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Prospective Tuberculosis Treatment: Peptides, Immunity and Autophagy

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Abstract

Tuberculosis (TB) is a world-leading infectious disease caused by *Mycobacterium tuberculosis* (Mtb). The current treatment lasts 6 months and has contributed to the development of multidrug resistant (MDR) strains that nowadays cause almost half a million deaths around the globe. Forty years of research have rendered only 1 new drug to treat the new MDR strains. In the current review we present emerging trends to treat TB particularly focused on natural and synthetic peptides. The ability of some of these peptides to display multifunctional roles in TB treatment, particularly immune system modulation through autophagy and direct antimicrobial activity against Mtb, may present advantages to control the impact of this disease. We review the mechanisms of action relevant in the development of multifunctional peptides that may lead to evaluate new ways to treat TB, a disease that has accompanied human society for centuries

Epidemiology and current treatment of tuberculosis

Tuberculosis (TB) is a chronic infectious disease caused by the bacillus *Mycobacterium tuberculosis*. TB is usually a pulmonary disease but can affect other sites as well (extrapulmonary TB). The disease is spread in the air and in general a relatively small proportion of people infected for the first time with *M. Tuberculosis* will develop active or progressive disease. TB is more common among men than women, and affects mostly adults in the economically productive age groups from developing countries.

TB is a worldwide health problem. Reports by the World Health Organization (WHO) indicate that there were 8.6 million new active cases and 1.3 million deaths during 2012 [1], equivalent to 125 active cases per 100,000 population. Indeed, TB ranks as the second leading cause of death from an infectious agent worldwide, after the human immunodeficiency virus (HIV). Moreover *M. Tuberculosis* is highly infectious: nearly one third of the world's population is latently infected and 10% of this population will develop active disease. An additional significant problem is the association with HIV infection, from 1.3 million deaths caused by TB in 2012, 0.32 million deaths where in HIV positive people [1]. These epidemiological observations hightligths the relevance of the immune system to control TB (see below). Thus, the HIV and TB epidemics create a public health problem of enormous proportions.

TB is an endemic disease in the developing world. The majority of TB active cases worldwide in 2012 were in the South-East Asia (29%), African (27%) and Western Pacific (19%) regions. India and China alone accounted for 26% and 12% of total cases, respectively. A smaller proportion of cases occurred in the Eastern Mediterranean Region (7.7%), the European Region (4.3%) and the Region of the Americas (3%). The TB incidence rate at country level ranges substantially, with around 1000 or more cases per 100 000 people in South Africa and fewer than 10 per 100 000 people in parts of the Americas, several countries in Western Europe, Japan, Australia and New Zealand [1].

Without treatment, TB mortality rates are high. In studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, approximately 70% died within 10 years; among culture-positive cases (but smear-negative), 20% died within 10 years. Fortunately there are efficient antibiotics to treat this disease. Treatment for new cases of drug-susceptible TB consists of a 6-month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide [2].

The World Health Organization (WHO) declared TB a global public health emergency in 1993. In the mid-1990s, WHO developed the DOTS strategy (direct observed therapy) to improve TB care and control at international level DOTS strategy conforms a short-course of chemotherapy, which comprises an intensive period of two months administering the four primary drugs followed by a period of four months of treatment with isoniazid and rifampicin. Within a decade, almost all countries had adopted this strategy and there was considerable progress; for example, in 2005 the numbers of TB cases reported were over 5 million and treatment success rates reached 85%. However and although TB can be controlled and cured by chemotherapy, treatment usually requires four specific drugs and 6 months of therapy, which produce significant compliance problems. The consequence of this is disease recrudesces and more important the arising of multidrug resistant (MDR) strains (see below).

