Check for updates

DR. STEFAN SCHÜLKE (Orcid ID : 0000-0002-9674-5116)

Article type : Review

Immune metabolism in allergies, does it matter? - A review of immune metabolic basics and adaptations associated with the activation ofinnate immune cells in allergy

Manuscript Acceptance Date: 28-Mar-2021

Short title: Immune metabolism and allergies

Alexandra Goretzki[1#], Yen-Ju Lin[1#], and Stefan Schülke[1*]

[1] Molecular Allergology, Paul-Ehrlich-Institut, Langen, Germany

[#] These authors equally contributed to this work

[*]**Corresponding author:**

Stefan Schülke, PhD

Paul-Ehrlich-Institute, Vice President´s Research Group: Molecular Allergology

Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

Phone: +49 6103 775209

Fax: +49 6103 771258

E-Mail: stefan.schuelke@pei.de

Declaration of funding:

This work was in part funded by the budget of the Paul-Ehrlich-Institut, Langen, Germany. AG was funded by the German Research Foundation (DFG SCHU2951/4). YJL was funded by the German Research Foundation (DFG SCHE637/4-1).

Conflict of interest statement:

The authors have no conflict of interest to declare.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ALL.14843

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

Word count:6625

This article is protected by copyright. All rights reserved

Abstract

Type I allergies are pathological, type 2 inflammatory immune responses against otherwise harmless environmental allergens that arise from complex interactions between different types of immune cells. Activated immune cells undergo extensive changes in phenotype and function to fulfill their effector functions. Hereby, activation, differentiation, proliferation, migration, and mounting of effector responses require metabolic reprogramming. While the metabolic changes associated with activation of dendritic cells, macrophages, and T cells are extensively studied, data about the metabolic phenotypes of the other cell types critically involved in allergic responses (epithelial cells, eosinophils, basophils, mast cells, and ILC2s) are rather limited. This review briefly covers the basics of cellular energy metabolism and its connection to immune cell function. In addition, it summarizes the current state of knowledge in terms of dendritic cell- and macrophage metabolism and subsequently focusses on the metabolic changes associated with activation of epithelial cells, eosinophils, basophils, mast cells, as well as ILC2sin allergy.Interestingly, the innate key cell types in allergic inflammation were reported to change their metabolic phenotype during activation, shifting to either glycolysis (epithelial cells, M1 macrophages, DCs, eosinophils, basophils, acutely activated mast cells), oxidative phosphorylation (M2 macrophages, longer term activated mast cells), or fatty acid oxidation (ILC2s). Therefore, immune metabolism is of relevance in allergic diseases and its connection to immune cell effector function needs to be considered to better understandinduction and maintenance of allergic responses. Further progress in this field will likely improve both our understanding of disease pathology and enable new treatment targets/strategies.

Keywords

Allergies, metabolism, glycolysis, immune metabolism, oxidative phosphorylation, Warburg effect;

