Metabolic exhaustion in infection, cancer and autoimmunity

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Abstract

It has become increasingly clear that changes in metabolism are not just consequences of T cell activation but rather are essential drivers of that process, shaping the extent and nature of differentiation and function. The process of T cell exhaustion has been linked to the outcome of chronic immune responses in multiple contexts including chronic infection, cancer and autoimmunity. Factors regulating the development and maintenance of exhaustion are of increasing interest as the target of therapeutic modulation. Recent work has shown T cell immunometabolism to be integral to the control and development of T cell exhaustion. Early metabolic changes are responsible for later emergence of exhaustion, do not simply reflect changes secondary to chronic activation and are modifiable. An increased understanding of this metabolic control promises to improve our ability to modulate T cell immunity to chronic antigen stimulation in multiple contexts.

Introduction

The ability to sustain T cell function despite persisting antigen stimulation contributes to the clearance of both chronic infection and cancer. However, where T cell immunity targets self antigen or excessively damages healthy tissue, maintaining robust responses can prove harmful rather than helpful. T cell exhaustion has emerged as a key mechanism through which CD8 T cells lose effector capacity during persistent stimulation, curtailing their ability to cause damaging immunopathology but also facilitating viral persistence or hindering tumour-targeted responses. A substantial body of work using murine models of chronic LCMV virus infection has identified the molecular, transcriptional and functional basis of T cell exhaustion. Recent studies have implicated early metabolic signalling during T cell activation in the later development of exhaustion. In doing so this work has highlighted the potential for metabolic signals to shape T cell differentiation, and has focussed attention on the potential of immunometabolic therapies in multiple contexts.

T cell differentiation and metabolism

The process of T cell activation and differentiation involves rapid proliferation, cytokine production and development of cytotoxic effector function. For this to happen, cells must balance increasing energy demands with an increasing need for substrates to allow growth and division. Bioenergetic needs of naïve or quiescent T cells are primarily met by mitochondrial oxidative phosphorylation (OXPHOS), an efficient means of generating ATP from a glucose substrate[1]. On activation, however, there is a switch to aerobic glycolysis[2] known as the 'Warburg effect', a comparatively inefficient process in which fewer ATP molecules are generated per molecule of glucose processed[3]. A T cells choice of aerobic glycolysis initially makes little sense as it is not only inefficient, but seemingly unnecessary – cells switch to glycolysis even when abundant oxygen is available. However, the trade off is that a greater number of metabolic intermediates are produced for anabolic growth, including those for nucleotide

and amino acid synthesis[4], maintenance of cellular redox balance (NAD+/NADH)[5] and acetyl-CoA production for lipid synthesis through citrate processing[6]. The switch to aerobic glycolysis is also required for the development of full effector function (IL-2 and IFN-γ release), as the glycolytic enzyme GAPDH otherwise restricts translation of IFN-γ mRNA[2].

However, effector differentiation is not the only goal of T cell activation and the switch to aerobic glycolysis is not complete[2]. Following antigen encounter and eradication an expanded, persistent population of memory T cells shows a faster, more robust response to subsequent activation (**Fig. 1**). For such memory to develop during a primary T cell burst, aerobic glycolysis-driven effector function must be balanced with other metabolic pathways including OXPHOS[7] and fatty acid oxidation (FAO)[8,9]. A central role for mitochondria in this balance has been highlighted by marked dynamic changes in their ultrastructure: T_{mem} show fusion of mitochondrial cristae into networks whereas their T_{eff} counterparts undergo fission and remain expanded[10]. The mature memory phenotype is also fuelled by an increased mitochondrial biomass[11] and spare respiratory capacity (SRC), driven by IL-15-mediated upregulation of carnitine palmitoyltransferase (CPT1a)[7]. CPT1a activity controls miotochondrial FAO and fosters the ability to generate a stronger, longer burst of both aerobic glycolysis and OXPHOS on restimulation[11]. However, it should be noted that the role of FAO has been demonstrated using pharmacological inhibition of CPT1a, the specificity of which has been brought into question by recent genetic deletion studies[12]. Much complexity surrounding the role of FAO in T cell differentiation remains to be unpicked.

FIGURE 1 Balance between T_{eff}/T_{mem} and metabolic pathways associated

It is to be expected that dynamic metabolic changes must occur to keep pace with the rapid changes in cellular function that accompany T cell activation[13]. However, these studies have together implicated cellular metabolism – and mitochondrial biomass and function in particular - as a key driver of T cell phenotype, rather than simply reflecting changes in energy demand during differentiation[8,14]. With immunometabolism taking centre stage, it has now become clear that the development and maintenance of T cell exhaustion are also under metabolic regulation.