In the last year MDR strains have increased in frequency afflicting around 450,000 people worldwide and producing 170,000 deaths [1]. In fact, MDR-TB has been identified as a significant problem in every region under the WHO coverage [1]. Treatment of MDR-TB disease is resource intensive and usually requires combination of second line drugs that are more expensive, more toxic, and less effective than drugs used in standard therapy. This problem has accelerated the efforts for new TB drug development and during the last decade has been an intense work in the development and evaluation for regimens to shorten the duration of treatment and reduce the likelihood of the development of resistance [3]. Currently, there are 10 new drugs in

clinical trials and after more than 40 years 1 new anti-TB drug has been approved by the FDA at the end of 2012, this drug is bedaquiline a selective ATP-synthase inhibitor [4].

Regarding protection conferred by vaccination, the Bacille Calmette–Guérin (BCG) vaccine was developed a century ago and is one of the most widely used vaccines globally. Vaccination at birth with BCG is widely applied as part of the Expanded Programme on Immunization of the WHO and billions of people have been vaccinated since 1921 [5]. Except for tuberculous meningitis in children, the capacity of BCG to protect against TB is debated, because randomized clinical trials have provided estimates ranging from 80% to no protection [6]. Several explanations have been suggested for these variations in the protective efficacy of BCG, such as antigenic differences among vaccines, interaction with environmental mycobacteria, nutritional or genetic differences in trial populations and differences in trial methodologies [7–9]. However, there is a lack of compelling evidence in favour of any of these proposed mechanisms. Yet, recent developments in nano-carriers might provide some improvements in the developing of effective vaccination strategies against tuberculosis [10].

Multidrug-Resistant Tuberculosis

The WHO defines MDR to TB caused by strains resistant to at least isoniazid (INH) and rifampicin (RMP), the two most common first line-anti-TB drugs used worldwide.

The mechanisms involved in the development of MDR and extensively drug-resistant (MDR-TB) are complex and determined by the mycobacterium, the host, and iatrogenic factors. Considering that MDR-TB rates are increasing, especially in low-income countries and in high-populated cities, it is important to develop and apply public health programs in TB endemic areas and a comprehensive management structure, including drug management. To increase treatment success of MDR-TB, there are several areas that must be emphasized, for instance, the development of new drugs to reduce the time of treatment and at the same time increase the effectiveness to reduce bacillary loads; furthermore, it is needed the proper follow up of the patients, including monitoring and evaluation [11]. Although in the last decade fluoroquinolones have been used for the treatment of MDR strains, It is likely that *M. Tuberculosis* will develop resistance to this antibiotic, therefore fluoroquinolones and new upcoming drugs must be protected, and their use in the treatment of non-MDR-TB cases must be strongly discouraged and preferably strictly regulated [12,13]. The current rising epidemic of fluoroquinolone-resistant MDR-TB fuelled by careless and indiscriminate use of fluoroquinolone must be used as an experience to both eliminate this practice in current TB treatments and in the controlled administration of novel anti-TB drugs.

In the last few years a new sort of strains has emerged due to the antibiotics evolutive pressure: extensively drug-resistant TB (XDR-TB). XDR-TB strains are defined as any multidrug-resistant strain that is also resistant to any fluoroquinolone and any of the second-line injectable drugs, such as capreomycin, kanamycin, or amikacin. From 2006, when the first report on XDR-TB was published, until the end of 2012, 92 countries had reported the presence of at least one case of XDR-TB. Recently the term totally drug-resistant TB was proposed to define TB cases with a resistance profile beyond XDR-TB, in which the strain would be virtually resistant to all available first- and second-line drugs; however, epidemiological studies are still not abundant on these new class of resitant strains [14]. More recently, it has been shown that resistance to antibiotics may emerge from the natural competition between strains of bacteria that share a niche, such as in the case of *Staphylococcus aureus* [15], highligthing an additional source of pressure for the emergence of natural drug resistance in bacteria.