Accepted Article

This article is protected by copyright. All rights reserved

List of Abbreviations

2-DG: 2-deoxy-glucose

ACL: ATP-citrate lyase

Ag: antigen

AIT: allergen immunotherapy

Akt: proteinkinase B

Alum: aluminum hydroxide

Arg1: arginase 1

Atg: autophagy-related gene

ß-hex: beta hexosaminidase

BMMCs: bone marrow-derived mast cells

CHI3L1: chitinase-3-like protein 1

DC: dendritic cell

DCA: dichloroacetate

ECAR: extracellular acidification rate

ECRS: eosinophilic chronic rhinosinusitis

EoE: eosinophilic esophagitis

ERK: extracellular signal-regulated kinase

FA: fatty acid

FAO: fatty acid oxidation

FAS: fatty acid synthesis

FcεRI: high affinity IgE receptor

GDH: glutamate dehydrogenase

GM-CSF: granulocyte macrophage colony-stimulating factor

HA: hyaluronan

HDM: house dust mite

HIF-1a: hypoxia inducible factor 1a

IDH: isocitratedehydrogenase

IFN-γ: interferongamma

ILC2: innate like lymphocytes type II cell

IκKε: inhibitory κ B kinase-epsilon

Jmjd3: Jumonji domain-containing protein D3

JNK: c-Jun N-terminalkinase

LDH: lactatedehydrogenase

LPS: lipopolysaccharide

This article is protected by copyright. All rights reserved

Accepted Article

LT: leukotriene
M2PK: M2-type pyruvate kinase
MAPK: mitogen-activated protein kinase
MS: mass spectrometry
mTOR: mammalian target of rapamycin
mTORC1/2: mTOR complex 1/2
NLRP3: NLR family pyrin domain containing 3
NMR: nuclear magnetic resonance
NO: nitric oxide
NOX: NADPH oxidase
OCR: oxygen consumption rate
oxLDL: oxidized low-density lipoproteins
OxPhos: oxidative phosphorylation
PAMP: pathogen associated molecular pattern
PDH: pyruvate dehydrogenase
PDHK(1): pyruvatedehydrogenasekinase (1)
PG (D2): prostaglandin(D2)
PGC-1β: peroxisome proliferator-activated receptor γ co-activator 1 beta
PI3K: phosphoinositide 3-kinase
PKM2: pyruvate kinase M2
PM: polymerized allergoids conjugated to mannan
PUFA: poly-unsaturated fatty acid
RBL-2H3: rat basophil leukemia cells
ROS: reactive oxygen species
SAM: S-adenoysylmethionine
ST-2: IL-33 receptor
STAT5/6: signal transducer and activator of transcription 5/6
TIM3: T cell immunoglobulin and mucin-domain containing protein 3
TSLP: thymic stromal lymphopoietin
TLR: Toll-like receptor
Treg: regulatory T cell
VHL: von-Hippel-Lindau

Accepted Article

This article is protected by copyright. All rights reserved

1. Introduction

Allergic diseases are an increasing health and economic problem in developed countries withIgE-mediated type I hypersensitivity disorders reported to affect more than 25% of the population(1,2).

Immunologically, onset and maintenance of type I allergies are caused by exaggerated type 2-mediated immune responses, directed against otherwise harmless environmental antigens. The main effector cells involved in establishment and elicitation of allergic reactions are Antigen Presenting Cells (Dendritic cells (DCs) and macrophages), Th2 cells, IgE-producing plasma cells, eosinophils, basophils, mast cells, and innate like lymphocytes type II cells (ILC2s).Additionally, lipid mediators derived from arachidonic acid such as prostaglandins (PGs, e.g. PGD4, PGE2) and leukotrienes (LTs, e.g. cysLT1), or the peptide hormone histamine (derived from histidine) are produced by activated immune cells within minutes, act locally through specific receptors expressed on many target cells, and are usually quickly metabolized(3,4).These molecules are prominently involved in the hallmark features of allergic inflammation byinducing potent and bronchoconstriction/long-lasting bronchospasm, immune cell recruitment, airway inflammation, -hyperresponsiveness, and -remodeling(3–5).

Activated immune cells can undergo extensive phenotypicchanges in order to fulfill their effector functions. Therefore, activation, differentiation, proliferation, migration, and mounting of effector responses require metabolic reprogramming: For example, T cells, DCs, macrophages, or neutrophils were shown to undergo distinct metabolic changes upon activation(6–9). To understand the mechanisms underlyingthese metabolic changes and their functional consequencesis the aim of a new research field termed“immune metabolism”. Box 1 summarizes the methods commonly used in immune metabolic studies.

This review will briefly introducethe fundamental principles of cellular energy metabolism, its connection to immune cell effector function, and then discuss the metabolic adaptations associated with the activation of allergy-related effector cells of the innate immune system.

2. Immune metabolism

2.1 Cells can use glucose to generate energy either via oxidative phosphorylation or glycolysis

Exposure of immune cells to different stimuli may require cells to prioritize biosynthesis of certain molecules or reactive oxygen species (ROS) over the simple production of energy in the form of ATP(10). To allow for this flexibility, cellular metabolism is a complex network of interconnected catabolic and anabolic pathways, most of which converge at the mitochondrion(10).

This article is protected by copyright. All rights reserved

Glucose, fatty acids, and amino acids are the main cellular energy sources which are used by the cell's metabolic pathways to generate both energy in the form of ATP or reduction equivalents and important biosynthetic intermediates needed for cellular function.

Under normal metabolic conditions, cells take up glucose via specialized glucose transporters from their surroundings. Inside the cell, glucose is activated by addition of an energy-rich phosphate group to generate glucose-6-P,which issubsequently cleaved in a multistep process in the cytoplasm to form two C3 bodies,finally resulting in the generation of two molecules of pyruvate(11)(Fig. 1A). Pyruvate is then imported into the mitochondrion where it is metabolized in the Krebs (also called TCA) cycle as acetyl-CoA by pyruvate dehydrogenase (PDH).

