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Immuno-Oncology Crosstalk and Metabolism

Springer

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ISBN 978-981-16-6225-6 ISBN 978-981-16-6226-3 (eBook)
https://doi.org/10.1007/978-981-16-6226-3

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Acknowledgments

We would like to thank all the contributors, the publishing team, colleagues, and mentors for the successful completion of this book. Editing a book is harder than we thought and more rewarding than we could have ever imagined. None of this would have been possible without the contributors who worked hard in coming up with interesting book chapters and the publishing team that gave a final shape to this book. We want to thank EVERYONE who ever said anything positive to us or taught us something. We heard it all, and it meant something. Finally, we want to thank our families for their constant support.

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Immune Cell Metabolism and Function

1

Ajay Dixit and Mahendra Singh

Abstract

Immune cells are highly dynamic by nature and rely on metabolism to adapt to different conditions. Many recent studies have shown the importance of immune cells and their metabolism in the pathogenesis of many diseases. Cellular metabolism acts as a guiding force to regulate immune cell activation, differentiation, and cellular behavior, thus regulating the extent of the immune response. Here in this chapter, we have discussed different metabolic signatures and pathways that control the activation status of immune cells and how the change in the metabolic status affects the immune response, disease pathobiology, and homeostasis especially in cancer.

Keywords

Metabolism · Immune-metabolite · Glycolysis · Lipids · Amino acids · Redox

1.1 Introduction

Immune cell metabolism refers to the study of changes in intracellular metabolic pathways that take place in immune cells during the process of immune activation that results in the alteration of their function. Recent studies of metabolic pathways in immune cells particularly in the last several years have clearly demonstrated a complex interplay between immunity and metabolic reprogramming, which presents

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M. A. Macha et al. (eds.), *Immuno-Oncology Crosstalk and Metabolism*,
https://doi.org/10.1007/978-981-16-6226-3_1

an extra layer of complexity for us to understand the role of the immune system in health and disease. Most relevant metabolic pathways to the immune cells are glycolysis, tricarboxylic acid (TCA) cycle, pentose phosphate pathway, fatty acid synthesis, fatty acid oxidation, and amino acid. Immune cells have characteristic metabolic pathways which are specific their lineage and phenotype. For example, fatty acid synthesis and glycolysis are key features of lipopolysaccharide (LPS)-activated macrophages, while interleukin-4 (IL-4)-activated macrophages primarily depend on the use of oxidative phosphorylation and fatty acid oxidation to generate energy. Similarly, T cells have their own characteristic metabolic pathways: for example, memory T cells are characterized by oxidative metabolism, where as effector T cells are highly glycolytic in nature.

Metabolism of these immune cells is so important that enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), pyruvate kinase isoenzyme M2 (PKM2), and enolase as well metabolites like succinate and citrate have important role in promoting specific event in immune cell activation process. Recent advances in the field of immunotherapies especially in the area of immune oncology have provided a body of evidence suggesting that small molecules that can target metabolic pathways and have potential to alter the phenotypes of immune cells, are now being studied and developed as possible therapeutic intervention strategies.

Interestingly, Early discoveries of cellular metabolic pathways were historically conducted on cells of lymphoid origin such as lymphocytes. Usually when lymphocytes prepare themselves for an immune response they undergo a high degree of activation and proliferation. However, the characterization of energy metabolism of other cell types such as myeloid cells and natural killer (NK) cells has also received an increasing amount of attention. A large number of studies have demonstrated that myeloid cells undergo unique metabolic reprogramming after stimulation of the Toll-like receptor 4 (TLR4) using LPS [1, 2].

Myeloid cells undergo a switch from oxidative phosphorylation to glycolysis despite the abundance of oxygen. It is important to note that activated lymphocytes also exhibit similar metabolic signature. These observations prompted researchers to hypothesize that activated myeloid cells exhibit a general characteristic feature such as “Warburg effect” (aerobic glycolysis) during the induction of innate immunity [3, 4].

Myeloid cells are one of the major stromal populations in the tumor microenvironment (TME) and comprised of macrophages, myeloid-derived suppressive cells (MDSCs), and dendritic cells (DC) [5–7]. Myeloid cells support the development and maintenance of immunosuppressive microenvironment in various cancers for which these cells have to undergo necessary metabolic adaptations and [8, 9] and due to the fact that myeloid cells play an important role in the activation of both adaptive and innate immune responses, we will first discuss the metabolic diversity underlying the myeloid mediated immune responses while specifically emphasizing how change metabolism alters myeloid cells-generated immune response.