Alternative treatment for tuberculosis: antimicrobial peptides

Because of the growing and spreading of new MDR-TB strains and its co-evolution with HIV, an urgently need for developing novel compounds and drugs with direct antimicrobial activity and immunomodulatory properties has emerged. Although many proposals have arisen in the last decade, antimicrobial peptides (AMPs) remain to be the best option because of their versatile activity; promoting both direct *M. Tuberculosis* killing through several mechanism and immunomodulation. AMPs are small cationic molecules of a variable length mainly composed by polar-hydrophilic, nonpolar-hydrophobic and positively charged amino acids. This special conformation gives the molecules amphipathic and cationic properties providing them with a partial positive charge; these features are key factors to provide antimicrobial activity [16]. AMPs are broadly distributed in nature. They are an important part of the innate immune response of several living organisms including humans. For instance, it has been observed that either deficiencies or over production of these peptides lead to several infectious and non-infectious diseases which has been reviewed elsewhere [17]. There are more than 40 AMPs in human and two groups (defensin and cathelicidins) are relevant for TB.

The antimicrobial mechanisms of AMPs are conserved among families; when the peptides are at a high concentration, they can insert into the bacterial membrane, causing alterations in the lipid bilayer and making it permeable, hence triggering bacterial death [18,19]. However, this is not the only mechanism of action known for AMPs; it has been shown that members of the buforines and cathelicidins family are able to cross the membrane and, in the cytosol, they can bind to DNA and RNA by electrostatic charges, interfering with vital processes [20]. On the other hand, there are peptides such as mersacidin that inhibits cell wall synthesis by interacting with peptidoglycan precursors [21]. Some other peptides, such as PR 39, HNP 1 and 2, inhibit the synthesis of very important proteins for bacterial viability [18]. Hepcidin, on the other hand, besides damaging the bacterial cell membrane, also decreases the iron levels and down-regulates both protein and mRNA expression of the iron-response element [22] (Figure 1).

It is not clear yet whether AMPs are produced by hosts infected with Mtb in an attempt to eliminate the bacilli during primary infection, but several approaches have been implemented to understand how these innate immunity molecules participate during progressive and latent TB. Although this is not the scope of the present review, it is noteworthy to summarize the studies that catapult the use of AMPs as candidates for TB treatment.

Several antimicrobial peptides from different species have been tested for their activity against *M. Tuberculosis* and so far human neutrophil defensins, synthetic rabbit defensin, and porcine protegrin had the ability to kill *M. Tuberculosis* including clinical isolates [23]. These in vitro observations were consistent with results obtained in animal models of TB: the AMPs tested (HNP-1 and HNP-3) had potent antimicrobial activity against *M. Tuberculosis* in vivo [24,25]. Although these findings encourage translating the use of these

peptides in humans as therapeutics, researchers found two important limitations on these peptides. First, the massive production of the peptides was very costly and second, there is not enough knowledge to determine any secondary effect derived from the physiopathological role of AMPs during TB. Since then, many important studies have emerged answering keystone questions that backup the use of antimicrobial peptides for the treatment of pulmonary TB. For instance, now it is known that defensins are over-produced by lung epithelial cells during *M. Tuberculosis* infection promoting its elimination [26]; this was confirmed through the use of a well-documented TB experimental animal model where susceptible animals that developed TB showed poor expression of defensins whereas resistant mouse strain showed a high and efficient production of defensins [27]. Defensins were the first group described in *M. Tuberculosis* infection and soon after the only cathelicidin in humans, LL-37, was evaluated. This peptide is necessary for *M. Tuberculosis* elimination in infected macrophages [28,29]; importantly, the proper production of LL-37 by macrophages depends on the presence of vitamin D [30]. These observations add to the fact that overexpression of LL-37 eliminates *M. Tuberculosis* during in vitro infection [31]. Now is known that many other molecules besides vitamin D might induce AMPs promoting *M. Tuberculosis* elimination such as L-isoleucine or butyrate and these findings have been reviewed elsewhere [32].