The Krebs cycle itself is a sequence of biochemical reactions that is used by aerobic cells for the oxidative degradation of organic substances in order to generate both energy andbiosynthetic intermediates (depicted in Fig. 1A).Finally pyruvate is degraded via the Krebs cycle, resulting in the generation of CO_2 and energy in the form of the reduction equivalent NADH(12).

Besides the usage of glucose-derived pyruvate, the Krebs cycle can also be fueled by either fatty acids degraded into acetyl-CoA molecules or glutamate derived from amino acid metabolism, which is metabolized into α-ketoglutarate by the enzyme glutamate dehydrogenase(13) (GDH, Fig. 1A). Finally, acetyl-CoA generated from citrate (via the ATP-citrate lyase, ACL) can be used for either lipid production in the mitochondrion or epigenetic modification of selective genes via histone acetylation in the nucleus(6,14–16).

NADH generatedfromthe Krebs cycle is subsequently used in a process called oxidative phosphorylation (OxPhos) at the inner membrane of the mitochondrion(12).Here, oxygen molecules are used as terminal electron acceptors to form a proton gradient over the inner mitochondrial membrane that drives the generation of ATP via the ATP synthase complex(12). Therefore, mitochondrial activity results in the consumption of oxygen, which can be measured as oxygen consumption rate (OCR)(Fig. 1A).In addition, oxygen can be consumed independently of OxPhosor by certain enzymes in the cytoplasm(17–19).

2.2 Energy production via glycolysis in the cytoplasm is a hallmark feature of both cancer cells and activated immune cells

Under certain conditions, cells can decide to predominantly produce lactate from pyruvate via the consumption of NADH by the enzyme lactate dehydrogenase (LDH) in the cell's cytoplasm(20)(Fig. 1B). Biochemically, this shift towards lactate production is mediated via hypoxia inducible factor 1a (HIF-1a)-dependent up-regulation of different pyruvate dehydrogenase kinases (PDHK)(21).

Inside the cell, the generated lactate cannot be further metabolized and is subsequently secreted out of the cell, acidifying the extracellular environment(22). This pathway is common for anaerobic

This article is protected by copyright. All rights reserved

conditions, but was also detected nearly 100 years ago under aerobic conditions in cancer cells(19,23,24). This “aerobic glycolysis”, first detected by Otto Warburg, is therefore known as the Warburg effect(24). It can be readily observed as yellow coloring of the culture medium and measured as extracellular acidification rate (ECAR; Fig. 1B).

To date, it is known that the Warburg effect not only occurs in cancer cells, but also in either strongly proliferating cells or activated immune cells characterized by a high energy demand(22,25).Therefore, this shift towards glycolysis is also used to fulfill the rapid energy demand of activated immune cells.

Indeed, a metabolic shift towards increased rates of glycolysis, often associated with reduced rates of OxPhos, was repeatedly described as an early step in the maturation, migration, differentiation, and pro-inflammatory activation of innate immune cells(10). Of note, OxPhosand glycolysis have different energy yields: while complete oxidation of glucose in the mitochondrion results in the generation of 36 mol of ATP/mol glucose, glycolysis only generates 2mol of ATP/mol glucose(26)(Fig. 1). However, glycolysis allows for a faster rate of energy generation while also providing important biosynthetic intermediates needed for immune cell effector function(27–29). In addition, the speed of glycolysis is further increased by the induced upregulation of glycolytic enzymes via HIF-1a(30). Thereby, while being less efficient, glycolysis can, for short periods of time,result in comparable energy production rates to full mitochondrial oxidation of glucose(20).

Increased rates of glycolysis are likely also a relevant metabolic change in allergic patients: Ostroukhova and colleagues reported serum lactate to be increased in clinically stable asthmatic patients, suggesting an increase in glycolytic activity(31). In line with this, activated T cells from asthmatic patients both expressed higher levels of the lactate-generating enzyme PDK1 and produced higher amounts of lactate compared to non-allergic controls(31). Here, lower concentrations of lactate (up to 0.25 mM) promoted, while higher concentrations (>0.25 mM) inhibited T cell proliferation(31). Mechanistically, inhibition of lactate generation by the PDK inhibitor dichloroacetate (DCA) blocked T cell proliferation as well as production of the cytokines IL-5, IL-17, and interferon gamma (IFN-γ), while stimulating production of IL-10 and induction of Foxp3(31). In a mouse model of ragweed-induced asthma DCA also suppressed both airway inflammation and hyperreactivity(31). In summary, these data suggest aerobic glycolysis (promoting T cell activation) to be increased in asthma. In line with these results, lactic acidosis is frequently reported in adult patients with acute severe asthma, particularly in an intensive care setting(32–34).