1.2 Macrophages

Macrophages are found in almost every tissue type and are crucial for maintaining immunity and homeostasis [10]. As the name suggests they are the *big eaters*; they detect, engulf, and destroy pathogens. Additionally, they act as specialized antigen-presenting cells where they present the digested antigens on MHC II to activated adaptive immunity. Interstingly, Macrophages are naturally highly plastic in nature and can change their phonotype based on the cues in the tissue microenvironment and can originate in tissues either from yolk sac during gestation period ex: tissue resident macrophages [11] or bone marrow ex: monocytes derived macrophages, which accumulates in tissues during inflammation via CCL-2/CCR-2 pathways [12]. Of note, Tissue-resident macrophages can maintained by self-replication and are longed lived whereas monocyte-derived macrophages are terminally differentiated and short lived [13, 14].

Phenotypically they are traditionally classified into two major types: M1 and M2. M1 macrophages known also as classically activated and are activated by IFN-γg and/or LPS, known for their proinflammatory nature while M2 macrophages also known as alternatively activated and are obtained by activating by interleukin (IL)-4 and IL-10 and have immunosuppressive and wound-healing functions [15, 16]. However, many recent studies have challenged this over -simplistic dichotomous classification and suggests that macrophages display rather broad spectrum of phenotype representing various activation stages which may not fit in this dichotomous M1 and M2 phenotypic classification [17, 18]. Future studies are required to understand how these diverse macrophages effects disease progression and outcome.

While the role of macrophages in adaptive and innate immunity has been known for over a century and macrophage biology is an extensive area of research in many pathological conditions such as atherosclerosis [19], diabetes [20], and pancreatitis [21], their role in cancer biology has been recognized more recently as much as that macrophages are considered to be the seventh hallmark of the cancer [22]. This chapter mainly focuses on macrophage metabolism in tumors. Macrophages are considered as the largest myeloid cell population that are known to infiltrate many types of solid tumors [6] which is why they are also commonly referred as tumor-associated macrophages or TAMs. Almost all stages of tumorigenesis like initiation, progression, immunosuppression, metastasis, as well as resistance to therapies are known to involved TAMs [23–28]; hence, it is an active area of research in immune oncology [29]. TME is rich in metabolite which are generated as result of myriad of biochemical pathways operating in cancer and stromal cells. TAMs compete with other cell types especially cancer cells for available metabolites and thus are forced to reprogram their metabolism in order to survive and maintain their phenotype. Although many biochemical pathways and metabolites posses the ability to modulate immunity but for simplicity sake we have focused on major pathways/metabolite as consequences of carbohydrates, amino acid and lipid metabolism.

1.2.1 Carbohydrate Metabolism

Glycolysis is one of the most simple metabolic pathways where glucose is converted into pyruvate and generates two molecules of ATP in the process. Besides ATP, glycolysis also produces several intermediate metabolites required for ribose, amino acids, and fatty acids metabolism supporting cells' basic needs. The pyruvate generated at the end of glycolysis usually enters mitochondria and participates in oxidative phosphorylation to produce more ATP molecules.

Macrophage plasticity is supported by metabolic shift between glycolysis and oxidative phosphorylation [30]. Proinflammatory M1 macrophages show high dependency on glycolysis [31] whereas M2 cells with anti-inflammatory functions are more dependent on oxidative phosphorylation (OXPHOS) [32] suggesting that a shift between different glucose metabolic pathways is necessary to support a particular phenotype. Most tumors are hypoxic in nature in which Hif-1a, a transcription factor central to hypoxic response in all types of cells, plays an important regulatory role. Hif-1a regulates many key enzymes involved in glucose metabolism like pyruvate dehydrogenase kinase 1 (Pdk1), glucose transporter 1 (Glut1), phosphoglycerate kinase 1 (Pgk1), glucokinase (Gck), lactate dehydrogenase, and pyruvate kinase isozymes M2 (Pkm2) [33]. The upregulation of enzyme Glut1 plays a crucial role in supporting the glycolytic activity of M1 macrophages [34]. Besides, there are more such enzymes which play important roles in all this, for example lactate dehydrogenase which converts pyruvate to lactate and takes it away from mitochondria, and pyruvate dehydrogenase kinase which is known for inactivating pyruvate dehydrogenase and limiting pyruvate entry into the Krebs cycle, thereby reducing OXPHOS. Further, the conversion of pyruvate into lactate is very important for maintaining NAD+ levels and its flux through the glycolytic pathway.