Hence, natural AMPs from humans held the promise to be effective therapeutics to fight TB and other infectious diseases, yet the production of these peptides is cumbersome and expensive. In the last few years several groups worldwide have searched for alternatives to simplify their production; one of these strategies aims to develop synthetic peptides derived from natural AMPs, with the purpose of increasing amphiphacity or by increasing their net positive charge. These modifications have increased the efficiency of these AMPs against bacteria and fungi [33,34]. Recently our group has tested these semisynthetic peptides as promising antimycobacterial compounds in a mouse TB model. Some of these peptides showed good activity to eliminate mycobacteria both in vivo and in vitro [35,36]. Another alternative is the use of antimicrobial peptides produced by bacteria such as the lantibiotics, which are AMPs synthetized by Gram-positive bacteria that are characterized by the presence of post-translationally modified amino acids in their structure, such as lanthionine and methyl lanthionine. The most studied lantibiotic is nisin A. The mechanism of action of this AMP involves the joining of a cell wall precursor to lipid II, allowing pore formation and at the same time inhibiting biosynthesis of the bacterial cell wall. Nisin A and its synthetic derivatives nisin S and nisin T are efficient lantibiotics against *M. Tuberculosis* and non-TB bacteria, and they constitute interesting compounds for clinical studies [37].

AMPs are known mainly because of their antimicrobial activity, however AMPs are not limited to this function. In fact, several authors claim that instead of antimicrobial some of these peptides are immunoregulators, promoting pro-inflamatory and/or anti-inflammatory cytokines, immature dendritic cells maturation through TLR4, chemotaxis [32] and apoptosis (see below). Based on this information some antimicrobial peptides have been modified to increase or decrease immunoregulatory activities, whereas antimicrobial effects remains the same and next we review some recent findings.

Innate Defense Regulator Peptides (IDRs)

IDRs are synthetic immunoregulatory and anti-infective peptides that are based on the sequences of natural human and non-human AMPs [33,34]. These synthetic peptides were designed to selectively modulate the innate immune response to infection, without the potential side effects (mast cell degranulation and enhancement of apoptosis) observed for certain AMPs. In recent studies, it has been demonstrated that the protective activity of IDRs could be solely based on their immunoregulatory properties and that this protection is efficient even in animals infected with MDR strains [35,38]. Besides this immunoregulatory property, the low potential of microbial resistance, lower toxicity and requirement of fewer doses, suggest that IDRs could be used as a treatment and as an adjuvant, as well as for conventional drug-sensitive, but mainly MDR. Several in vitro and in vivo experiments have tested the efficacy of IDRs in experimental TB with pathogenic and MDR strains. In a murine model of progressive pulmonary TB, the intratracheal administration of the IDR peptides E2, E6 (peptides modified from a bovine antimicrobial peptide, bactecin) and CP26 (a synthetic peptide comprising the amphipathic region of cecropin A and the hydrophobic N-terminal of the bee venom peptide melittin), during late disease in mice infected with drug-sensitive *M. Tuberculosis* or MDR strains significantly reduced lung bacillary loads; however, there was no reduction in the inflammatory infiltrate (pneumonia) compared with control non-treated mice [36]. Further experiments showed that the use of others IDRs, such as HH2 or 1018, not only decreased bacillary loads but also pneumonic areas. Conversely, the use of recombinant antimicrobial peptides such as human β-defensin-2 and/or human neutrophil peptide led to an evident reduction in the bacillary loads but a marked pneumonia caused by the non-controlled immune-stimulatory activity of these peptides [35]. Therefore the creation of new synthetic peptides, which modulate specifically immune function, represents a new venue to explore in the treatment of TB.

In this sense, understanding the mechanism to induce regulation of the immune system to fight TB may be relevant to target the action of new compounds. Recent reports have shown that autophagy may be relevant in fighting bacterial infections, particularly TB. The relation between autophagy and immune systems has been reviewed elsewhere [39-41] and in the next section we will review the role of peptidic and non-peptidic compounds in autophagy and their association in TB.

Autophagy: a new mechanism to treat tuberculosis

Autophagy is a highly conserved process occurring inside cells where cytoplasmic constituents including long-lived proteins, protein aggregates, organelles and invading pathogens are sequestered within double-membrane bound compartments that are delivered to the lysosomes for degradation and the products are recycled [42].