Exhaustion and metabolism

T cell exhaustion is associated with progressive dysfunction characterised by limited proliferation, cytokine production and effector capacity that is driven by persistent, high levels of antigen exposure and a relative lack of costimulatory 'help' signals[15]. Exhaustion was originally identified in the murine LCMV model of chronic viral infection but has since been identified during numerous other chronic infections, in the tumour microenvironment and during autoinflammatory responses[16,17]. Its development controls persistence and outcome of cellular immunity to persistent antigen and has been associated with disease outcome in each case[15,17]. More recent work has demonstrated that exhaustion is also associated with significant bioenergtic compromise in the setting of both chronic viral infection and the tumour microenvironment[18-20]. The observed metabolic dysfunction precedes the development of exhaustion *per*

se and has been mechanistically linked to early inhibitory PD1 signals. However, this is only one part of a complex cascade modulating metabolism and the balance of effector function, cellular survival and exhaustion. To date, studies have focussed on catabolic metabolism, mitochondrial function and on pathways controlling them. Consequently, this review is similarly focussed, but this should not be taken to preclude the potential for downstream synthetic pathways to also play an important role.

In the LCMV model of chronic viral infection, late emergence of T cell dysfunction is preceded by both transcriptional and functional changes suggesting glucose deprivation, with suppression of both glycolysis and OXPHOS[18,21]. Glucose transport is limited in early exhaustion with downregulation of GLUT1-mediated transport. However, alterations in glucose uptake were seen to be modest, prompting a search for changes in mitochondrial mass and function. This showed that mitochondria of exhausted cells (Texh) are larger, but dysfunctional. Increased mitochondrial biomass was accompanied by a paradoxical reduction in mitochondrial function (Oxygen consumption rate, OCR) and by increases in both mitochondrial depolarisation and reactive oxygen species (ROS) production relative to their effector counterparts (Teff). Ultrastructural changes in the mitochondria, including elongation of cristae, were also apparent[18] although it is less clear how these relate to ultrastructural changes already known to differentiate classic memory and effector cell populations[10]. It is challenging – particularly in vivo - to quantify the degree of metabolic switching required to induce these mitochondrial changes (as it is with associated parameters such as spare respiratory capacity (SRC))[7], and thus to understand their importance.in directing T cell fate.
$CD8^+$ tumour-infiltrating lymphocytes (TIL) also exhibit an exhausted phenotype[22,23] that limits anti-tumour responses, and can be reversed by cancer immunotherapy with impressive results[16]. Like Texh in chronic LCMV infection, TIL exhaustion in multiple murine models shows evidence of glucose deprivation and mitochondrial dysfunction. As in Texh in the LCMV model, reduced TIL glucose uptake was matched by reduced mitochondrial function (OCR) and increased depolarisation[19,20]. However, while bioenergetic changes of Texh were broadly similar in both TIL and chronic viral models, there were also differences. Whereas Texh in LCMV infection showed greater mitochondrial biomass and increased ROS production[18], TILs showed a reduction in both[19]. The apparent contribution of PD1 signaling to metabolic dysfunction was also different in the two contexts. Early metabolic dysfunction of Texh in LCMV-cl13 infection responded to either genetic deletion of PD1 (adoptive transfer of transgenic *Pdcd1*$^{-/-}$ LCMV-specific P14 T cells) or to PDL1 blockade[18,19]. However, mitochondrial biomass was not substantially altered by PD1 blockade in TILs, even though the blockade resulted in effective tumour regression[19]. These context-specific differences in metabolic features of exhaustion require further investigation but some explanations can be offered based on our increasing appreciation of metabolic factors controlling T cell effector function, survival and exhaustion.

Metabolic substrate availability and exhaustion: Goldilocks and the 3 substrates

Emerging evidence has implicated several metabolic pathways as important players in the development of T cell exhaustion. However it should be noted that, as with their contribution to T cell differentiation more broadly, their respective roles are actually integrated, with changes in one influencing many others.

(FIGURE 2: metabolic substrates and T cell exhaustion)

Hypoxia and exhaustion

Both metabolic and effector dysfunction of TIL is clearly driven by and restricted to the tumour microenvironment[19,20,22,23]. Local metabolic substrate availability is an important factor shaping exhaustion and may also explain some of the variation in metabolic parameters between exhaustion models (**Fig. 2a**).