2.3 A shift towards aerobic glycolysis results in a ”disrupted”Krebs cycle and the production of important immune cell effector molecules

This article is protected by copyright. All rights reserved

In addition to workingunder conditions of low oxygen availability, the switch to Warburg metabolism also provides Krebs cycle intermediates which are critically needed in activated and proliferating immune cells(20,25,35–37)(Fig. 1B).

Here, especially the accumulation of citrate, cis-Aconitate, and D-isocitrate (caused by a decreased expression of the enzyme isocitrate dehydrogenase (IDH, Fig. 1B), which normally converts D-isocitrate to α-ketoglutarate(38)), resulting from a "disrupted" Krebs cycle, was suggested to facilitate the generation of both directly and indirectly antimicrobial molecules such as prostaglandins, nitric oxide (NO), ROS, or itaconate, all of which are important immune effector molecules (reviewed in (39)) (Fig.1B). Moreover, succinate accumulating in lipopolysaccharide (LPS)-stimulated macrophages was shown to promote glycolysis and IL-1β production via the stabilization of HIF-1a(reviewed in(40))(Fig. 1B).Finally, fumarate was also shown to boost TNF and IL-6 production upon LPS re-stimulation of β-glucan-trained macrophages, potentially via inhibition of lysine-specific demethylase 5,resulting in increased levels of H3K4me3 at the promoters of Tnf and Il6(41)(Fig. 1B).

Therefore, changes in the metabolic phenotype can have profound effects on the ability of the respective cell to fulfill its effector function.

2.4 Metabolic adaptions in innate immune cell and trained immunity

Myeloid cells (monocytes/macrophages, NK cells, innate-like lymphoid cells) were shown to display an increased reactivity after re-stimulation with both the same or different stimuli, also well after the initial stimulation(42–46).These results suggested, that functional reprogramming of innate immune cells upon their initial activation can result in the development of innate memory-like responses which change the response of the respective cells towards a second challenge(43). Here, compared to the initial response, subsequent immune responses to the second challenge can either be enhanced (termed "trained immunity") or suppressed ("trained tolerance")(43,47–49). This system allows innate immune cells to react differently to future infections.

Stimuli described to trigger trained immunity include among others β-glucan, the tuberculosis vaccine BCG, uric acid, oxidized low-density lipoproteins (oxLDL), parasites, or viral pathogens(41,46,50–54).Moreover, in a model of atherosclerosis, Christ and colleagues showed sterile triggers in the form of a "Western Diet"(see also Box 2 for a summary of the connection between diet and inflammation) to activate innate immunity, NOD-like, and IFN- signaling pathways, resulting in long-lasting, NLR family pyrin domain containing 3 (NLRP3) inflammasome-dependent trained immunity in myeloid cells(55). While systemic inflammation induced by a "Western Diet" was largely blunted by returning to a control diet, myeloid cell-induced innate immune responses, potentially contributing to the establishment and maintenance of

This article is protected by copyright. All rights reserved

inflammatory disease, remained augmented(55).

Mechanistically, “innate immune memory’’ arises from both epigenetic (accumulation of H3K4me1, H3K4me3, or H3K27Ac modifications(46,50,56)) and metabolic changes (including increased aerobic glycolysis, glutaminolysis, cholesterol metabolism, and fatty acid synthesis, reviewed in(46)) in the respective cells that can last for prolonged periods of time and protect from secondary infections(55,57,58). The long-lasting nature of epigenetic modifications allows cells to stay in the “trained” state characterized for example by an unfolded chromatin structure with exposed promoter- and enhancer regions allowing for an easier activation of immune-related genes(46).

In this context, the induced metabolic changes not only provide the energy needed for innate immune cell activation but also enzymes, cofactors, and substrates required for epigenetic modifications (e.g. by providing acetyl-CoA via ATP-citrate lyase (which converts citrate into acetyl-CoA) for histone acetylation, S-adenoysylmethionine(SAM) for histone methylation, or α-ketoglutarate for activation of dioxygenases (reviewed in(46)).