Another important pathway is pentose phosphate pathway (PPP) which is involved in maintaining M1 phenotype. PPP is important for maintaining NADPH pool inside the cell. The oxidative phase of PPP converts $NADP^+$ to NADPH., Enzyme NADPH oxidase utilizes NADPH to generate reactive oxygen species (ROS). ROS regulates several functions in macrophages including but not limited to phagocytosis, bacterial killing, and polarization [35]. Mitochondrial ROS also help in the secretion of cytokines like TNF-α, IL-6, and IL-1β. In a recent study led by Bulua et al., it has been demonstrated that defects in mitochondrial ROS result in reduction in inflammatory cytokine production after LPS stimulation suggesting a role for ROS in maintaining inflammatory phenotype. Unsurprisingly, M2 macrophages have lower capacity to generate ROS [36].

The deletion of gene encoding 6-phosphogluconate dehydrogenase (PGD), which converts 6-phosphogluconate into ribulose 5-P, is known to reduce proinflammatory response in macrophages in a medical condition called hypercholesterolemia indicating the important role of PPP [37]. In a study by Haschemi et al. [38], it has been shown that carbohydrate kinase-like protein, CARKL, catalyzes the conversion of sedoheptulose into sedoheptulose-7-phosphate which is an orphan reaction in the PPP. This is how CARKL helps the refocusing of cellular metabolism to a high redox state upon physiological or artificial downregulation. These investigators also

reported that CARKL-dependent metabolic reprogramming is required for both M1- and M2-like macrophage polarization. Overexpression of CARKL in macrophages results in defective M1 polarization as well as dampened inflammatory response [38]. CARKL promotes the non-oxidative steps in the pentose phosphate pathway, which can in turn lead to increased ribose-5P production, required for nucleotide and UDP-GlcNAC synthesis. UDP-GlcNAC is essential for the process of N-glycosylation of the key M2-specific proteins such as CD206, which is abundantly expressed on the surface of M2 macrophages. M2 macrophages express higher levels of the glycolytic enzyme 6-phosphofructo-2-kinase B1 (PFKFB1), which breaks down fructose-2,6-bisphosphate [39], an activator of glycolysis to fructose-6-phosphate, resulting in reduced glycolytic rate.

1.2.2 Amino Acid Metabolism

Both M1 and M2 macrophages utilize arginine metabolism differently [40]. M1 macrophages metabolize L-arginine into nitric oxide (NO) and L-citrulline. The NO generated in this process has tumoricidal properties. On the other hand, M2 macrophages upregulate ARG1, which catalyzes the conversion of L-arg to L-ornithine, and polyamine synthesis [41]. Polyamines are known to support tumor growth [42].

ARG1 is a urea cycle enzyme that has long been known as a marker of alternatively activated macrophages. ARG1 converts L-arginine into ornithine and urea. Even though the function of macrophage-derived ARG1 is not fully well understood, recently a large number of reports have emerged which indicate that in hypoxic conditions lactate can mount the expression of the ARG1 gene [43, 44]. A genetically engineered mouse model in which ARG1 has been knocked out in their macrophages develops significantly smaller tumors than their wild-type counterparts, suggesting that tumor progression can be influenced by macrophage-derived ARG1 [45]. Interestingly, in the same study it was shown that TAMs have increased expression of all enzymes from the urea nitrogen cycle, as compared to the tumor cells.

In another study, TAMs either isolated from glioblastomas or co-cultured with cell lines derived from glioblastoma were reported to have increased expression of genes pertaining to glutamate transport and its metabolism. This is of particular importance because this shows that glioblastoma tumor microenvironment contains large amounts of glutamate [46]. Furthermore, tryptophan metabolism, L-arginine-derived metabolites, and cysteine/cysteine play important roles by mediating the immunosuppressive activity of MDSCs. Therefore, production of high levels of NO may also help mediate the immunosuppression of MDSCs [1].

1.2.3 Lipid Metabolism

Lipids play critical roles during macrophage polarization [47] and metabolic reprogramming in macrophages is linked to their activation. M1 macrophages can kill pathogens by sustaining inflammatory responses, mediated by their reliance on aerobic glycolysis and fatty acid biosynthesis. While glycolysis is a way of producing ATP at a faster pace in the M1 macrophages, but fatty acids act as precursors for the synthesis of inflammatory mediators in the M1 macrophages. On the contrary, anti-inflammatory M2 macrophages mediate the resolution of inflammation and tissue repair, switching their metabolism to fatty acid oxidation and oxidative phosphorylation. However, discoveries in recent years have challenged this classical view and suggest towards a rather complex metabolic network during macrophage activation. It has been shown that lipid metabolism plays a critical role in the activation of both M1 and M2 macrophages. A body of work demonstrates that inflammasome activation in M1 macrophages essentially occurs owing to the fatty acid oxidation while glycolysis plays a crucial role in fueling fatty acid oxidation in M2 macrophages [48].