It has long been known that the tumour microenvironment is hypoxic and that this favours tumour growth[24]. However, recent work has revealed the importance of hypoxia in driving the metabolic dysfunction of exhaustion. The tumour suppressor gene *Vhl* does not interact with Hif (hypoxia inducible factor) subunits under hypoxic conditions, allowing Hif to translocate to the nucleus and mediate transcription of target genes. Genetic deletion of *Vhl* unleashed Hif activity during persistent T cell responses resulting in fatal immunopathology during chronic LCMV infection but improving survival in an experimental melanoma model as T cells maintained effector function instead of exhausting[25]. This work further supports the prevailing theory that exhaustion has evolved as a mechanism by which chronic infection is 'tolerated' rather than risk fatal injury during an attempt to clear it[17]. It also highlights the importance of the hypoxia/Vhl/Hif pathway in controlling T cell effector function during persistent antigen stimulation. A metabolomic study of Vhl-deficient murine $CD8^+$ T cells (with consequent high levels of Hif activity) subsequently identified S-2-hydroxyglutarate (S2HG) as a key immunometabolite controlling persistent CTL activity in both immunisation and tumour models[26]. S2HG levels are increased by TCR stimulation in a Hif-dependent fashion but may also be influenced by other metabolic pathways including glutaminolysis promoting both effector differentiation and persistence[26]. It also appears that the contribution of hypoxic Vhl/Hif signalling to exhaustion is distinct from the contribution of classic inhibitory receptors. In the absence of Vhl (P14 $Vhl^{-/-}$ in the LCMV-cl13 model), classic inhibitory receptors such as LAG3 and PD1 were still induced during chronic infection but exhaustion did not develop, resulting in lethal immunopathology driven by an aggressive $CD8^+$ CTL response.

Hypoxia is a constant presence in conditions of rapid T cell infiltration, proliferation and activation whether this is in the TME, virally infected tissue or in the context of autoinflammatory disease. It is clear that the T cell metabolic response to this hypoxic microenvironment is an important determinant of its ability to sustain an effector response and consequently of disease outcome. However, the exact impact of hypoxia on T cell effector function remains unclear with some studies suggesting that limiting Hif expression (by knockdown rather than genetic deletion) can improve rather than attenuate effector capacity[27].

Glucose and exhaustion

T cell activation is characterised by aerobic glycolysis, the switch away from OXPHOS despite the presence of sufficient oxygen to maintain it[13]. However, a

lack of glucose availability in – or a lack of uptake from - the microenvironment does have important consequences for persistence of T cell effector function (**Fig. 2b**). Some studies have suggested that limiting glycolysis can favour OXPHOS-mediated memory formation and enhanced persistent effector function[28], although others have demonstrated a need for ongoing glycolysis to maintain effector function[29]. This apparent discrepancy reflects a need for glycolysis-driven effector function to be counterbalanced by a concurrent need for memory formation, driven by alternative metabolic pathways such as Hif, FAO and OXPHOS. At least some glycolysis is clearly required for initial T cell activation and for the development and maintenance of proliferation and effector function[2,13]. Similarly, even in the setting of enforced constitutive glycolysis (achieved through conditional deletion of *Vhl*) a long-lived memory population does develop, albeit skewed towards an effector-memory phenotype[29]. A balanced contribution of both effector-associated glycolysis and memory-promoting metabolism such as FAO and OXPHOS is required for optimal T cell-mediated control of pathogen or tumour[30]. Skewing the response too far in either direction could hamper persistent effector function.

Texh cells do show an early reduction in GLUT1-mediated glucose uptake and early gene expression profiles consistent with glucose deprivation[18,19,31]. When TIL or Texh cells from chronic LCMV infection were exposed to conditions of glucose deprivation, they showed attenuation of both TCR and NFAT signalling with associated defects in Ca^{2+} flux, akin to those charcterising T cell anergy[31,32]. Altered levels of the glycolytic metabolite phosphoenolpyruvate (PEP)[31] in Texh also contribute to the observed metabolic and effector dysfunction and do not simply reflect secondary changes in metabolism. Glycolysis-induced PEP accumulation was identified as necessary to sustain both Ca^{2+} and NFAT signalling – and hence effector function - following TCR activation, with a loss of IFN-γ and CD40L expression when it was suppressed[31].

This same study also reminds us that cells are continually competing for metabolic substrates in their local microenvironment – and it is not just a T cells ability to take up and use a substrate that determines metabolic fitness, the competing ability of neighbouring cells may be just as important. Overexpression of the enzyme hexokinase-2 in tumour cells increased their rate of glycolysis with evidence of glucose deprivation developing in TILs as a consequence[31]. This competition for substrate has been elegantly demonstrated in the TME, where tumour cell-driven changes in the availability of glucose were reflected in a reduction in TIL effector capacity[20]. A reduction in tumour cell glycolysis may at least in part explain the reversal of TIL exhaustion and the success of 'checkpoint' inhibitory receptor blockade[20]. The degree to which such competition for substrate contributes to exhaustion in the context of either chronic infection or autoimmune responses is less clear, although is very likely to play a role.

