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mTOR, linking metabolism and immunity

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Abstract

mTOR is an evolutionarily conserved serine/threonine kinase that plays a critical role in cell growth and metabolism by sensing different environmental cues. There is a growing appreciation of mTOR in immunology for its role in integrating diverse signals from the immune microenvironment and coordinating the functions of immune cells and their metabolism. In CD8 T cells, mTOR has shown to influence cellular commitment to effector versus memory programming; in CD4 T cells, mTOR integrates environmental cues that instruct effector cell differentiation. In this review, we summarize and discuss recent advances in the field, with a focus on the mechanisms through which mTOR regulates cellular and humoral immunity. Further understanding will enable the manipulation of mTOR signaling to direct the biological functions of immune cells, which holds great potential for improving immune therapies and vaccination against infections and cancer.

Keywords

mTOR; rapamycin; T cells; B cells; metabolism; autophagy

Introduction

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that controls cell proliferation and metabolism in response to a diverse range of extracellular stimuli such as the availability of nutrients, growth factors and stress [1, 2]. Deregulation of the mTOR signaling pathway has shown to be closely associated with cancers, metabolic diseases as well as aging [1]. A growing body of evidence suggests that mTOR regulates functional outcome in a wide range of immune cells, including T cells, B cells, dendritic cells, macrophages, neutrophils, mast cells and natural killer cells [3]. Here we review the recent progress regarding the role of mTOR in the regulation of T cell and B cell responses and discuss the potential mechanism of mTOR as a metabolic checkpoint in influencing an immune response. We highlight the potential for improving vaccine efficacy and anti-tumor therapy by pharmacological targeting of the mTOR pathway.

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mTOR signaling cascades

mTOR is physiologically active in complex with accessory proteins that determine the functional outcomes of mTOR signaling. The two currently recognized multi-molecular signaling forms of mTOR, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), are differentially activated by distinct extracellular and intracellular signals [4], as illustrated in Figure 1. mTORC1 comprises mTOR and four subunits, including the scaffolding protein, regulatory-associated protein of mTOR (RAPTOR), DEP-containing mTOR-interacting protein (DEPTOR), mammalian lethal with Sec13 protein 8 (mLST8) and the Proline-Rich Akt Substrate 40kDa (PRAS40). mTORC2 comprises mTOR and five subunits, including the scaffold protein Raptor-Independent Companion of TOR (RICTOR), mammalian stress-activated protein kinase interacting protein 1 (mSIN1), DEPTOR, mLST8 and the Protein Observed with RICTOR (PROTOR) [4].

mTORC1-signaling events are associated with cellular growth and proliferation in response to a variety of environmental cues, such as growth factors and nutrient availability, as well as immune-regulatory signals [1]. Classically, growth factors, cytokines or other co-stimulatory signals activate PI3 kinase (PI3K), leading to activation of the protein kinase Akt. Akt in turn activates mTOR through inhibition of the mTORC1 repression factor Tuberous Sclerosis Complex (TSC). TSC is a hetero-dimeric complex, comprising the TSC1 and TSC2 subunits, which functions as a GTPase-activating protein by inhibition of the GTP-binding protein Rheb. Rheb is an essential component of mTORC1 activation. Upon de-activation of TSC, the active, GTP-bound Rheb interacts with mTORC1 to promote signaling. Akt can further phosphorylate PRAS40, relieving it from inhibiting mTORC1. Reciprocal to the activating signals from Akt, a decrease in the ATP/ADP ratio can activate AMP-activated Protein Kinase (AMPK) which in turn inhibits mTOR activity by phosphorylation of TSC2 and/or RAPTOR [1].

