from animal studies showed that CD8 memory cells can be bystander activated to produce IFNγ in the absence of cognate antigen, what can be beneficial for the host [57]. Recently, it was shown that monocytes and dendritic cells contribute to inducing bystander activation of CD8 T cells leading to IFNγ and granzyme B production [58].

Chu et al. showed that target cells express NKG2D ligands following bacterial infection and demonstrated that BA-CTLs directly eliminate these target cells in an innate-like, NKG2D-dependent manner. Selective inhibition of BA-CTL-mediated killing led to a significant defect in pathogen clearance. According to author, these

data suggest an innate role for memory CD8 T cells in the onset of de novo generated, antigen-specific CD8 T cell response [59].

Neutrophils

Neutrophils are the major cellular component of the innate system. They are the critical, primary defense against invading microorganisms. They provide rapid, non-specific response to infectious challenge and are an important interface between innate and adaptive immune system. Neutrophils are short-living granulocytes derived from pluripotent hematopoietic stem cells in the bone marrow [60]. The majority of hematopoiesis is linked to granulopoiesis, and almost 60% of leukocytes within the bone marrow are granulocytes precursors [61]. There are two major population of granules present in mature neutrophils-primary (azurophilic, peroxidase positive), which are first to develop during granulopoiesis, containing myeloperoxidase (MPO) and proteolytic enzymes (cathepsins, proteinase-3, elastase), antimicrobial defensins and bactericidal/permeability-increasing protein [62].The next type of granules-specific granules (peroxidase negative)-which mature late during differentiation, contain a functionally important membrane proteins (lactoferrin, collagenase), receptors for chemotactic peptides, cytokines, opsonins, adhesion proteins [62]. Matured neutrophils are released into the bloodstream, where they circulate for 10-24 hours before they migrate into tissue, where they last for next 1-2 days before apoptosis and being cleared by macrophages [63,64]. Neutrophils released into systemic circulation form the majority of the circulating leukocyte cell population. Under normal conditions the number of mature neutrophils is almost constant and during infection can be increased in the circulation by up to 10-folds.

Recruitment of neutrophils

A dynamic part of circulating neutrophils rolls along the walls of postcapillary veins using transient interactions with endothelial cells, searching for signs of tissue damage, inflammation or invading microorganisms and for the presence of host- and/or pathogen-derived chemotactic signals or chemoattractans [65,66]. In presence of invading pathogens, a different host cells (monocytes, macrophages) secrete potent inflammatory mediators and neutrophils chemoattractans (LTB4, IL-8, GCP-2) which bind specific surface neutrophils receptors. These signals direct neutrophils out of intravascular space to sites of infection within tissues. The one of the neutrophil chemotactic molecule is the C5a complement cascade component [66]. N-formyl peptides or phenol-soluble modulins (PSMs) are able to recruit neutrophils directly [67]. Another important fact is the priming of neutrophils described in the early 1980's. Its classical definition is the ability of a primary agonist, at sub-stimulatory concentrations, to enhance the production of superoxide in response to secondary stimulus [68]. Priming occurs on almost all levels of neutrophil function-adhesion, phagocytosis, secretion of cytokines, synthesis of leukotriene, degranulation [66]. Priming is induced by cytokines, chemokines, growth factors, lipid-derived signaling molecules, and physical cell-cell contact and adhesion [66]. Upon recognition of chemotactic signals and/or neutrophil priming, neutrophils exit peripheral circulation by transmigration across endothelial wall, a process called extravasation [62].

Neutrophils activation

The physiological role of neutrophils is directed towards the eradication of invading pathogens that have disrupted front-line structural immune defence. A correct response results in eradication of the microorganism and restoration of tissue homeostasis.