Amino acids, survival and effector differentiation

Enhanced T cell survival in an exhausting tumour microenvironment has also been shown through increased uptake and metabolism of L-Arginine, that is usually specifically depleted during T cell activation[33]. Increased L-Arginine correlated with improved T cell survival with differentiation skewed towards the replicative, $CCR7^+$ central memory (T_{cm}) phenotype (**Fig. 2c**). Once again,

availability of the metabolic substrate controlled the response as restoring depleted L-Arginine levels improved cytotoxic capacity both in vitro and in vivo[33]. Once again, enhanced memory differentiation was associated with attenuation of effector function, in this case a reduction in IFN-γ production (although, consistent with memory differentiation, equivalent levels were produced on secondary restimulation)[33]. Cellular uptake of other amino acids (AA), including leucine[34] and glutamine[35] via the AA transporters slc7a5 and ASCT2 respectively, are important for initiation, maintenance and integration of TCR signals with downstream metabolic sensors mTOR. They have not as yet been investigated in the context of exhaustion but their expression and AA availability is likely to also play a role. The critical AA transporter CD98, a downstream target of the metabolic regulator c-Myc[36] and a heterodimer incorporating slc7a5, does show elevated expression on memory but not exhausted $CD8^+$ T cells during chronic infection[37].

Together, these data make it clear that competition for metabolic substrate within the local microenvironment is a critical factor influencing the balance of effector function and T cell persistence during exhaustion[19,20,31].

Metabolic exhaustion in different models: the same but different

The majority of studies to date consistently demonstrate metabolic dysfunction preceding exhaustion in different contexts. Furthermore, the observations are not restricted to murine models of disease. TIL from human cancer have been shown to have a similarly dysfunctional metabolic phenotype[19,38] while human $CD8^+$ T cells specific for chronic persistent (HBV)[39,40] but not acute (influenza)[40] or recurrent virus infections (CMV)[39] have been shown to have similar limitations in glucose uptake and mitochondrial biomass.

However, while bioenergetic changes of Texh appear broadly similar in both TIL and chronic viral models, there are also differences. Whereas Texh in chronic LCMV infection showed greater mitochondrial biomass and increased ROS production[18], TILs showed a reduction in both[19]. The apparent contribution of PD1 signalling to metabolic dysfunction was also different in the two contexts. Early metabolic dysfunction of Texh in LCMVcl13 infection responded to either genetic deletion of PD1 (adoptive transfer of transgenic *Pdcd1*$^{-/-}$ LCMV-specific P14 T cells) or to PDL1 blockade[18,19]. However, mitochondrial biomass was not substantially altered by PD1 blockade in TILs, even though the blockade resulted in effective tumour regression[19].

Sampling of T cells at different stages of exhaustion (a dynamic process reflecting a continuum of dysfunction[41]) and from microenvironments comprising different substrate availabilities may explain at least some of the variance observed. However, other factors may also contribute. Texh cells are known to be heterogeneous, with two principal subsets defined by both their level of PD1 expression and reciprocal expression of the transcription factors Tbet and Eomes[30]. The $Tbet^{hi}Eomes^{lo}PD1^{int}$ subset show greatest proliferative potential, are the lineage precursors of their more cytotoxic $Tbet^{lo}Eomes^{hi}PD1^{hi}$ counterparts and are the dominant subset responding to PD1-PDL1 blockade[42]**.** In LCMV, the $Tbet^{lo}Eomes^{hi}PD1^{hi}$ subset was shown to have more severe metabolic dysfunction consistent with its 'terminally exhausted' phenotype. The distribution of these subsets amongst TIL is not currently clear [19,38] but the lack

of .a metabolic response to PD1 blockade[19] suggests they are likely to be enriched for 'terminally exhausted' cells. The observation that adoptive transfer of TIL into an acute viral infection setting (VV-OVA) fails to restore their function, is consistent with it[19]. Despite this, a bioenergetic revival following PD1 blockade, such as is seen in chronic infection[18], may still occur in tumour models and contribute to revival of tumoricidal function. However, it may be occurring out with the tumour microenvironment in less terminally-exhausted T cells and consequently not be visible within it.

Exhaustion is a spectrum rather than a binary state[15] and both its extent and the nature of its induction may differ depending on the context. TILs show an exhausted phenotype only within the tumour microenvironment, remaining fully functional in the periphery of tumour-bearing mice[15,19] or in human cancer[22,38]. Indeed, TIL metabolic dysfunction could not be reproduced on co-culture of $CD8^+$ T cells with isolated tumour cells in vitro[19] indicating an important and complex role of the microenvironment in its induction. By contrast, exhaustion is apparent between different mice rather than between different anatomical locations in the same mouse[43]. Those receiving the clone 13 strain of LCMV develop chronic infection and exhaustion, while those receiving the highly related Armstrong strain have robust memory differentiation following an acute, self-terminating infection.
It is clear that T cell bioenergetics vary markedly between different tissues during infection[18,19] and also between distinct tumour models[19], highlighting the importance of context for their development and maintenance. Given evidence demonstrating the importance of local microenvironmental signals, substrate availability and the phenotype of neighbouring cell types (both immune and stromal), it is less surprising that, in different contexts, T cells come to be exhausted by different paths.