Recent results suggest trained immunity to not only be relevant in infectious diseases, chronic inflammation, and cancer, but also in allergy and allergen-immunotherapy.For example,aluminum hydroxide (Alum) was shown to influence the metabolic state of human DCs changing their responses towards immune activating non-oxidized mannan in an allergen-immunotherapy setting: Benito-Vilalba and colleagues reported Alum to interfere with the allergy suppressing properties of allergoids conjugated to non-oxidized mannan(59). Here, Alum suppressed polymerized allergoids conjugated to mannan (PM)-induced PD-L1 expression and IL-10 production from human DCs and impaired PM-induced functional $Foxp3^+$ Treg cells, while at the same time increasing Th1-, Th2-, and Th17-cytokine production(59). Moreover, Alum suppressed the PM-induced induction of Tregs in mice(59). Mechanistically, Alum inhibited PM-induced mammalian target of rapamycin(mTOR) activation thereby blunting glycolytic shift, ROS production, and DC capacity to generate functional Treg cells(59).

Eljaszewicz*et al.* also showed subcutaneous allergen immunotherapy (AIT) for aeroallergens to result in dynamic changes in the systemic innate immune response, suggesting trained immunity to also contribute to the immunologic changes associated with successful AIT one year into the therapy(49). In their study, AIT induced time-dependent changes in both composition and phenotypes of circulating ILCs, monocytes, and DC subpopulations: ILC2 cells displayed reduced frequencies in patients undergoing AIT, while ILC1 cells increased over time, suggesting that AIT limited the number of pathology-driving ILC2- in favor of ILC1 cells(49). In addition, numbers of both plasmacytoidDCs and $CD11c^+$ myeloid cells increased and monocytes shifted from pro-inflammatory (non-classical) towards anti-inflammatory phenotypes (HLA-DR low, intermediate monocytes) during the course of AIT(49). Therefore, AIT resulted in changes in the development of

This article is protected by copyright. All rights reserved

DC subsets with increased regulatory potential and the development of phenotypes, frequencies, and composition of immune cells with higher similarity to healthy controls(49).

Taken together, cells undergo metabolic changes during both allergy and AIT. In the next section we will go into more detail concerning the different cell types.

3. Metabolic changes in different types of immune cells

Our current understanding of the immune metabolic processes underlying allergic reactions is still limited. While the metabolic changes associated with activation of DCsand macrophages are meanwhile well described (see (39)), data about the metabolic phenotypes of the other innate immune cell types critically involved in allergic responses are limited. Therefore, this review will briefly recapitulate the current knowledge in terms of DC and macrophageimmune metabolism and subsequently focus on the metabolic changes associated with activation of epithelial cells, eosinophils, basophils, mast cells, as well as ILC2s (the metabolic phenotypes and signaling pathways discussed in the review are illustrated in Figs. 2 to 4).

Also the acute activation and differentiation of T and B cells,as well as the formation of memory cells, which are not the focus of this review,are paralleled by distinct changes in cellular metabolism that are still not completely understood (reviewed in(60–62)).

3.1 Epithelial cells

Epithelial cells are the first line of contact with allergens. Limited reports are describing the relationship between epithelial cell metabolism and different allergic diseases, such as asthma, house dust mite (HDM)-induced allergic airway inflammation, and eosinophilic esophagitis(EoE)(63,64).

Mitochondrial dysfunction and metabolic reprogramming are important for disrepair and fibrosis in lung disease(65). Xu *et al.* reported increased mitochondrial numbers in bronchial epithelial cells and enhanced levels of OxPhos, partly driven by reprogramming of arginine metabolism, in the airway epithelium of patients with asthma(66). Studies in *in vivo*animal models have suggested allergen-induced lung inflammation to be in part caused by ROS- and NO-dependent mitochondrial damage and reduced ATP production in the airway epithelium(67,68) (Fig. 2B). Moreover, Rodriguez-Perez *et al.* demonstrated decreased fatty acid desaturase activity in humans with asthma(69). Additional experiments using a HDM murine model and *ex vivo* bronchial epithelial cells from asthma patients suggested inhibition of fatty acid desaturase to promote both airway hyper-responsiveness and reduce antiviral responses(69)(Fig. 2B), demonstrating fatty acid metabolism to be involved in asthma pathology.