At the site of infection the neutrophils bind and ingest invading microorganisms by phagocytosis. There are two main mechanisms that account for microbicidal properties of neutrophils: the production and reaction of free radical species, and the coordinated release of proteolytic and antimicrobial granules contents. The neutrophils contain the NADPH oxidase enzyme, that when activated, catalyses the transport of electrons to molecular oxygen with production of superoxide anion as the final effect. The superoxide anion exerts strong anti-microbial properties. The free radical can be released outside the cell, or within the cell in the phagosome. The release of the contents of primary and secondary granules is of significant anti-microbial meaning. The granules contain MPO, lactoferrin, lysosomes, NGAL. MPO (myeloperoxidase) is an enzyme which forms cytotoxic hypochlorus acid (HOCL) after reaction of chloride anion with hydrogen peroxide. MPO oxidises tyrosine residue to form the cytotoxic species, the tyrosyl radical. Some evidence suggests that MPO can act as paracrine signaling molecule causing neutrophils survival. Due to common origin, neutrophils and macrophages have some functions in common (phagocytosis, similar kinetic behavior during infections, antimicrobial immune-modulatory activities) [69]. Activated neutrophils release factors attracting monocytes/macrophages (MIP-1α, MIP-1β) [70], and NK cells [71]. Neutrophils may influence macrophages differentiation into pro- or anti-inflammatory subtype [72]. Interferon-γ released from activated neutrophils induces macrophages activation [73]. Neutrophils release MPO which is taken up by residential macrophage expressing macrophage mannose receptors (MMRs). MPO and MMR interaction leads to release of ROS (reactive oxygen species) and pro-inflammatory cytokines (IL-6, IL-8, TNF-α, IL-1, GM-CSF) by macrophages. Releasing TNF-α, IL-1β, G-CSF and GM-CSF at site of infection macrophages increase survival of recruited neutrophils from 6-12 h to 24-48 h [74]. IL-17 cytokine is an element of innate immune system released by CD4+ TH 17, NK cells and neutrophils. IL-17 acts on neutrophils increasing their number, survival and recruitment at the site of infection [75]. Neutrophils are transporting vehicle for intracellular pathogens delivering antigens to DCs and participating in activation of T-cell immune response by DCs [76]. Neutrophils and macrophages reaction during infection leads to the production of peptide innate defense regulator 1 (IDR1), which exerts activity similar to defensins or cathelicidins [77]. This peptide activates antimicrobial activity of macrophages [78]. Along with the activation of innate or adaptive immune cells, neutrophils by releasing arginase or ROS may deactivate NK cells or T cells activation by depleting the extracellular L-arginine levels required for T cell activation [79,80].

NETs/ETosis

Neutrophils regulate severity of infection by forming neutrophil extracellulary traps (NETs) [81]. NETs are formed as a result of extracellular release of neutrophil nuclear contents and bind to Gram-negative and Gram-positive bacteria. Due to high serine protease content NETs can degrade bacterial virulence factors and kill bacteria extracellularly [82]. The one of the two mechanisms of NETs formation includes recognition of LPS or bacteria by platelets or neutrophils. This mechanism reveals rapidly, so NET formation takes only minutes after exposure. This process is connected with vascular obstruction, endothelial and hepatic cells damage observed during sepsis [83]. NET formation by neutrophils helps in containment of infection, decreasing inflammation by releasing anti-inflammatory lipoxins and lowering pathogen load [81,84]. Phagocytosis primes the neutrophil to undergo apoptosis - a response called phagocytosis-induced cell death (PICD) [85,86].

Angermeyer et al. according to own results stated that NETosis

in ICU patients correlates with positive blood cultures and bacterial infections. NETosis was also found up to 48h before increase of CRP and clinical signs of sepsis. He concluded that it might useful to include the quantification of NETosis into regular screening of seriously ill patients in the ICU [87].

ETosis

Other cells such eosinophils and mast cells also released extracellular traps (ETs) composed by DNA and microbial proteins [88]. It seems to be a more general mechanism, so the release of intracellular DNA to the extracellular milieu was renamed as ETosis, meaning death with release of DNA extracellular traps

Neutrophil activation is not only involved in the activation of innate or adaptive immune response but also suppresses overwhelming function of both elements of the immune system. A correct response results in eradication of the microorganism and restoration of tissue homeostasis.

Neutrophils are part of leukocytes family, which is one of the elements of scoring system(s) used for septic patients' clinical status and prognosis assessment (APACHE II score). According to the SSC Guidelines leukocytes count and/or the number of the immature forms are one of diagnostic criteria for sepsis [89].

Conclusions

The rapid response to infection after pathogen recognition is one of the essential features of innate immune system. This reaction is non-specific but strictly controlled by cells, mediators and receptors that comprise the innate part of immune system. This system is not only a first-line "fighter" but provides information about invading pathogens and is necessary for shaping next step adaptive immune response.

The new view of innate immune system functioning in reaction to infection includes cellular elements being classified as "adaptive" with "innate" ways of response, and reveals new strategies used by "old" neutrophils in killing invading pathogens. These interface cells properties and/or new capabilities in protective manipulating in TLR-signaling in infection/sepsis might offer topics for more advanced research and new preventing and therapeutic tools.

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