However, metabolism of macrophages during activation is way too more complex than it has been thought; therefore, in order to unravel the metabolic signature of macrophages more studies are needed. It is important to note that most studies in this regard have been conducted in mouse models while many differences exist between human and murine macrophages in terms of gene expression signatures and corresponding metabolic pathways activated during polarization. This makes the extrapolation of these research findings difficult from mouse models to human subjects, especially in order to determine as to whether or not the reprogramming of macrophage polarization by metabolic interventions would be helpful in the treatment of human diseases [1].

Environmental cues are also crucial in order to determine the course of cell metabolism in macrophages. This is particularly important for those tissue macrophages which encounter a specific set of environmental signals. Furthermore, the presence of chronic inflammation may alter the tissue microenvironment, not only in promoting the influx of macrophages but also in affecting the metabolic signature of resident macrophages. Therefore, the tissue microenvironment plays an important role in determining the chronicity or severity of various diseases characterized by inflammation. For example, recent observations made in tumor-associated macrophages (TAM) have shown that the metabolic routes used by TAMs are greatly influenced by tumor cell-derived compounds such as lactate, which instigates proinflammatory reprogramming and prompting tumor angiogenesis [49]. Recent studies indicate that in addition to TAMs, tissue microenvironment-dependent metabolic rewiring of immune cells accumulating in the vessel wall or in the joints promotes inflammation and disease progression in certain diseases such as atherosclerosis and rheumatoid arthritis [50, 51]. There is a great body of evidence that suggests that targeting lipid metabolism in macrophages might improve the outcome of metabolic diseases as well as it could be a key to therapeutic strategies in tumor tissues [52–54].

1.2.4 Others

In a recent study, an age-related increase in the production of lipid messenger prostaglandin E2(PGE2) was observed in the mouse model of Alzheimer's disease. PGE2 binds to receptor protein EP2 on the cells, which in turn results in suppression of oxidative phosphorylation and glycolysis pushing macrophages into an energy-deficient state limiting the beneficial functions of macrophages and increase in inflammation. Inhibition of EP2 restores the function of macrophages and protects the aging brain [55].

1.3 Myeloid-Derived Suppressor Cells (MDSCs)

Myeloid-derived suppressor cells are a group of immature myeloid cells which are highly heterogenous in nature that possess potent immune-suppressive properties. MDSCs are comprised of two major subsets: polymorphonuclear (PMN)-MDSCs and monocytic (M)-MDSCs. Phenotypically, murine PMN-MDSCs are characterized as CD11b+Ly6ClowLy6G+, while M-MDSCs are CD11b +Ly6ChiLy6G−. Human PMN-MDSCs are instead defined as HLA-DRlow/− CD14−CD11b+CD15+, while M-MDSCs as CD11b+CD14+HLA−DRlow/− CD15− [56, 57]. However, it is difficult to discriminate proinflammatory cells such as neutrophils and inflammatory monocytes from immunosuppressive PMN- and M-MDSCs as all these cells share cellular origin and phenotypic markers.

Suppression of antitumor immunity by MDSCs is well established [58, 59]. In the patients with cancers, MDSCs' occurrence is regulated by a network of transcriptional regulators which promote immature and immunosuppressive activation of myeloid cells. STAT3 was the first transcription factor that was characterized for its capability to drive MDSC expansion and accumulation [60]. NF-κB and JAK/STAT signaling is known to upregulate immunosuppressive molecules such as inducible nitric oxide synthase (iNOS) (in M-MDSCs), arginase 1 (ARG1) (in PMN-MDSCs), and reactive oxygen species (ROS) [61]. Several cytokines and chemokines (e.g., G-CSF, CCL2, GM-CSF, CXCL1) induce the mobilization of MDSCs from bone marrow to the peripheral lymphoid organs and to the TME [62], where they promote tumor immune evasion. Further, MDSCs are known to express higher level of PD-L1 which inhibits T-cell activity [63, 64]. The role of AMPK in regulating MDSC immunosuppressive functions has also been reported. It has been shown that increased level of phosphorylation and activation of adenosine monophosphate-activated protein kinase leading to reduction in NO production also reduce IL-6 levels and inhibit MDSC migration suggesting that AMPK can be a potential drug target to reduce immunosuppressive behavior of MDSCs in tumors [65].