The mTORC1 signaling pathway controls the expression of a diverse range of genes that promote cellular growth and proliferation. The most well-characterized of these pathways is the phosphorylation of p70-S6 kinase (S6K) and eukaryotic initiation factor 4E binding protein (4E-BP), which promotes protein translation [5, 6]. Active mTORC1 also down-regulates the autophagy pathway and promotes lipid biosynthesis as well as mitochondrial biogenesis. Enhanced mitochondrial biogenesis was observed upon increased mTORC1 activity by genetically deleting the mTORC1 repression factor, TSC1 in hematopoietic stem cells [7]. Signaling events involving mTORC2 are less well-characterized. mTORC2 signaling has been associated with cell survival and cytoskeleton organization [8, 9]. The finding that Akt is also a downstream target of mTORC2 implies both forms of active mTOR function in a synchronized manner towards the same physiological outcomes [1].

mTORC1 is a specific target of rapamycin. Rapamycin is a small molecule drug, derived from *Streptomyces hygroscopicus*, that binds a 12-kDa FK506 binding protein (FKBP12) to form the rapamycin-FKBP12 complex, which is an inhibitor of mTORC1 [10]. In addition to the blockade of mTORC1 activity, prolonged rapamycin exposure is implicated in inhibiting mTORC2 [11]. Clinically, rapamycin is used as an immune-suppressive agent for the prevention of allograft rejection. The immunosuppressive effect has been largely attributed to the inhibition of T cell proliferation [12]. However, recent observations of immunostimulatory and immunoregulatory effects of rapamycin have indicated a broader influence of mTOR in controlling the biological activities of immune cells [13–16].

mTOR in the differentiation of effector CD4 T cells and regulatory T cells

Upon antigen stimulation, naïve CD4 T cells can differentiate into several distinct subsets, including Th1, Th2, Th17, follicular helper T cells (Tfh) and regulatory T cells (Treg). Each

subset has a unique transcription factor critical for specific lineage differentiation, i.e., T-bet for Th1, Gata-3 for Th2, ROR γ t for Th17, Bcl-6 for Tfh, and Foxp3 for Treg, respectively [17]. In addition, differentiation into these lineages is coordinated by distinct downstream cytokine-mediated transcription factors, known as Signal Transducer and Activator of Transcription (STATs) and associated cytokines, such as IL-12/STAT4 (Th1), IL-4/STAT6 (Th2) and IL-6/STAT3 (Th17) [18, 19]. Recently, accumulated evidence from several studies highlight that mTOR activity is closely associated with distinct CD4 T helper lineage differentiation [20, 21]. It has been shown that mTOR-null CD4 T cells were unable to differentiate into Th1, Th2 and Th17 cells under appropriate skewing conditions [22]. This was largely due to impaired phosphorylation of corresponding STATs in response to different cytokine stimuli, leading to the inability of inducing the expression of lineage-specific transcriptional factors [14, 22]. It has been further demonstrated that mTORC1 and mTORC2 have differential role in lineage differentiation of CD4 T cells [23]. Upon conditional deletion of the specific upstream activator for mTORC1, Rheb, the CD4 T cells were incapable of developing to either Th1 or Th17, while Th2 cell differentiation from these Rheb-deficient cells was unperturbed [23]. Conversely, when RICTOR, the essential component of mTORC2, was conditionally deleted, RICTOR-null CD4 T cells failed to differentiate into Th2 cell type, but Th1 and Th17 lineage differentiation remained intact [23]. Accordingly, corresponding lineage-specific STATs were inactivated by mTORC1 or mTORC2 inhibition [23].

Thus these studies suggest that mTORC1 and mTORC2 selectively regulate Th1, Th2 and Th17 differentiation. Paradoxically, in an earlier study, it was shown that both Th1 and Th2 differentiation were abolished when RICTOR was deleted specifically in CD4 T cells [24]. This discrepancy was regarded as a possible consequence by using different conditional deletion system of RICTOR gene (Lck-Cre versus CD4-Cre), which leads to the deletion of RICTOR at different stage of CD4 T cell development [25]. Overall, these data clearly demonstrate that mTOR signaling regulates the differentiation of Th1, Th2 and Th17 subsets. Distinct from Th1 and Th17 cells, Tfh cells are specialized in providing help to B cells for optimal antibody production [26]. Despite extensive studies of mTOR in other CD4 T cell lineages, its role in the regulation of Tfh differentiation remains to be elucidated.

