Fungal Host Defence

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Humans have developed complex immune systems that defend against invading microbes, including fungal pathogens. Many highly specialized cells of the immune system share the ability to store antimicrobial compounds in membrane bound organelles that can be immediately deployed to eradicate or inhibit growth of invading pathogens. These membrane-bound organelles consist of secretory vesicles or granules, which move to the surface of the cell, where they fuse with the plasma membrane to release their contents in the process of degranulation. Lymphocytes, macrophages, neutrophils, mast cells, eosinophils, and basophils all degranulate in fungal host defence. While anti-microbial secretory vesicles are shared among different immune cell types, information about each cell type has emerged independently leading to an uncoordinated and confusing classification of granules and incomplete description of the mechanism by which they are deployed. While there are important differences, there are many similarities in granule morphology, granule content, stimulus for degranulation, granule trafficking, and release of granules against fungal pathogens.

Keywords: granule ; degranulation ; trafficking ; host defence

1. Introduction

Lack of effective therapy is largely responsible for the high mortality [1]. The therapeutic options for fungal infections are limited and associated with toxicities, which has led to an interest in immune therapeutic approaches [2]. One such therapeutic target is granule-dependent release of antifungal molecules used in host defence.

Immunity is a sophisticated, coordinated system consisting of highly specialized innate and adaptive immune cells that play vital roles against fungi. Both innate and adaptive immune cells are involved in fungal host defence, such as against organisms among the Ascomycota (*Aspergillus fumigatus, Candida albicans*), Basidiomycota (*Cryptococcus neoformans*), and Zygomycota (*Rhizopus oryzae*). NK cells, eosinophils, mast cells, neutrophils, and T cells boast intracellular membrane bound vesicles, which store compounds that can be immediately deployed for host defence. These intracellular compartments have been called “secretory vesicles”, “secretory lysosomes”, or “granules”. These organelles form when products of the trans-Golgi network are packaged into transport vesicles. Transport vesicles move the cargo to an endosome that undergoes acidification and processing of the cargo leading to formation of the secretory vesicles. Secretory vesicles contain molecules that induce fungal cell death or stasis when immune cells engage an invading pathogen.

Immune cells not only act independently, but also work in a complex manner by releasing factors and cytokines that signal and/or prime each other to effectively clear infections. Depending on the immune cells, granules are deployed in different ways. The immune cell can bind to the pathogen and antimicrobial compounds are released directly onto the pathogen. Alternately, immune cells can bind to another host cell that contains the pathogen. In this case, the antimicrobial compounds are released in a directed way through an immunological synapse (IS) between the immune cell and the host cell containing the pathogen, leading to death of the microbe. Immune cells may not bind directly to the pathogen, but receive signals from the pathogen or surrounding cells, causing release of antimicrobial compounds in a non-directional way in the vicinity of the pathogen. Finally, granules are released onto the pathogen surface when it is trapped in an extracellular matrix made up of DNA. Granules are also recruited to phagosomes that contains the engulfed pathogen, but this intracellular pathway will not be the subject of this review.

The mechanisms and machinery by which granules are trafficked within immune cells and released on to the pathogen vary depending on the immune cells and target pathogens. However, the immune cell subtypes share similarities in activation, signaling, and granule trafficking towards the plasma membrane.

2. Granule Characteristics in Different Immune Cell Subsets

Despite common features, secretory vesicles are described and classified differently for each immune cell. Granules are usually classified by size, morphology, and density using electron microscopy. If the buoyant densities of granules differ, they can be separated by centrifugation, which allows proteomic approaches to identify constituents. NK cells have three types of granules: type 1, type 2, and intermediate [3], which are grouped by their morphology (Table 1). Type 1 granules are 50–700 nm in diameter and filled with a dense core surrounded by a thin layer of vesicles [4]. Type 2 granules are 200–1000 nm in diameter and characterized by multiple vesicles and membrane whorls [3]. Intermediate granules have dense cores and multiple vesicles and are less abundant than type 2 granules [5]. Type 1 granules are fully mature while other types represent different stages of granule development [3]. Different components of the granules contain different constituents. The dense core contains cytolytic proteins, while the multivesicular domains contain lysosomal proteins (Table 2) [4]. By contrast, the granules of CD8+ T cells have not been separated by morphology. Rather, granules are characterized in one group with variable granule morphology that resembles the spectrum of granules in NK cells ranging from 100 to 1300 nm [6]. Granules in cytotoxic T cells can be separated by sucrose gradients (Table 1), which allows for separation of different proteins in granules of different buoyant density [7].

Table 1. Granule types and contents in various immune cells.