1.3.1 Carbohydrate Metabolism

MDSCs have been demonstrated to exhibit the Warburg effect while they are going through their maturation with high glucose and glutamine uptake rates as well as reduction in their oxygen consumption rate (OCR). Approximately 95% of the total ATP generated in the MDSCs is obtained through a glycolysis-dependent mechanism [66]. The metabolic reprogramming of cancer cells such as the use of aerobic glycolysis (the Warburg effect) affects the tumor microenvironment and infiltrating immune cells through changes in glucose metabolism. M-MDSCs are known to get differentiated into M1- or M2-like TAMs and to TNF-α and inducible nitric oxide synthase (iNOS)-producing dendritic cells (DCs) in the tumor microenvironment, while monocytes also convert into monocytic-MDSCs (M-MDSCs) [67]. While going through maturation and activation, tumor-derived MDSCs exhibit an increase in central carbon metabolism, including glycolysis, PPP, and TCA cycle. Granulocytic-MDSCs (G-MDSCs) have also been demonstrated to utilize both glycolysis and OXPHOS in mouse models of various types of cancers [68].

Tumor-derived MDSCs show upregulation in the glycolysis, and its metabolite produced during this process known as phosphoenolpyruvate could protect MDSCs from apoptosis and contribute to their survival [66]. Owing to the high uptake rates of glucose in both tumor cells and MDSCs, immune cells do not have any metabolic plasticity in order to acclimatize to the condition of low oxygen tension and limited glucose availability, which could result in immune cell dysfunction and death, indirectly facilitating tumor escape and progression.

In addition to glycolysis, another metabolic activity in these cells which is known as glutaminolysis plays an important role in ensuring an adequate supply of intermediates and energy during tumor progression. A recently conducted study demonstrates that glutaminolysis supports the maturation and immunosuppressive function of MDSCs through iNOS activity in vitro [69]. Thus, a growing body of studies now point to the high metabolic plasticity of immune cells, which can change their differentiation and function according to the context required.

1.3.2 Amino Acid Metabolism

In TME, MDSCs in the presence of IFN-γ show higher uptake of L-Arg by inducing the cationic amino acid transporter 2 (CAT2), iNOS, and ARG1. Further, depletion of L-Arg by G-MDSCs blocks CD3zeta expression in T cells leading to the inhibition of antigen-specific T-cell proliferation. Ablation of CAT2 impairs L-Arg uptake and reduces immunosuppressive and pro-tumoral activities of MDSCs [70]. MDSCs are known to help sequester L-cysteine, thereby causing its deprivation in the tumor microenvironment. L-cysteine deprivation also decreases the expression of CD3zeta and inhibits T-cell proliferation. Thus, MDSCs can effectively block the activation of T cells by sequestering cysteine, as T cells lack the cystathionase required to convert methionine to cysteine [71]. Metabolites derived from L-Arg metabolism

such as tryptophan and cysteine play an important role in regulating the immunosuppressive activity of MDSCs [72].

1.3.3 Lipid Metabolism

A subset of MDSCs that infiltrate tumors undergo both metabolic and functional reprogramming to become highly immunosuppressive cells so as they could support tumor growth. MDSCs express lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) that in turn enables these cells to specifically associate with endoplasmic reticulum (ER) stress and lipid metabolism, which possess potent immunosuppressive activity promoting T-cell-suppressive functions [72, 73]. Further, high PPAR-γ activity restrains ROS production in G-MDSCs [74], thereby helping in the processes of impairing cancer cell proliferation and metastasis.

1.4 Dendritic Cells

Dendritic cells (DCs) are a subtype of antigen-presenting cells which undertake a job on capturing antigens coming from tumors or pathogens and presenting these antigens to the T cells so as to invoke immune response. Cellular processes such as the development, polarization, and maturation of DCs are controlled by the metabolism of DCs as metabolic pathways provide energy support for these cellular processes as well as for the functions of DCs [75]. However, the immune functions of DCs in tumor microenvironment (TME) are generally inhibited. Abnormal metabolism of tumor cells results in metabolic changes in TME, such as hyperglycolysis, lactate and lipid accumulation, acidification, and tryptophan deprivation, leading to the limited function of DCs and occurrence of tumor immune escape [76]. Metabolic regulation of DCs in combination with immunotherapy can strengthen the ability of antigen presentation and T-cell activation of DCs, improve the existing antitumor therapy, and overcome the defects underlying DC-related therapies in the current stage, which has great potential in oncology-focused therapies.

Glucose is vital for migration of DCs to C-C motif chemokine ligand 21 (CCL21). Blocking glycolysis results in the destruction of the optimal migration of DCs to the draining lymph nodes. Activated form of DCs depends on glycolysis and PPP to maintain their energy production and membrane integrity; they also provide elements for the generation of an inflammatory mediator, and sustain their ability to migrate [77]. Inhibition of glycolytic pathway impairs various functions of DCs including antigen presentation, T-cell stimulation, and cytokine production [78]. Surface of DCs does have MHC II proteins; upregulation in the expression of MHC II on the surface of DCs requires molecule redistribution of endocytic compartments via lysosome tubulation [79], which also needs energy support.