In contrast to effector CD4 lineages, Foxp3⁺ T regulatory cells (Tregs) maintain tolerance to self-antigens and suppress effector functions of other T cell subsets [27]. Rapamycin treatment or genetic ablation of mTOR drives the preferential expansion of Tregs *in vitro* and *in vivo* [22, 28]. These data suggest that mTOR activity antagonizes Treg differentiation. Accumulating evidences suggest that CD4 T cell differentiation is critically coupled to cellular metabolic state [29–31]. It has been postulated that Treg differentiation depends more on fatty acid oxidation and mitochondrial respiration as an energy source, whereas effector T cells display highly glycolytic metabolic demand [30, 32]. Consistent with this point, mTOR promotes glycolysis, but not fatty acid oxidation, by selectively increasing the translation of glycolysis related proteins [33, 34]. In addition to the regulation of metabolic state, rapamycin inhibition of mTOR or mTOR deficiency has also been reported to increase responsiveness to TGF- β , leading to elevated Treg expansion [22, 35].

mTOR in B cell response

In contrast to extensive studies in T cells, the function of mTOR in B cell responses has received minimal attention. In a T cell-dependent B cell response, naïve B cells become activated and migrate from the B cell follicle to the T-B border, whereby these cells will interact with cognate CD4 T cells [36]. Subsequently, T cell-helped B cells will either differentiate into short-lived extra-follicular plasma cells or migrate into B cell follicles to initiate a germinal center (GC) reaction [37]. Germinal centers are unique tertiary

compartments within B-cell follicles, supporting rapid proliferation and somatic hypermutation/affinity maturation of activated B cells [38]. The consequence of a GC reaction is to establish a high-affinity, long-lived memory pool that contains memory B cells in lymphoid tissues and plasma cells in bone marrow [39]. Therefore, B cell responses involve a series of cellular events, including migration, growth/proliferation and differentiation. As mentioned, mTOR regulates almost all of these events in many other cell types, including CD4 T cells. However, little is known regarding how mTOR coordinates these events in B cell responses (Figure 2). Since mTOR integrates various signals to dictate the fate of CD4 T cell differentiation, it is of great interest to investigate whether mTOR signaling also determines the differentiation outcomes of activated B cells at the T-B border or within the germinal center. Furthermore, it is important to dissect whether mTOR mediated metabolism is linked to B cell differentiation and function.

To date, there are very limited publications regarding mTOR regulation of B cell immunity. Several earlier studies have demonstrated that the inhibition of mTOR with rapamycin blunted B cell proliferation and plasma cell differentiation [40–44]. However, most of these experiments were performed *in vitro* using strong BCR or TLR agonists, which may not recapitulate the complexity of B cell responses under physiological conditions. Recently, two mouse models with altered mTOR activity by genetic targeting have been used to study role of mTOR in B cell function [45, 46]. In the mTOR hypomorphic mouse, B cells with reduced mTOR activity showed decreased differentiation into plasma cells in response to antigen stimulation [45]. In contrast, conditional deletion of mTOR inhibitory TSC-1 molecule in B cells leads to constitutively hyperactive mTOR signaling, and also results in defects in plasma cell differentiation after immunization [46]. The observed discrepancy of these studies could be explained by few possibilities: first, in both studies the development of naive B cells was compromised prior to the antigen stimulation due to changes introduced in their mTOR activity [45, 46]; secondly, optimal antibody responses may require a defined range of mTOR activity, i.e., either too high or too low mTOR activity could be suboptimal for plasma cell differentiation. Future studies should employ systems where the mTOR signaling pathway could be specifically targeted at different stages of B cell response, including B cell activation, GC reaction, and memory B cell/plasma cell differentiation. Since antibody responses are the correlate of protective immunity for many successful human vaccines [47], a better understanding of the role of mTOR in B cell responses will facilitate the rational design of future vaccines.

mTOR in CD8 T cell memory development

Recent work provided insight into how mTOR could control the outcome of memory CD8 T cell differentiation [48–50], however the mechanism by which mTOR-signaling pathways sense and induce metabolic changes to influence the fate of immune effector cells has yet to be determined. Upon recognizing cognate antigen, CD8 T cells undergo robust proliferation followed by rapid contraction, whereby most effector cells undergo apoptosis, leaving only a small proportion to become long-term protective memory cells [51, 52]. In the lymphocytic choriomeningitis virus (LCMV) acute infection model, the administration of rapamycin during the expansion phase following viral infection markedly enhanced the number of virus-specific memory CD8 T cells. These findings were attributed to an increased number of effector cells committed to become memory precursors. Furthermore, rapamycin treatment during the contraction phase accelerated conversion of effector CD8 T cells in becoming long-lived memory cells, characterized by the re-expression of CD62L, CD127 on the cell surface and an increased level of the anti-apoptotic molecule, Bcl-2 [16, 49]. These memory cells exhibited both superior recall ability as well as enhanced protective capability, and the effect of rapamycin was identified as being intrinsic to the CD8 T cells, acting through the down-regulation of mTOR activity [49]. Likewise, LCMV-specific CD8 T cells,