	NK Cells	CD8+ T Cells	Mast Cells	Eosinophil	Neutrophil
Types	Type 1 Granule (fully Formed) 50–700 nm Contains a dense core surrounded by thin layer of vesicles Type 2 Granule 200–1000 nm Contains multiple vesicles and membrane whorls Intermediate Granule Contains dense cores and multiple vesicles, less abundant than type 2 granules	CytotoxicGranule 100–1300 nm Exists in tiny droplets, dark-core bodies surrounded by a thin membrane, or large granules containing small internal vesicles	Type 1 Granule MHC class II, β-hexosaminidase, lysosomal membrane protein (LAMP)-1/2, Mannose- 6-phosphatereceptors (M6PR) Type 2 Granule MHC class II, β-hexosaminidase, LAMP-1/2, M6PR, Serotonin Type 3 Granule β-hexosaminidase, serotonin	Primary Granule: 500–1000 nm Lack crystalline core Secondary (Specific) Granule: 500–1000 nm Contain distinctive dense crystalline core that is surrounded by a less dense matrix and enclosed by a trilaminar membrane	Primary Azurophilic Granule electron dense 500–1000 nm Secondary SpecificGranule 200–500 nm Tertiary (gelatinase)Granule Mean size of 187 nm
Content	In all granule types: Perforin Granzymes Defensins 1–3 LL-37 Granulysin FasL and TRAIL	In all granule types: Perforin Granzymes Defensins 1–3 LL-37 Granulysin FasL and TRAIL May be separated by granule density	No distinct difference in content between granule types but are: chymase, tryptase, mast cell carboxypeptidase A3 (CPA3), β-hexosaminidase, histamine, granzyme	Primary Granule: Charcot–Leyden crystal protein (galactin-10) Secondary Granule: eosinophil peroxidase (EPO) major basic protein (MBP) eosinophil cationic protein (ECP) eosinophil-derived neurotoxin (EDN)	Primary Granule: neutrophil elastase, myeloperoxidase (MPO), defensins, cathepsin G, proteinase 3 Secondary Granule: lactoferrins, defensins, BPI, MPO, lysozyme, LL-37 Tertiary Granule: matrix metalloproteinases, azurocidin, lysozyme

Contents listed are an up-to-date comprehensive list of molecules necessary for cell death.

Table 2. Pathways and modes of degranulation in various immune cells.

	NK Cells	CD8+ T Cells	Mast Cells	Eosinophil	Neutrophil
Pathway	ERK2 ➔ JNK1 ➔ MTOC, granule polarization and cytotoxicity ITAM dependent and independent signaling ➔ MAPK cascade ➔ NK cell effector functions	TCR ➔ LCK/ZAP70 ➔ LAT/PLCγ/ITK ➔ PIP2 ➔ IP3 ➔ Ca2+ influx ➔ degranulation	Surface receptors (CCR1, TLR4, KIT, or FcεRI). G-protein, MyD88, Jak/STAT, ➔ Lck-phos ➔ LAT-phos ➔ PLCγ ➔ degranulation	CCR3 ➔ G-protein/Lyn, Fgr, Hck ➔ PI3K ➔ Akt ➔ BAD ➔ MAPK ➔ Ras ➔ RAF ➔ MEK1 ➔ ERK ➔ BAD	Microtubule assembly: selectins/integrins ➔ Pyk2 ➔ Vav ➔ paxillin granule mobilization: surface receptors (GPCR, Fc-R, PPRs) ➔ PI3K/PLC/SLP-76/Vav complex ➔ Rac and PIP3
Mode	Cytotoxic degranulation through direct contact of target cells	Cytotoxic degranulation through direct contact of target cells	Anaphylactic/cytotoxic degranulation Phagosomal granule fusion and degranulation	Piecemeal degranulation Intact granule exocytosis and EETosis Phagosomal granule fusion and degranulation	Cytotoxic degranulation Phagosomal granule fusion NET formation and degranulation onto NETs

Granulocytes (neutrophils, eosinophils, and mast cells) have more than one type of granule and may contain different cytolytic contents. Mast cell granules are distinguished by their membrane proteins and serotonin rather than their microscopic appearance (Table 1). Type I and II mast cell granules all contain proteins of the major histocompatibility complex (MHC) class II, β-hexosaminidase, lysosome-associated membrane protein (LAMP)-1 and 2, and mannose 6-phosphate receptor (M6PR), while type III granules lack MHC class II, LAMP-1, LAMP-2, and M6PR. Type I granules, in contrast to type II and III have serotonin [11].

Eosinophils have two types of granules: primary and secondary specific (Table 1) [12]. Sizes range from 500 to 1000 nm [13]. The secondary specific granules have a distinctive dense crystalline core that is surrounded by a less dense matrix and enclosed by a trilaminar membrane [14]. The primary granules are smaller than the secondary specific granules and lack a crystalline core [9]. Granules in all immune cell types appear as distinct, electron dense membrane bound intracytoplasmic organelles that can also be seen on light microscopy. Granules are of similar size (50–1300 nm), with most in the range of 200–500 nm.

Neutrophils have three types of granules: primary azurophilic, secondary specific, and tertiary gelatinase granules (Table 1). These granules are classified by their sizes and intensity on electron microscopy as well as their granule content. Primary granules are electron dense and range from 500 to 1000 nm [15]. Secondary granules range from 200 nm to 500 nm and tertiary granules have a mean diameter of 187 nm [15][16].

3. Conclusions

While immune cells deploy secretory vesicles for direct cytotoxic activity against fungal pathogens, they also employ the contents of secretory vesicles to signal a vast array of other responses that lead to the ultimate clearance of the fungal invasion. Further studies are needed to fully understand granule contents and detailed mechanisms used in response to fungal invasion in different immune cells.

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