Fatty acid metabolism has been shown to be critically involved in the development, maturation, and function of the DC [80]. Because of its integration with

mitochondrial function, the fatty acid synthesis (FAS) affects the derivation of DCs, which can not only block monocyte-derived DC formation from human PBMCs but also prevent the generation of DCs in primary and secondary lymphoid organs. FAS also helps decrease the expression of MHC II leading to the increased CD40 expression on the DC surface. Further, oxidized lipoproteins can accumulate in the tumor-resident dendritic cells via scavenger receptor-mediated internalization where they form lipid droplets [81, 82]. Unfortunately, no effective way has been developed as of now to modify metabolic pathways in the DCs. However, there is a great potential for new therapies in this direction. Future studies so as to understand metabolic pathways in the DCs may provide new insights into the more effective treatment of tumors.

1.5 T Cells

The ability of T cells especially CD8 T cytotoxic T cell to detect and eliminate neoplastic cells in the body has been the main focus of cancer immunology research in the past few years. Metabolic studies in T cell have shown that the function of T cells is critically dependent on their ability to metabolically adopt in the tumor microenvironment. Differentiation of naïve T cells into various forms of T helper is metabolically demanding and the inability of T cell to adopt could hamper this process, thus effecting physiological and pathological Th1 and Th2 immune responses [83].

Many recent studies have pointed out that naïve T lymphocyte activation process requires a dramatic change in their metabolism [84–87]. Therefore, investigating the signaling pathways and clues that regulate these metabolic changes and their functional consequences in T-cell development and activation has been a focus of T-cell response research during the recent years.

1.5.1 Carbohydrate Metabolism

T-cell activation alters cellular metabolism characterized by an increased glucose uptake and glycolysis [88], which has also been reported in cancer cells. However, in cancer cells, this altered metabolic program is primarily driven by cellular dysregulation due to the alteration in genes due to many mutations, whereas in T cells this well-regulated physiological response is essential for proper functionality. As in naïve T cells the requirement for macromolecule is minimal; hence, the cellular metabolism is geared towards the production of energy for basic needs, which is mostly by oxidative phosphorylation. However, upon activation, the naïve T cells increase their glucose uptake and aerobic glycolysis [89] to meet the increase in energy demands.

The increased uptake of glucose upon activation/antigen encounter is regulated by PI3K (phosphoinositide 3-kinase)–Akt and dependent on mTOR- and MYC-regulated pathways [90, 91]. Naïve T cells largely depend on oxidative

catabolic metabolism and undergo a dramatic shift from catabolic to anabolic metabolism upon activation. During this process T cells that fail to undergo this metabolic reprogramming do not gain effector functions suggesting the importance of the metabolic shift. Tumor cells learn to utilize glucose more efficiently in hypoxic conditions. Once activated the effector T cells use the glycolytic pathway to support their rapid growth and production of various effector molecules. Besides T-cell, tumor cells also rely on glucose for growth and survival. Thus, tumor cells and activated T cells compete for the available pool of glucose in TME. However, as observed in most cases, tumor cells outcompete immune cells for glucose by over-expressed higher levels of glucose transporter GLUT1. Indeed, higher expression of GLUT1 in cancer cells correlates with lower CD8 T-cell infiltration in many cancers such as PDA [92], renal cell carcinoma [91], SCC [93], and ovarian cancer [94]. Thus, inadequate levels of glucose and other nutrients hamper T-cell proliferation [95, 96], cytokine production [97, 98], and TCR signaling [99]. Therefore, the ability of tumor cells to take up glucose more efficiently results in their increased proliferation which ultimately suppresses antitumor immunity. Apart from ATP production, the intermediates generated during glucose catabolism via aerobic glycolysis enter in many pathways for the synthesis of proteins, nucleic acids, and lipids. Thus, glucose metabolism is an essential key regulatory pathway in T-cell activation and function.

1.5.2 Amino Acid Metabolism

Besides glucose, tumor and immune cell also compete for amino acids and acetate which are utilized in various other pathways. Glutamine metabolism regulates T-cell responses.

Effector T cells increase the glutamine uptake upon activation and can differentiate into Treg if glutamine availability is limited. Effect of glutamine metabolism differs between T-cell subsets which is metabolized by the enzyme glutaminase (GLS) to glutamate. Glutamine activates CD4 Th17 and pharmacological inhibition or genetic deletion of GLS has been shown to reduce inflammation in various inflammatory diseases such as airway inflammation [100], inflammatory bowel disease [101], and psoriasis [102]. Activated T cells show decreased proliferation and cytokine secretion when exposed to lower glutamine concentrations [103], therefore suggesting that decreased glutamine levels can hinder immune responses in situ.