expanded in the presence of rapamycin *in vitro*, generated more memory CD8 T cells after adoptive transfer into mice [53]. These CD8 T cells treated with rapamycin had an elevated level of oxidative phosphorylation, resulting in enhanced cell survival upon the withdrawal of growth factors. In contrast, increased activity of mTORC1 in CD8 T cells by the addition of IL-12 resulted in a reduced number of memory CD8 T cells [50]. Equivalent results were observed by down-regulating a negative regulator of the PI3K/Akt pathway, PTEN, using a microRNA cluster, miR17–92 [54]. In both studies, the defect in memory differentiation could be rescued by rapamycin treatment.

The importance of fatty acid metabolism in memory CD8 T cells was demonstrated using T cell-specific knockout of a Tumor Necrosis Factor (TNF) receptor-associated factor (TRAF6). The TRAF6-deficient cells mounted a robust immune response, but failed to survive as memory cells [48]. Similar to naïve T cells, memory cells are quiescent and catabolic, employing fatty acid oxidation and autophagy to meet cellular metabolic demands. AMPK, which regulates fatty acid metabolism, exhibited reduced activity in the TRAF6-deficient effector CD8 T cells upon the withdrawal of growth factors. A drug that activates AMPK, Metformin, was able to restore the memory population in the TRAF6-deficient CD8 T. Furthermore, administration of the mTOR inhibitor, rapamycin, further improved memory cell development [48]. Rapamycin has shown to improve lipid metabolism in cells and this may facilitate memory formation.

Together, these data improve our understanding of the metabolic requirements and regulation of memory CD8 T cells. To understand the mechanism by which mTOR inhibition contributes to improve CD8 T cell memory would involve a careful examination of all downstream functions. Rapamycin and its derivatives have been shown to improve CD8 T cell memory in non-human primates upon vaccinia vaccination and have enabled better protection in mouse tumor models [48–50, 55–57]. Oncogenic activation of mTOR supports tumor cells in their fast growth, proliferation and survival. Hence, targeting of the mTOR pathway using small molecule inhibitors as an anti-tumor therapy has attracted intense interest. Further investigation of the effects of mTOR inhibition on the immune response will assist the development of novel therapeutic strategies that combine the benefits of inhibiting tumor growth and enhancing anti-tumor immunity.

mTOR in T cell metabolism and functions

In eukaryotic cells, mTOR functions to sense the nutrient availability and to translate this information into an appropriate cellular response. When energy and nutrients are ample, mTOR is active, thereby signaling downstream pathways in promoting translation and biosynthesis and suppressing autophagy for the recycling of nutrients [4]. Conversely, low mTOR activity indicates an insufficient energy supply, which attenuates biosynthesis and increases autophagy. In the context of the immune response, mTOR also integrates immunological stimuli and metabolic signals to influence downstream biological processes. Previous studies have shown that co-stimulatory molecules such as CD28 could activate mTORC1 activity via the PI3K/Akt pathway [58]. Cytokines such as IL-2 and IL-4 could also activate mTOR via the same upstream signaling pathway [59, 60]. Conversely, inhibitory signals reduce mTORC1 activity as seen in PD-L1 engagement of PD-1 on regulatory T cells [61].