Autophagy is important for the innate immunity and pathogen clearance since bacteria and viruses are vulnerable to degradation by this process [43]. Yet, some pathogens have developed strategies to evade autophagy. For instance, Mycobacterium infects permissive macrophages while evading microbicidal ones; this is accomplished by using cell-surface-lipids to hide underlying pathogen-associated molecular patterns and at the same time related phenolic glycolipids induce the recruitment of permissive macrophages [44]. The death is avoided by preventing the normal maturation of the autophagosome into a degradative and microbicidal compartment, and transforming it into a compartment that resembles an early endosome [44,45].

Particularly, *M. Tuberculosis* remains intact in the autophagosome of macrophages by interfering with autophagolysosome biogenesis [46], which involves the inhibition of the fusion between the autophagosome and the lysosome mediated in part by mycobacterial lipids that mimic mammalian phosphatidylinositols and inhibit phosphatidylinositol 3-phosphate (PI3P)-dependent membrane trafficking mechanisms. This blockage can be overcome by the activation of cellular autophagy by different ways, including starvation, drugs, microRNA and peptides [47].

Non-peptidic inducers of autophagy

Starvation

Gutierrez and collaborators demonstrated that stimulation of autophagic pathways by starvation in macrophages causes the maturation of the mycobacterial autophagosomes into autophagolysosomes inducing their acidification, overcoming the trafficking block imposed by *M. Tuberculosis* and culminating in bacterial death [48]. Similar lysosomal mediated killing has also been reported for Streptococcus, Shigella, Legionella, and Salmonella [49-52]; note that in these cases, autophagy may be induced by the bacteria itself.

Vitamin D

It is known that the active form of vitamin D (1, 25-dihydroxyvitamin D3) activates a direct antimicrobial pathway in human macrophages inducing autophagy [53]. This autophagic pathway involves the generation of the peptides cathelicidin and defensin B4, which exert direct antimicrobial activity against *M. Tuberculosis* [29,54,55].

miR-155

The microRNA miR-155 accelerates the autophagic response in macrophages, thus promoting the maturation of mycobacterial phagosomes and reducing the number of intracellular bacteria [56].

Statins

These molecules are cholesterol-lowering drugs but they also can modify immunologic responses. The use of statins in murine TB infection studies showed an increased host protection, with reduced lung burdens and improved histopathologic features. These results have been explained considering that statins might counteract *M. tuberculosis*–induced inhibition of autophagosomal maturation and promote host-induced autophagy, increasing the host protection against TB [57].

ATP

Stimulation of human macrophages with ATP promotes the acidification of Mycobacterium-containing autophagosomes and subsequent killing of *M. tuberculosis*. The acidification of autophagosomes is mediated by ATP stimulation of P2X7, a plasma membrane receptor for extracellular ATP, which is upregulated on mature macrophages [58].

Rapamycin

This drug has been used to induce autophagy and enhance vaccine efficacy against TB in a mouse model. Rapamicyn-induced autophagy enhanced the presentation of the immunodominant mycobacterial antigen Ag85B by macrophages infected with *M. tuberculosis*. Furthermore, rapamycin increased localization of the mycobacteria within autophagosomes and lysosomes [59].

Peptidic inducers of autophagy

Reports of peptides that induce autophagy and some of them with activity against *M. Tuberculosis* are described next.

Apoptosis inhibitor of macrophages (AIM)

AIM is a scavenger protein secreted by tissue macrophages, which enhances macrophage mycobactericidal activity, upregulates the production of reactive oxygen species, increases mRNA levels of the antimicrobial peptides cathelicidin and defensin 4B and acidifies the mycobacterial autophagosomes, leading to bacterial death [60].

DRAM

Damage-Regulated Autophagy Modulator is a lysosomal protein that is induced during DNA damage by p53; in this context, the expression of DRAM leads to macroautophagy and is required for p53-mediated death [61].