Early T cell metabolism may control exhaustion, but what controls early T cell metabolism?

Multiple lines of evidence support the assertion that metabolic pathways control the development of T cell exhaustion, although exact in vivo mechanisms remain to be fully elucidated. But what signals control those metabolic pathways early after activation? A series of elegant studies have now identified major signalling pathways controlling the balance between effector and memory differentiation. More recently, these pathways have been investigated in both TIL and chronic infection models of T cell exhaustion, indicating an important role there also.

During T cell activation and proliferation the intracellular serine/threonine kinase mTOR (mechanistic target of rapamycin) integrates diverse environmental signals including those derived from nutrient, cytokine and TCR signalling pathways[44]. Effector differentiation is favoured by TCR-triggered PI3K/Akt activity, driving mTOR-dependent[14] inactivation of the transcription factor FOXO1[45]. FOXO1 serves to repress effector function and promote memory formation by increasing expression of the transcription factor Eomesodermin and by concurrently repressing Tbet[46]. In the presence of TCR signals, such FOXO1 inactivation tips this balance towards Tbet-mediated effector differentiation (**Fig. 3**). It is also clear that many signals feed into this complex

junction, including those dependent on the local availability of metabolic substrates considered above, including hypoxia, glucose, immunometabolites such as S2HG, PEP and amino acids such as L-arginine[25,29,31,33].

FIGURE 3: metabolism integrates inhibitory and TCR signalling in exhaustion

Inhibitory receptor control: PD1 and T cell metabolism

It is now becoming apparent that early inhibitory receptor signalling in activated T cells can modulate T cell metabolism to influence the later development of exhaustion. It has long been known that T cell exhaustion is driven by high levels of sustained TCR signalling[41,47]. However recent work has shown that, despite ongoing antigen exposure and TCR triggering, downstream PI3K/AKT/mTOR signalling in exhaustion is not similarly sustained[18,48] – in other words, persistent antigen may drive exhaustion but this does not correspond to persistent downstream TCR signals. PD1 expression is induced on antigen-stimulated cells by TCR signals through NFATc[49-51]. However, PD1 ligation is capable of 'desensitising' the TCR signal, allowing a degree of ongoing nuclear FOXO1 activity that in turn sustains both further PD1 expression and ongoing TCR desensitisation[48]. This establishes a self-reinforcing cycle that restrains development of full effector function with an associated switch away from aerobic glycolytic metabolism[48,52].

These data suggest that persistent PD1 signalling actually facilitates T cell survival in the face of persistent antigenic stimulus. By desensitising TCR signals, PD1 may actually serve to maintain a degree of bioenergetic fitness in the face of persistent antigen exposure. We have seen that exhausted T cells do show evidence of metabolic dysfunction[18,19]. However, without persistent PD1 signals this could be much worse. PD1 can therefore act to facilitate an ongoing, if attenuated, T cell response in the context of persistent TCR activation[48]. This system is complex, however – genetic deletion of PD1 can counterintuitively allow the later accumulation of 'terminally exhausted' cells (*Pdcd1*$^{-/-}$)[53]. However, the details of how PD1 facilitates survival with attenuated function remain unclear and its effects are likely to vary with the time post stimulation (early versus late, see discussion of PGC1a below).

This role for PD1 in T cell differentiation also makes evolutionary sense. It is likely that exhaustion has evolved as a means of carefully balancing control of chronic infection with the risk of causing damaging immunopathology[17]. During acute T cell activation, a feedback loop is established whereby strong TCR signals drive Akt -mediated cytotoxicity (**Fig. 3**) but also drive PD1 expression, limiting that TCR signalling. Should TCR signals persist, they will be desensitised by PD1, allowing preservation of bioenergetic fitness sufficient to sustain a longer-term, albeit more restrained, T cell response.