Naïve T cells and activated T cells have drastically different metabolic signatures [62]. mTOR signaling in both cell types illustrates this dichotomy by variously facilitating increased nutrient uptake and energy production. Naïve T cells employ catabolic metabolism, which utilizes lipid oxidation and autophagy to provide energy and substrates for basal levels of biosynthesis (Figure 3). Upon receiving activation signals via TCR and

costimulatory receptors, naïve T cells must ready themselves to undergo a metabolic reprogram in preparation of the surge in bioenergetic demands required for a vigorous clonal expansion [63–65]. Activated T cells switch from catabolic to anabolic in their metabolism, utilizing aerobic glycolysis and glutaminolysis as a major supply of ATP as well as metabolic intermediates for fueling the fast synthesis of proteins, lipids, nucleotides and other biosynthetic products. mTORC1 has been shown to be involved in the up-regulation of enzymes involved in glycolysis, glutaminolysis and lipid synthesis, as well as in the pentose phosphate pathway to increase cell surface expression of the glucose transporter, GLUT-1, and the glutamine transporter, SNAT-2 [34, 63, 66]. This global metabolic reprogramming is achieved through controlling transcription factors such as hypoxia-inducible factor (HIF-1 α), sterol-regulatory element binding protein (SREBP) and c-Myc [34, 63]. mTORC1 also directly up-regulates protein production by phosphorylation of the translational factor 4E-BP and the S6 kinase, as well as in promoting amino acid uptake [5, 67]. These findings emphasize the critical role for mTORC1 in the generation of effector T cells. This notion would appear to contradict the observed enhancement in the memory precursor population at the expansion phase of CD8 T cell response upon rapamycin treatment [49], however these physiological features are directly associated with the extent of mTOR inhibition and are rapamycin dose-dependent. Large quantities of rapamycin inhibited, rather than promoted, CD8 T cell expansion in the LCMV model [49].

Following antigen clearance, the transition phase of effector to memory T cell differentiation presents another major metabolic challenge, involving the reversal from anabolic to catabolic state. The molecular events underlying this metabolic switch are not well characterized. Memory T cells are quiescent in nature, using lipid oxidation and autophagy for energy production. mTOR signaling promotes the glycolytic metabolic program. Inhibition of this pathway by exposure to rapamycin resulted in a metabolic bias towards oxidative phosphorylation and produced a larger memory pool [48, 49]. All these data strongly suggest an effective switch in metabolism towards lipid oxidation during the contraction phase is likely to produce a better memory pool.

The metabolic processes and mTOR signaling activities of T cells vary according to environmental stimuli and activation state. Likewise, the influence of rapamycin upon mTOR varies accordingly. Indeed, more memory CD8 precursor cells were formed upon exposure to rapamycin during the expansion phase, suggesting mTOR influences cellular commitment immediately following activation [49]. However, rapamycin had no influence upon the commitment to T-cell fate when administered during the contraction phase, but accelerated the conversion of memory precursor cells to the central memory phenotype. In light of these findings, differences in mTOR-mediated signaling activities may be a critical component in the differentiation of subsets of effector CD8 T cells, i.e. into a majority that are committed to apoptosis and a distinct population of cells that persist to become the memory population. The precise mechanism of how changes in metabolic status could quantitatively and qualitatively improve the memory T cell pool and dictate T cell fate still warrants further investigation.

Autophagy in T cell differentiation

The mechanisms through which downstream effectors of mTOR influence T cell function and differentiation warrant further investigation. One such physiological function of significant interest is the autophagy pathway. AMPK and mTORC1 have shown to reciprocally control the activity of this pathway through regulation of the mammalian autophagy-initiation kinase, Ulk1 [68]. Active mTORC1 directly down-regulates autophagy activity, whereas AMPK promotes it. Autophagy is critical to maintain metabolic homeostasis in quiescent T cells, not only as a nutrient recycle pathway but also for the removal of

protein aggregates, damaged organelles and invading pathogens [69–72]. The deletion of autophagy-related genes, such as *atg5* and *atg7*, has resulted in compromised T cell development and reduced survival/proliferation upon activation [69, 72]. A potential role for autophagy in T cell activation was based on the finding that autophagosomes accumulated upon T cell activation *in vitro* [72, 73]. Although these findings initially appeared contradictory, given that enhanced mTOR activity upon TCR stimulation would likely result in an anabolic metabolism and inhibition of autophagy activity, upstream AMPK was shown to be active in activated T cells, which could promote autophagy activity [74]. Furthermore, a recent study clearly demonstrated a role of autophagy during TCR activation, whereby autophagy was shown to moderate TCR downstream signaling by degrading Bcl10, an adaptor in TCR-induced activation of NF- κ B signaling cascade [75]. Degradation of Bcl10 via the autophagy pathway was highly selective, in which Malt1, a direct binding partner of Bcl10, was not degraded. These findings draw a parallel with degradation via the ubiquitin-proteasome machinery.