FLIP derivated peptides

DED1, an α-helix ten amino-acid (α2) peptide and DED2, an α-helix twelve amino acid (α4) peptide, are two domains of the protein FLIP (FLICE-like inhibitor) capable of binding FLIP itself and Atg3, effectively suppressing Atg3–FLIP interaction without affecting Atg3-LC3 interaction, resulting in robust mammalian cell death with autophagy [62].

Muramyldipeptide

This peptide acts over the nucleotide-binding oligomerization domain–containing-2 (NOD2) protein in dendritic cells inducing autophagy [63].

Tat–beclin 1

Levine and colleagues designed a peptide (Tat-beclin 1) composed with a region from the protein Beclin 1 which is necessary to induce autophagy. To promote cell permeability of this Beclin 1 peptide, it was linked to the HIV-1 Tat protein via a G2 linker (YGRKKRRQRRRGGTNVFNATFEIW). In vitro experiments showed Tat–beclin 1 induced a 10–50-fold reduction titers in HeLa cells infected with the Sindbis virus (SINV), Chikungunya virus (CHIKV), West Nile virus (WNV) and this was not due to the cytotoxicity of the peptide. HIV-1 replication in human monocyte-derived macrophages was also substantially inhibited; increased autophagosome and autolysosome numbers, as well as enhanced protein degradation, were seen in Tat-Beclin 1-treated HeLa cells. Tat-beclin 1 interacts with a previously unknown negative regulator of autophagy, GAPR-1 (also known as GLIPR2). This confirmed that Tat–beclin 1 is an inducer of autophagy. Finally, Tat-beclin 1 showed antibacterial activity in an in vitro model of *Listeria monocytogenes* infection; yet, the reduction of bacteria counts was reported only for a *L. monocytogenes* strain that

lacked the autophagy evasion protein, ActA, thus the real advantage of such peptide in treating bacterial infections remains to be elucidated [64].

Non-Peptidic inducers of autophagy	
Name	Mechanism of action
Starvation	mTOR is inhibited and ATG13 is dephosphorilated during autophagy.
Vitamin D	Activates an antimicrobial pathway (cathelicidin and defensin B4) in human macrophages that induces autophagy.
miR-155	Promotes the maturation of autophagolysosomes.
Statins	Stop the inhibition of autophagosomal maturation and promote host-induced autophagy.
ATP	Stimulates receptor P2X7 and induces the acidification of autophagosomes to kill bacteria.
Rapamycin	Enhances the presentation of the mycobacterial antigen Ag85B by macrophages and induces autophagy.
Peptidic inducers of autophagy	
Name	Mechanism of action
Apoptosis inhibitor of macrophages (AIM)	Upregulates the production of reactive oxygen species, increases mRNA levels of antimicrobial peptides (cathelicidin and defensin 4B) and acidifies the autophagolysosomes, leading to bacterial death.
Damage-Regulated Autophagy Modulator (DRAM)	p53 target; expression of DRAM induces macroautophagy and is required for the cell death induced by p53.
FLIP-derived peptides	Bind FLIP itself and Atg3, suppressing Atg3–FLIP interaction without affecting Atg3-LC3 interaction, resulting in cell death with autophagy.
Muramyldipeptide	Acts over the nucleotide-binding oligomerization domain–containing-2 (NOD2) protein in dendritic cells inducing autophagy.
Tat–beclin 1	Interacts with a previously unknown negative regulator of autophagy, GAPR-1, to induce autophagy.
Indolicidin, SPFK and 27RP	These peptides induce ionic interactions with lipophosphoglycans on the parasite's surface, inducing dissipation of membrane potential and the balance of intracellular pH. Cells treated with these peptides show signs of autophagy.