These new insights into metabolic control of T cell exhaustion have important implications for the design of therapies aimed at its modulation. This schema may also initially seem at odds with observations that PD1 blockade can promote restoration of cytotoxicity in both TILs[16] and in chronic viral infection[54]. However, PD1-driven desensitisation of TCR signals and preservation of some metabolic fitness serves to keep T cells in the fight – the response to persistent antigen is, after all, more of a marathon than a sprint. Reversing exhaustion

through inhibitory receptor blockade shows much promise as a therapeutic approach in chronic infection or cancer[23,54,55]. PD1 blockade can clearly induce increased cytotoxicity of exhausted $CD8^+$ T cells. However, if this occurs through promoting 'terminal' differentiation of more cytotoxic $Tbet^{lo}Eomes^{hi}PD1^{hi}$ cells at the expense of a more durable and metabolically-fit $Tbet^{hi}Eomes^{lo}PD1^{int}$ population[30,53], such therapy could effectively undercut the immune systems capacity to maintain an ongoing response. The predicted result would be eradication of the targeted antigen (whether tumour or virus) in some cases where induced cytoxicity was sufficient, but perhaps a 'deeper' state of subsequent exhaustion where it isn't and residual metabolic fitness was lost through inhibitory receptor blockade.

What is less clear at this stage is exactly how this perpetuating cycle is established in the first place. While the microenvironmental signals discussed above are important, costimulatory signalling is very likely to also contribute. A lack of costimulatory signals during TCR triggering results in a hyporesponsive state termed anergy[32]. Costimulatory signals, such as those received through CD28 binding[56,57] or cytokines such as IL-2 (ref [58]), are critical for upregulation of glucose, AA transporters and the FAO regulator CPT1a that facilitate the metabolic switch underlying T cell activation[13,57]. A failure of such initial costimulation results in comparative metabolic quiescence and anergy[59]. Exhaustion is distinct from anergy: T cells have received sufficient stimulatory signals to activate, but they continue to receive them and become dysfunctional. However, inadequate costimulation (rather than absent costimulation as in anergy) also contributes to development of exhaustion[60,61] and is likely to promote the early PD1-driven cycle of bioenergetic compromise promoting later exhaustion (**Fig. 3**).

Other inhibitory receptors

Just as the precise nature of metabolic dysfunction of exhausted cells appears different in different contexts (see above), so the mechanism by which it develops may also be distinct. A range of coinhibitory receptors are highly expressed on exhausted T cells and play distinct but synergistic roles in driving that phenotype[49]. Mitochondrial dysfunction of TILs is apparent in the tumour microenvironment of mutliple mouse models[19,20,31] and in human cancers[19,38]. However, the degree of metabolic dysfunction does not directly parallel the concurrent level of inhibitory receptor expression[19,26] in each context. Exhaustion can clearly develop in the absence of specific inhibitory receptor expression[18,53] and inhibitory receptor expression can be present without the development of exhaustion, for example where Vhl/Hif signals are absent[25]. Further, whereas metabolic changes are important drivers of exhaustion early during TCR signalling, exhaustion can be at least partially reversed at later stages with increased cytotoxicity despite no clear modulation of metabolism[18,19]. Together, these data suggest that multiple, redundant signals must contribute to the development of exhaustion and its associated metabolic dysfunction. The summated input from these cell surface and local environmental signals contribute to the resulting degree of exhaustion.

mTOR and metabolism in exhaustion

During T cell activation mTOR acts to integrate multiple signals including from metabolic, cytokine and TCR signalling pathways[44]. Perhaps unsurprisingly, mTOR activity has therefore been shown to have a central role in T cell metabolism and, more recently, in exhaustion. It was, however, initially a surprise when the immunosuppressive drug rapamycin, an inhibitor of mTOR, was found to promote T cell memory differentiation and the associated switch away from aerobic glycolysis towards FAO metabolism[8,14]. It has been used for some time as an immunosuppressive agent capable of limiting TCR-driven activation and proliferation. However, mTOR has since been shown to directly promote phosphorylation and inactivation of FOXO1, promoting effector differentiation through downstream Tbet-mediated effects[45,46,62,63] .
Early mTOR blockade in the LCMV-cl13 chronic viral infection model also modestly improved mitochondrial fitness of exhausted cells (depolarisation and size[18]) suggesting that chronic mTOR signals downstream of the TCR can contribute to the metabolic changes of exhaustion, even if TCR signals are downtuned by PD1. It has been observed that blocking mTOR actually impairs TIL response to PD1 blockade, suggesting that ongoing mTOR signalling to at least some degree may be needed to preserve effector function[18,19].

The evidence for mTOR involvement in the metabolic phenotype of exhaustion highlights the complex, dynamic balance between 'terminal' effector function and T cell persistence. While mTOR signalling may in part drive the cell away from mitochondrial metabolism toward anaerobic glycolysis, these changes are a necessary part of effector differentiation. Both are required for adequate ongoing T cell responses to persistent antigen and the degree to which either path is inhibited impacts on the resulting phenotype[30].