The initial growth of activated T cells is characterized by an increase in cellular size in preparation for the subsequent proliferative burst. During the proliferation stage, autophagy could be less critical, whereby cells use glycolysis as an alternative and more efficient source to fuel the biosynthesis required for fast proliferation. Autophagy is possibly inhibited by high-mTOR activity during the expansion phase, where signaling through the TCR is still active. Upon transition to the memory phase, mTOR activity returns to a basal level, and T cells again revert to catabolic metabolic machinery, where autophagy will again be required for housekeeping functions amongst memory cells. It is tempting to speculate on the significance of autophagy in memory cells which are quiescent, similar to naïve cells, wherein autophagy is important for catabolic metabolism. One important question is whether autophagy also involved in mediating the contraction phase, whereby effector T cells transition to become memory cells. Whether this conserved nutrient-recycling pathway is required for cellular survival and function in the absence of activating and co-stimulatory signals has yet to be determined.

Conclusion

Numerous lines of evidence suggest mTOR plays a central role in regulating the biological outcomes of immune cell stimuli. However, much remains unknown with regard to the level, type and specific downstream targets of mTOR signaling in the immune responses. A key question concerns how mTOR-mediated metabolism is coupled to immune functions. Furthermore, it is critical to determine whether synergistic or individual downstream pathways of mTOR activity are required in shaping a given immune response. Collectively, a greater appreciation of how mTOR pathways influence various immune responses will lead to more precise and specific manipulation of mTOR signaling, thus facilitating the development of novel strategies for vaccine development and cancer therapy.

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Highlights

- mTOR facilitates cross-talk between metabolic and immune programming.
- mTOR is critical for CD4 and CD8 effector and memory T cell differentiation.
- There is a need to understand how mTOR regulates B cell responses.
- Autophagy and other pathways under control of mTOR influence immune cell functions.
- Manipulation of mTOR signaling pathways holds promise for immunotherapy.

Figure 1. mTOR signaling cascade

The mTOR kinase forms two functionally distinct complexes: mTORC1 and mTORC2. mTOR senses environmental cues, including growth factors, nutrients and immune signals, through mTORC1 and mTORC2. mTORC1 controls cellular activities, such as autophagy, protein translation as well as cell growth and metabolism, while mTORC2 regulates cytoskeleton organization, cell survival as well as metabolism. Rapamycin forms complex with FKBP12 and inhibits the kinase activity of mTORC1. Inhibition of mTORC2 requires high doses or prolonged exposure. Arrows indicate activation signal whereas bars correlates to inhibition.

Figure 2. mTOR and B cell responses

In a T-dependent B cell response, naive B cells become activated and differentiate into either short-lived plasma cells (SLPCs) or germinal center (GC) B cells. With help from antigen-specific cognate Tfh cells, GC B cells undergo rapid proliferation and extensive somatic hypermutation, leading to the eventual differentiation of long-lived plasma cells (LLPCs) and memory B cells (MBCs). mTOR may have potential roles in integrating various immune and environmental signals in dictating different outcomes at various stages of B cell differentiation as highlighted in the boxed area.

Figure 3. Metabolic changes of antigen-specific CD8 T cells at various stages of response to an acute infection

Along with co-stimulatory signals, TCR signaling in engaging a cognate antigen presented by MHC-I can activate naïve CD8 T cells. Naïve and memory T cells are quiescent, using mainly fatty acid oxidation and autophagy for basal levels of energy and biosynthesis. Activated T cells and effector T cells have distinct metabolic signatures. In order to meet the bioenergetic demand of subsequent clonal expansion, naïve T cells undergo metabolic reprogramming, switching from mitochondrial oxidation to glycolysis, glutaminolysis and pentose phosphate pathway. During contraction phase, where effector T cells transition to become long-lived memory T cells, the metabolic program reverts from effector-like (anabolic) to memory-like (catabolic). This metabolic switch is less well characterized. It is yet to be determined if this process is immediate or gradual, and whether processes such as autophagy contribute to this transition. Green represents catabolic metabolism whilst red represents anabolic metabolism. The gray pyramid represents the level of antigen.