Table 1: Inducers of autophagy relevant to TB treatment

Indolicidin, SPFK and 27RP

These antimicrobial peptides were tested against *Leishmania donovani*, exhibiting a 50% antileishmanial activity. Their mechanism of action involves ionic interactions with lipophosphoglycans on the parasite's surface, inducing dissipation of membrane potential and the balance of intracellular pH with extracellular environment. By the use of transmission electron microscopy, extensive intracellular damage including cytoplasm vacuolization and degeneration of cellular organization without disruption of the plasma membrane was observed. Indolicidin and the two peptides derived from Seminalplasmin, SPFK and 27RP, induce cell death in *L. donovani* via a non-apoptotic process by activating the pathway(s) of autophagic cell death [65].

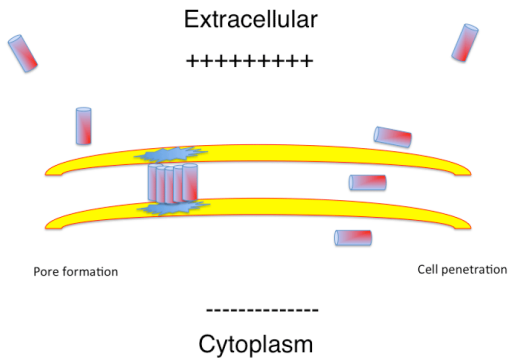

Figure 1: Mechanisms of action on membranes of antibacterial peptides. Biological membranes from both eukaryotes and prokaryotes separate the intracellular from the extracellular spaces; the image represents a biological bilayer membrane by two yellow archs delinated with red lines. A property of these membranes is the presence of an electrochemical potential that it is usually larger for bacterial cells than for eukaryotic cells; the image represents this electrochemical potential by the + and – symbols on both sides of the membrane. Antibacterial peptides are cationic and amphipatic molecules; in the figure antibacterial peptides are represented as cylinders with two colors, red indicates the cationic and blue the hydrophobic charges that in combination with the cationic character renders amphipathicity to these peptides. Antibacterial peptides show some specificity to kill bacterial cells by making pores on their membranes (represented in the image by a blue explosion at the membrane), presumably because these maintain a large chemoelectrical potential at their membrane that is sensed by the cationic peptide. Yet, in membranes with low chemoelectrical potential such as eukaryotic membranes, these peptides may be able to penetrate into them disrupting the membrane. Furthermore, some antibacterial peptides are ligands to the chemokine receptor CCR6 and TLR-4, promoting mobilization and activation of IDC. This multi-functionality may allow antibacterial peptides two kill bacteria and elicit intracellular response on eukaryotic cells, particularly immune system cells.

Mechanisms of action of pro-autophagy and antimicrobial peptides

The use of peptides have some advantages over other molecules decribed above, among others because some peptides besides inducing autophagy also provoke the expression of antibacterial peptides (e.g. AIM), or are antibacterials themselves (e.g. Indolicidin, SPFK and 27RP) which increase their activity in one single molecule. Particularly, small peptides such as indolicidin, SPFK or 27RP are potentially useful therapeutic molecules because they have two activities in a small number of amino acids (13, 12 and 27, resp) overcoming the difficulties of production and displaying polypharmacologic properties.