Amino acid L-arginine (Arg) is also known to play a critical role in regulating antitumor T-cell immunity. Arginine is metabolized by two key enzymes, namely nitric oxide synthase (NOS) and arginase (ARG1). Arginine metabolism affects T-cell differentiation [104] suggesting that arginine levels within the TME can promote T-cell dysfunction. Therefore, manipulating NOS and ARG activity in tumors is lucrative to enhance the efficacy of T-cell-based therapies. Local NO produced by intra-tumoral DCs augments adoptively transferred CD8 cytotoxic T cell [105] mediated tumor killing suggesting that NO can be pro- or antitumor

depending on the context. Higher arginine levels in tumors correlate with higher amount of suppressive TAMs [28] in TME. Arginine promotes T-cell effector function during the T-cell activation. Arginine also promotes their survival as well as differentiation into the memory T cell [104] suggesting that lower arginine levels can promote T-cell dysfunction with TME. Thus, manipulation of NOS and ARG1 activity within tumor sites could enhance the efficacy of T-cell-based therapies.

Tryptophan (Trp), an essential amino acid, also has important immune-physiological functions. Trp can be metabolized into different end products by the host or intestinal microbiota in the gastrointestinal tract. For instance, the intestinal microbiota can catabolize Trp to indoles and its many derivatives, which also play an important role in the regulation of intestinal immune tolerance. Trp is commonly catabolized by kynurenine pathway (KP) to produce kynurenine (Kyn) and other metabolites like 3-hydroxyanthranilic acid (KA), anthranilic acid (AA), 3-hydroxykynurenine (3-HK), xanthurenic acid (XA), and QA [106]. These KP metabolites can play a role in immune regulation. Trp-derived metabolite 3,3-′-diindolylmethane (DIM) has been shown to bind to aryl hydrocarbon receptor (AhR), a known regulator of T-cell response. AhR binding causes induction of FoxP3 expression in T cells which causes enhanced $FoxP3^+$ Treg generation [107]. Tregs have immunosuppressive properties and in turn regulate the function of various other immune cells specifically cytotoxic T cells to induce immune tolerance. Tregs play an important role in many inflammatory diseases as well as in many cancers. Higher Treg correlates with poor prognosis in many cancers. Hyperactivation of KP is reported in many cancers [108]. Higher expression of indoleamine 2,3-dioxygenase (IDO), as the key enzyme in Trp metabolism, correlated with poor prognosis in hematologic malignancies, breast cancer, lung cancer, glioma, melanoma, prostate cancer, and pancreatic cancer.

1.5.3 Lipid Metabolism

Lipids act as structural molecules and are required for the cell membrane formation, many recent studies have suggested its role beyond just structural molecule. The fatty acid metabolism in T-cell activation and differentiation is now well accepted in the field of T-cell biology. Fatty acid metabolism involves fatty acid synthesis (FAS) and fatty acid oxidation (FAO). FAS produces key lipids important for cell membrane formation, which supports cell proliferation, while fatty acid oxidation (FAO) generates ATP and many metabolic intermediates required for important physiological functions.

As observed in other cells, T cells can use fatty acids as an energy source by β-oxidation. Preferential FAO has been shown to regulate differentiation, fates and functionality of CD8+ memory T (Tmem) cells, and induction of CD4+ regulatory T (Treg). Activated T cells rely on FAS [109] where PI3K/Akt pathway activates sterol regulatory element-binding protein (SERBP)-1 which leads to upregulation of ATP citrate lyase (ACLY) and fatty acid synthase (FASN) [110], whereas naïve T cells and memory T cells rely on FAO to maintain basic functions such as membrane

functional integrity [111]. In some cases, FAO may also prevent the activation of Teff cell by upregulating programmed cell death protein 1 (PD-1) expression as well as by upregulating carnitine palmitoyltransferase 1A, one of the rate-limiting enzymes in FAO, leading to inhibition of IFN-γ secretion [111]. On the other hand, FAO also favors more Treg cell formation via MAPK activation [112]. Immunosuppression is one of the hallmarks of most solid tumors and Tregs are an important component of it. Treg promotes SERBP-1-dependent lipid metabolism in the tumor microenvironment which hampers $CD8^+$ T cell's ability to produce IFN-γ and kill tumor cells, thus supporting the generation of immunosuppression of tumor-associated macrophages. Besides SERBP1, peroxisome proliferator-activated receptor (PPARs) is also known to regulate lipid metabolism and promote immunosuppression in solid tumors. Increased PPAR-γ activity inhibits lipolysis, limits OXPHOS in T cells, and promotes differentiation of Tregs [113].