Recently, Qian *et al.* reported the induction of glycolysis in the lungs of asthmatic mice(70). Here,

This article is protected by copyright. All rights reserved

inhibitory κ B kinase-epsilon (IκKε)-dependent IL-1β production was shown to stimulate lactate generation via induction of glycolytic enzymes in primary mouse tracheal epithelial cells(70) (Fig. 2B). Furthermore, enhanced glycolysis was required for IL-1β- or IL-1α-mediated pro-inflammatory responses, HDM-induced release of thymic stromal lymphopoietin (TSLP), and granulocyte macrophage colony-stimulating factor (GM-CSF) from tracheal epithelial cells*in vitro*(70) (Fig. 2B). In line with this, primary nasal epithelial cells from asthmatics were shown to express higher levels of LDHA and produce more lactate than cells derived from healthy controls(70). A follow-up study from the same group suggested, that the dimeric form of pyruvate kinase M2 (PKM2), an enzyme which converts phosphoenolpyruvate to pyruvate, was increased in a murine *in vivo*modelofHDM allergy as well as nasal epithelial cells of asthma patients(64). PKM2 was essential for IL-1β-induced pro-inflammatory signaling, which led to allergic airway pathogenesis(64) (Fig. 2B).Another report showed that sputum lactate levels negatively correlated with lung function in asthmatics, suggesting that metabolic aberrations contribute to asthma development(70). Additionally, Yagi *et al.* found mevalonate biosynthesis and subsequent protein prenylation (a posttranslational lipid modification) fueled by glucose-derived citrate generation to play an important role in Th2 cell differentiation(30) (Fig. 2B). Taken together, these studies indicate, that the upregulation of glucose metabolism in epithelial cells during allergic disease and mediators derived from the activated epithelial cells could promote allergic inflammation (Fig. 2A&B).

In general, allergic diseases are characterized by a dysfunctional epithelial barrier. Several studies also indicate the importance of epithelial cell metabolism on maintenance of barrier integrity: Masterson *et al.* showed either HIF-1aknockdown or prolonged hypoxia leading to the suppression of both claudin-1 expression (a tight junction control protein) and epithelial barrier function by using *in vitro* 3D organotypic epithelial cultures(63) (Fig. 2B). Clinically, esophageal biopsies from EoE patients also showed a repressed HIF-1aand claudin-1 expression(63). Although limited reports are describing the metabolism of intestinal epithelial cells in food allergy, it was shown that glucose metabolism was critical to preserve the structure of the intestinal epithelial barrier(71). *In vitro,* ATP depletion in CaCo2 intestinal epithelial cells using either 2-deoxy-glucose (2-DG) or hypoxia conditions increased epithelial permeability associated with a loss of tight junction structure with derangements of perijunctional actin(71) (Fig. 2B).

In summary, the available studies suggest that changes in mitochondrial function, fatty acid metabolism, and glycolysis of epithelial cells are involved in allergic disease progression.

3.2 Platelets

In order to understand the pathomechanism and metabolic changes associated with allergic diseases, Obeso and colleagues investigated material from profilin allergic patients *ex vivo* that

This article is protected by copyright. All rights reserved

were grouped into either non-allergic controls, mild-, moderate-, or severe-allergics(72).
In this study, the progression to the severe allergic phenotype was characterized by a decrease in both carbohydrates (hexoses, pentoses) and pyruvate while lactate levels were increased in plasma(72). Furthermore, concentrations of lysophospholipids, sphingosine-1-phosphate, sphinganine-1-phosphate, and lauric-, myristic-, palmitic-, and oleic acids also increased with disease severity.
In contrast to this, significantly down-regulated transcripts in the severe allergic group were found to be associated with coagulation, platelet function, protein synthesis, histone modification, and fatty acid metabolism(72).Especially, in severe allergics, platelets displayed a downregulation of transcripts associated with formation of adhesion complexes (GP1BA, GP9, GP6, and SELP), platelet activation (P2RY12, ALOX12, and PTGS2), aggregation of complexes (ITGB3, ITGA2B), synthesis of 12-Hydroxyeicosatetraenoic acid and thromboxane A2 (ALOX12, PTGS2), and both shape change induction and granule secretion processes (MYL9, SDPR, RAB27B)(72).
Therefore, the results obtained by Obeso*et al.* suggest the transition to severe allergic phenotypes/inflammation to be either caused or accompanied by alterations in platelet function, reduced protein synthesis, and the switch to glycolytic Warburg metabolism(72).