FOXO signals and exhaustion: death, survival and metabolism

FOXO transcription factors integrate signals from growth factors, substrate availability and inflammation and in turn regulate cellular growth, differentiation and metabolism[64]. The balance of FOXO signalling is clearly important in directing $CD4^+$ regulatory T cell differentiation[65,66], however this balance also plays an important cell-intrinsic role in $CD8^+$ T cell differentiation and in directing metabolic dysfunction of exhaustion. FOXOs 1 and 3 are expressed in T cells[64]. FOXO1 activity promotes T cell survival and persistence[67,68] while FOXO3 activity limits survival through pro-apoptotic effects mediated by Bim and PUMA[69-71]. Ablating FOXO3 activity (*Foxo3*$^{-/-}$ $CD8^+$ T cells) restricts the contraction phase post-stimulation allowing greater persistence of effector $CD8^+$ T cells[72], facilitating clearance of chronic LCMV infection[73] and control of HIV in humans[74]. By contrast, FOXO1 signalling can maintain a pluripotent stem cell phenotype[75] and is required for differentiation of effector $CD8^+$ T cells into a functional memory subset[76] through its downstream modulation of the transcription factors Tbet and Eomes[46]. It is therefore likely that the balance of FOXO signalling following TCR signals will determine the balance of effector fate and cellular persistence during chronic antigen exposure.
However, the balance of FOXO-driven regulation is complex[64] and a complete picture of its contribution to the metabolic dysfunction of exhaustion has yet to emerge with several apparent contradictions persisting. For example, the role of FOXO3 in promoting apoptosis and effector differentiation is at odds with its

apparent ability to maintain PGC1a expression and metabolic fitness (described below). Similarly, despite its role in promoting stem cell-like persistence, FOXO1 also appears to be necessary for differentiation of the terminally exhausted $Tbet^{lo}Eomes^{hi}PD1^{hi}$ subset[48]. These apparent discrepancies may reflect the complex integration by FOXOs of multiple other pathways including hypoxic signalling[77], metabolic pathways[78] and nutrient availability[79]. A future challenge will be to fully understand the complex role of these transcription factors in regulating metabolism during exhaustion.

PGC1a: regulation at the heart of metabolic exhaustion?

The ability to sustain energy production during rapid cellular proliferation requires mitochondrial biogenesis and the induction of gene expression programmes regulating substrate uptake and use. This process is controlled by the transcriptional coactivator PGC1a[80] through control of both nuclear respiratory factors (Nrf) and mitochondrial transcription factor A (Tfam)[81]. It is now clear that PGC1a is central to the metabolic dysfunction of exhaustion in both LCMVand tumour models. PGC1a shows reduced levels of expression in both TIL[19] and Texh in the LCMV model[18], while its overexpression limits mitochondrial dysfunction [18,19], facilitating the emergence of polyfunctional CD8 T cells[18] that drive tumour infiltration and regression[19].

The FOXO family of transcription factors - both FOXO1[19,82] and FOXO3[19,83] - have each been shown to promote PGC1a expression. Conversely, PGC1a expression is repressed by TCR-driven Akt signalling[82] which in turn phosphorylates and inactivates FOXOs 1 and 3[81]. Therefore persistent antigen/TCR signalling may serve to maintain Akt signalling and promote effector differentiation, but metabolic fitness will also be curtailed as FOXO-driven PGC1a expression is restricted (**Fig. 3**).

It remains unclear, however, exactly how complex signals from multiple inhibitory receptors including PD1 are integrated to modulate PGC1a levels and whether this is the only or the most important transcription factor modulating early metabolic fitness. Absence of PD1 from the onset of a T cell response (through genetic deletion, *Pdcd1*-/-) facilitates a later expansion of exhausted cells in the absence of inhibitory signals restraining cytotoxicity and terminal differentiation[53]. This demonstrates that PD1 is not essential for the development of exhaustion and its expression at certain points may even limit its development. Genetic ablation of PD1 also results in higher expression of PGC1a[18] suggesting that, if PD1 is acting to preserve a degree of early bioenergetic fitness and to facilitate later survival despite ongoing stimulation, it is unlikely to be doing so through PGC1a. Other data showing delayed tumour progression after PD-1 blockade in the absence of metabolic changes in TIL also suggest additional mechanisms[27]. Further studies will be required to clarify the complexity of these signalling events.

Autoimmune disease, metabolism and exhaustion

T cell exhaustion regulates cellular immunity during the response to persistent antigenic stimulation, whether that is during chronic viral infection or in the tumour microenvironment. Exhaustion has also been inversely associated with a relapsing course of autoinflammatory disease, another instance of immune reactivity to persistent antigen (in this case, 'self'). A transcriptional signature

specific for exhaustion correlates with a reduced rate of relapse in multiple diseases[61] and features of T cell exhaustion correlate with favourable response during immunotherapy of type 1 diabetes[84]. Such observations are consistent with the evolution of exhaustion as a mechanism to limit immunopathology in the face of persistent TCR stimulation[17,41]. A broadly similar phenotype of exhaustion is apparent in the distinct contexts of chronic viral infection and the tumour microenvironment. These broad similarities are accompanied by specific differences, too, likely driven by distinct signals received in each context. It is likely that immunometabolism also plays a key role in control of effector, persistent or exhausted T cell responses in autoimmunity.