Besides fatty acids, cholesterol metabolism in immune cells in the tumor microenvironment can also play an important role in their functionality. Some recent studies show an increase in cholesterol content in activated CD8 T cells [114]. Activation of TCR is accompanied by increased activity of enzymes involved in cholesterol biosynthesis. ACAT-1 and ACAT-2 genes encode cholesterol esterification enzyme that converts free cholesterol to cholesteryl esters for the storage. ACAT-1 is expressed in $CD8^+$ T cells and plays an important role in the early stage of T-cell activation. ACAT-1 deletion leads to decreased cholesterol esterification but increased cholesterol biosynthesis of cholesterol results in increased membrane cholesterol in $CD8^+$ T cells [115] and the increase in membrane cholesterol enhances TCR clustering and efficient immunological synapse formation.

Intracellular cholesterol and its derivatives can inhibit Tc9 cell differentiation. SUMOylation of liver X receptor (LXR) decreases binding of P65 to the IL-9 promoter, thus reducing the expression of IL-9 [116]. Further, cholesterol in tumor-infiltrating lymphocytes (TILs) can upregulate the expression of endoplasmic reticulum stress receptor XBP1 [117], which may cause higher expression of the immune checkpoint causing weaker T-cell activity and decreased antitumor response.

1.6 Natural Killer (NK) Cells

Natural killer (NK) cells are the major subset of the innate immune system that are of the lymphoid lineage. NK cells are characterized by their cytolytic functions against tumor cells in TME or virally infected cells. NK cells constitute ~5–20% of the total population of peripheral blood lymphocytes and share many common surface markers with T cells. NK cell functionalities, as seen in other cell types, are regulated by their metabolism. Conversely, NK cell metabolism and its antitumor responses are impaired in TME due to metabolic competition with cancer cells. It has also been demonstrated that the immunosuppressive effect of the tumor microenvironment (TME) alone limits the antitumor potential of NK cells. In the TME, various tumor and tumor-associated cells produce and secrete factors like IL-6, IL-10, transforming

growth factor-β (TGF-β), prostaglandin E2 (PGE2), and indoleamine 2,3-dioxygenase (IDO) that may directly or indirectly dampen NK cell activation [118] by down receptors NKp30, NKp44, or NKG2D [119] and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [120]. Furthermore, HLA-E expressed on the surfaces of cancer cells can activate inhibitory receptors (CD94/NKG2A) on NK cells [121] which tilt the balance between activation/inhibition signals in NK cells.

TME strongly affects NK cell metabolism and regulates full effector functions. As seen in other cell types, reduced availabilities of glucose substantially impact NK antitumor activity. Cong et al. reported that glucose deprivation dampens NK cell antitumor activity. In their study decrease in glycolytic rates attenuated cytotoxicity and cytokine production which was due to enhanced fructose-1,6-bisphosphatase (FBP1), an enzyme that inhibits glycolysis [122]. The increase in glycolysis is regulated by sterol regulatory element-binding proteins (SREBP) which promote citrate–malate shuttle. Blocking the activation of SREBP protein or citrate–malate shuttle inhibited the interferon-γ production and NK cell cytotoxicity [123] suggesting a crucial function of SREBP regulating glucose metabolism and thus for NK cell effector function.

Besides glucose, amino acid metabolism also plays a role in NK functionality. Reducing arginine in the media has been shown to impair the proliferation and IFNγ in NK cells [124, 125]. Conversely, mTOR signaling, which is important for regulating glycolysis, is inhibited in leucine-depleted media [126] suggesting the important role of amino acid metabolism in NK biology. Experts in the field believe that it is crucial to explore the NK cell metabolism to determine the way it keep its antitumor activity intact in the metabolically restrictive TME. The more we delineate the finer details of immunometabolism of NK cells, the better we can understand the effector functions of the NK cells. Further studies are needed to determine the ways through which TME shapes NK cell metabolism, which could be targeted to improve NK cell-based immunotherapies.

1.7 Conclusion

Cancer immunotherapy is an encouraging and fast-growing therapeutic modality for human cancers which has increasingly sought attention of both biomedical researchers and patients. Despite the recent success of immune checkpoint inhibitors and CAR T-cell therapy more specifically in hematologic malignancies, the application of immunotherapy in solid tumors is still facing several obstacles resulting from the heterogeneous expression of antigens as well as the induction of immunosuppressive tumor microenvironment. Tumor cells do exhibit specific metabolic requirements in order to survive and proliferate so as to progress into bigger tumors. Within a microenvironment where immune cells share resources with tumor cells, survival of immune cells completely depends on competing metabolic pathways with tumor cells for their development and effector function. This competing shared microenvironment results in acidification, hypoxia, and nutrient depletion that in