3.3 Dendritic cells and macrophages

As part of the innate immune system, DCs and macrophages need to quickly react to invading pathogens in order to induce and orchestrate protective immune responses. In response to inflammatory signals, macrophages polarize into two cell types: (1) inflammatory M1-macrophages, driven by bacteria-derived pathogen associated molecular patterns (PAMPs) such as LPS and IFN-γ and (2) anti-inflammatory M2-macrophages activated by type 2 cytokines IL-4 and IL-13(73–75).
Several studies repeatedly reported the ability of DCs and M1-macrophages to switch their metabolism towards glycolysis,resulting in a “disrupted” Krebs cycle(76–80)(Fig.3A). This tendency enables these two cells to efficiently execute their particular effector functions, such as producing pro-inflammatory cytokines(81), phagocytosis in macrophages(82), cell migration(83), and $CD4^+$ and $CD8^+$ T cell activation(84)in DCs(Fig. 3B). Mechanically, HIF-1a is important for glycolytic metabolism in both cell types: HIF-1a-deficient macrophages lack both lactate- and ATP-production after LPS stimulation, and impairedglycolysiswas shown to profoundly reducethe clearance of bacteria and fungi*in vitro* and *in vivo*(85,86). In DCs, HIF-1a-deficiency leads to decreased expression of MHC-II, CD80, and CD86, resulting in impaired T cell stimulatory capacity(87,88)(Fig. 3B). Moreover, succinate and succinyl-CoA were shown to induce IL-1β production via stabilization of HIF-1a *in vitro*(81)(Fig. 1B).

This article is protected by copyright. All rights reserved

Besides HIF-1a, Krebs cycle-derived metabolites also play an important role in macrophage-mediated inflammation: fumarate was shown to induce TNF- and IL-6-production, citrate to induce ROS-, NO-, and prostaglandin-production *in vitro*(89)(Fig. 1B). Furthermore, the mTOR network was also reported to be involved in DC differentiation, glycolysis, and mitochondrial respiration both *in vitro* and *in vivo*, which all contribute to the regulation of immune responses by DCs(90)(Fig. 3B). In contrast to M1-macrophages and DCs, M2-macrophages show decreased glycolytic activity, paralleled by a higher basal mitochondrial OCR*in vitro*(38)(Fig. 3C). This metabolic reprogramming is mediated by the signal transducer and activator of transcription (STAT6)-dependent peroxisome proliferator-activated receptor γ co-activator 1 beta (PGC-1β) signaling pathway(91)(Fig. 3C). Knockdown of PGC-1β results in inhibition of fatty acid oxidation (FAO) and anti-inflammatory functions*in vitro*(91). In addition, α-ketoglutarate, both an intermediate of the Krebs cycle and the product of glutaminolysis (Fig. 1A), was shown to suppress NF-κB activation and restrict M1 polarization, while promoting M2-polarization via Jumonji domain-containing protein D3 (Jmjd3)-dependent epigenetic reprogramming *in vitro*(92)(Fig. 3C).

Although the metabolic profiles of activated DCs and macrophages are well reported, studies on the contribution of DC and macrophage immunometabolism in allergic diseases are still limited. Since M2-macrophages are activated by Th2 cytokines, they have been suggested to be involved in allergic diseases such as asthma(93,94). Jin*et al.* found that *in vivo*, recombinant *Trichinella spiralis* thioredoxin peroxidase-2, a protein that belongs to a family of antioxidant enzymes, could both promote M2 polarization and enhance Th2 responses(95)(Fig. 3C). Another *in vivo* study by Kim *et al.* showed, that chitinase-3-like protein 1 (CHI3L1), a glycoprotein that hydrolyzes chitin, was secreted from intestinal macrophages(96). Via mitogen-activated protein kinases(MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)phosphorylation, CHI3L1 led to M2 macrophage polarization and contributed to Th2-associated inflammation in food allergy *in vivo*(96)(Fig. 3C).

In contrast to these findings, Otobe*et al.* showed the induction of a M2 macrophage-phenotype to protect from local Th2 responses in a mouse model of 2,4-dinitrofluorobenzene-induced contact hypersensitivity(97). In CX3CR1-deficient mice, 2,4-dinitrofluorobenzene challenge resulted in decreased ear swelling and dermal edema, paralleled by an increased expression of M2 macrophage markers (e.g. arginase-1) in peritoneal macrophages isolated *ex vivo*(97).

Besides M2-macrophages, the release of pro-inflammatory cytokines and lipid mediators by M1-macrophages also plays a role in allergy. Tiotiu*et al.* analyzed macrophages in the sputum and found that in severe asthma patients, both macrophage number and their activation status were reduced compared to healthy controls. However, three gene modules involved in the regulation of inflammatory response (TNF-, TLR-, and NF-κB-pathways) were found to be enriched in severe

This article is protected by copyright. All rights reserved

asthmatics, suggesting these cells to closely resemble a M1-phenotype(98).