It is clear that metabolic 'switches' are not only important for the initiation of T cell activation[13] but are also responsible for controlling an ongoing balance of effector/memory differentiation and exhaustion[18,19]. Many studies have sought to directly compare measures of metabolic fitness in autoinflammatory disease, typically making comparison with uninflamed, healthy control tissues and sampling cells during or after active autoinflammation and immunosuppressive therapy[85]. In this context it is unsurprising to find altered metabolism as a secondary consequence of immune activation or therapeutic modulation – indeed it would be surprising not to find it. This highlights the need for appropriate control for variable proliferation and immune activation in models and assays of immunometabolism, such as those used during LCMV models (where adoptive transfer of small numbers of transgenic, LCMV-specific P14 T cells facilitates their direct comparison with host cells in an equivalent context[18,53]). Evidence to support a primary role of exhaustion-associated metabolic dysfunction in autoinflammatory disease is more limited.

Several recent genetic studies have, however, implicated exhaustion-associated metabolic pathways in the onset and progression of multiple autoimmune phenotypes. Perhaps the most striking example is the recurrent association of a variant allele in neutrophil cytosolic factor 1 (Ncf1) with onset or severity of multiple autoinflammatory diseases[86]. Ncf1 is a necessary for activation of NADPH oxidase and consequent production of superoxide during the respiratory burst. Reduction in ROS levels in the presence of a mutant allele leads to increased severity and relapse in mouse models of multiple sclerosis and rheumatoid arthritis[86,87] and shows association with multiple human autoimmune diseases[86]. Redox signalling can be controlled by PD1 signalling in T cells[88] and ROS generation has been implicated in the metabolic dysfunction of exhaustion[18,19]. However, a direct link between NCF1 function and exhaustion remains to be tested.

The process of T cell exhaustion has thus far been associated with the progression rather than onset of autoinflammatory disease[61,84], although exhaustion-associated inhibitory receptors have also been linked to broken tolerance[17]. A genome-wide association study of clinical outcome has identified a variant allele in FOXO3 associated with disease progression (but not susceptibility) in Crohn's disease, rheumatoid arthritis and malaria infection[89]. Alongside the association of T cell exhaustion with outcome in Crohn's disease[61] this suggests a possible role for FOXO3-driven metabolic control of T cell exhaustion in autoinflammatory disease. However, FOXO3 the Crohn's associated

variant allele has not as yet been functionally tested in T cells and a monocyte-intrinsic signalling pathway directing TGF-β and TNF production is at least partly responsible for the association[90].

Summary

During T cell activation changes in cellular metabolism are inevitably required to sustain the rapid changes in cell function. Increasing evidence suggests that metabolic changes do not simply react to changes in T cell differentiation but drive them. It is now apparent that the integration of complex early signals from a cells microenvironment is critical both for initiating activation and for regulating the nature of 'downstream' differentiation. These signals direct phenotypes of effector function and memory development, but also of T cell exhaustion during chronic antigen exposure.

In both chronic infection and tumour models where exhaustion is apparent, metabolic changes in T cells occurred rapidly, prior to the emergence of exhaustion *per se*, and were maintained into later stages. Multiple observations therefore confirm a prominent role for cellular metabolism in 'upstream' control of exhaustion rather than simply reflecting changes secondary to its development.

A wealth of evidence also indicates that signalling pathways promoting cellular persistence/survival can be dissociated from those controlling effector function. Signals promoting aerobic glycolysis during activation may enhance effector differentiation but this may be at the expense of memory potential. Conversely, the use of alternative pathways such as lipid metabolism at the expense of aerobic glycolysis may do the opposite. However, both processes are required in parallel to appropriately mount an effective, durable T cell response to persistent antigen[30]. As such, the metabolic balance between effector function and persistence represents another example of the 'Goldilocks' principle, in which effector and memory differentiation must be neither too much nor too little, but 'just right' in order to appropriately deal with infection without the development of immunopathology. Future challenges will include quantifying the extent to which each metabolic pathway contributes to T cell function, how each is controlled and how we might harness that knowledge to modulate metabolism and hence immunity. To achieve this, our understanding of T cell metabolism during human disease will have to catch up with the advances driven by informative mouse models of cancer and infection. However, modulating immunometabolism shows promise as a means of controlling chronic T cell responses in infection, cancer and autoinflammatory disease.

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