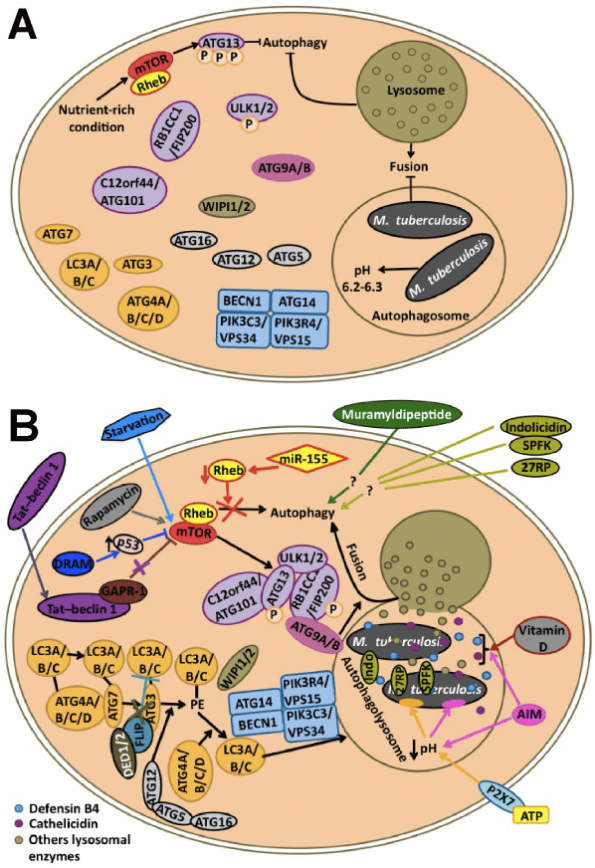

Figure 2: Mechanisms of autophagy inducers. A) In normal cells autophagy is regulated by mTOR. When mTOR is active, phosphorilates and inhibits ATG13 stopping the autophagic process. After having been phagocited by a macrophage, M. Tuberculosis detains the first autophagic stimuli stopping the maturation of the phagosome and preventing its fusion with lysosomes; it also limits the acidification of this vacuole (pH 6.2-6.3), allowing it to reside and multiply inside of the macrophage. B) If a second autophagic stimulus is sensed the autophagic process is restarted and M. Tuberculosis is killed. In the case of starvation or treatment with rapamycin, mTOR is inhibited and ATG13 and ULK1/2 are dephosphorilated starting the autophagic machinery. The microARN: miR-155 diminishes the translation of protein Rheb that together with mTOR inhibit autophagy. Statins induce the incorporation of LC3 proteins to the autophagic membrane improving the fusion with the lysosomes. ATP induces the acidification of phagosomes by the stimulation of the receptor P2X7. Vitamin D and AIM induce the synthesis of the antibacterial peptides: cathelicidin and defensin B4 and AIM acidificates the mycobacterial phagosomes. DRAM induces the expression of p53, which inhibits mTOR. DED1/2 inhibits the protein FLIP that normally prevents the interaction of ATG3 with LC3 for the elongation of the phagosome. Tat–beclin1 inhibits GAPR-1, an activator of mTOR. In the case of muramyldipeptide and the antibacterial peptides indolicidin, SPFK and 27RP it is know that they induce autophagy but the mechanism is unknown.

Beyond natural sources of pro-autophagyc peptides, it is possible to tinker with these to add antibacterial activity to them [66], rendering in this way peptides potentially useful in the treatment of mycobacterial infections. A challenge in these designed peptides is to target specific cells in an organism, avoiding undesired secondary effects. In the case of TB, a possible target would be macrophages. In the case of the peptide Tat–beclin 1 addition of the Tat peptide only improved the penetration of the Beclin peptide into cells in a non-specific way; such design may be targeted by linking the Beclin peptide to a ligand peptide, like Ellerby and collaborators did with their Hunter-Killer peptides [67]. However, it seems that the autophagy-induced by Beclin 1 was not efficient to treat bacterial infections (see above). Thus, it is also important to take into account in these designs the pathway used to induce autophagy (Figure 2).

On the other hand, we have recently pointed out that some cell-penetrating peptides (CPPs) may display direct antimicrobial activity [68] and such peptides may be used to improve the chances of pro-autophagyc peptides such as Tat-Beclin 1, to treat antimicrobial infections. In such case, it is important to consider that some specificty may be lost if the penetrating mechanism is not mediated by receptor-mediated endocytosis or by the emergence of new activities observed when multiple activities are combined into a single peptide [68]. That is, in designing new synthetic peptides useful in the treatment of TB and/or MDR-TB the direct antibacterial mechanism of action of these peptides has to be taken into account as well as the penetrating (Figure 1) and the pro-autophagyc mechanisms (Table 1 and Figure 2). Future research in this direction may provide new tools for the treatment of TB in the developing world.

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