Moreover, fragmented hyaluronic acid, which plays a role in the pathogenesis of asthma(99), can polarize macrophages towards a M1-phenotype through cytosolic phospholipase A2 group IVA activation and arachidonic acid release *in vitro*(100)(Fig. 3B*)*, indicating a link between allergic inflammation and lipid metabolism in macrophages.

In DCs, during metabolic stress, Elesela and colleagues found Sirtuin 1, an NAD^+ dependent deacetylase, to activate AMPK, resulting in phospho-acetyl-CoA carboxylase-mediated inhibition of fatty acid synthesis *in vitro*(Fig. 3B). This process altered the T cell differentiation induced by the respective DCs from Th2/Th17- towards Th1-responses both *in vitro* and *in vivo*, suggesting Sirtuin 1-regulated metabolism to have an important role in DC function(101). Besides, Sinclair *et al.* reported that mTOR-deficient $CD11b^+$ DCs showed metabolical reprogramming, resulting in disrupted FAO. When intranasally challenged with HDM*in vivo*, mTOR-deficient $CD11b^+$ DCs induceda neutrophilic Th17 response instead of eosinophilic Th2 inflammation(102)(Fig. 3B).

Taken together, metabolic pathways impact both DC- and macrophagefunction and fate, which also influences induction and maintenance ofallergic reactions.

3.4 Eosinophils

Data describing the immune metabolic status of eosinophils are sparse. Early on Sher *et al.* reported polyhydroxyalkanoateS-stimulated eosinophils to increase both hexose monophosphate shunt activity and glycolysis*in vitro*(103).Recently, Porter and colleagues compared human eosinophils and neutrophils in terms of metabolic phenotype*in vitro*(104): while eosinophils and neutrophils displayed similar glycolytic capacity, eosinophils exhibited significantly greater basal mitochondrial respiration, ATP-linked respiration, maximum-and spare respiratory capacity(104). These results suggest, that (compared to neutrophils) eosinophils display a greater metabolic flexibility, with the ability to use either glycolysis, glucose oxidation, orOxPhos(104) (Fig. 4A). Concordantly, Peachman*et al.* have calculated the number of mitochondria in neutrophils to range from 5-6 mitochondria per cell while this number was 5 to 6 times higher in eosinophils (24–36 mitochondria/eosinophil)(105).

Interestingly, eosinophils from atopic and non-atopic subjects were shown to have comparable OCR upon priming*in vitro* with IL-5 and stimulation with the chemotactic factor N-formyl-methionyl–leucylphenylalanine(104).

Accordingly, Jones *et al.* confirmed human eosinophils to display metabolic plasticity(19). Here, human eosinophils stimulated *in vitro*with either IL-5, IL-3, or GM-CSF increased glycolysis while also enhancing glutaminolysis with subsequent degradation via theKrebs cycle(19) (Fig. 4B). Interestingly, IL-5-driven activation of both increased glycolysis and mitochondrial activity in

This article is protected by copyright. All rights reserved

eosinophils was dependent on STAT5/PI3K/Akt-signal transduction (Fig. 4B), resulting in NADPH oxidase(NOX)-dependent ROS production(19). This ability of eosinophils to compensate for reduced ROS-production and Krebs cycle intermediates by increasing glycolysis confirms their metabolic plasticity.

Increased glycolytic activity by cytokine-stimulated eosinophils was accompanied by accumulation of both intra- and extracellular lactate and therefore the generation of a local acidic microenvironment(19). Eosinophils and T cells were shown to efficiently fulfill their effector functions in acidic environments(106,107), suggesting that metabolic changes can also induce, maintain, or boost the function of other immune cells.

Autophagy (Greek for “eating of self”) is a both highly conserved and regulated catabolic process responsible for the turnover of macromolecules and organelles via the lysosomal degradative pathway(108). In mammals it is regulated by mTOR (mTOR activation results in autophagy inhibition), PI3K, and numerous autophagy-related (Atg) proteins(108).

The contribution of autophagy to eosinophil development and activation is largely unknown (reviewed in(108)). Interestingly, eosinophil development seems to be differentially regulated by the mTOR complex: while autophagy induction by rapamycin-induced inhibition of mTOR complex 1 (mTORC1) effectively blocked eosinophil differentiation and attenuated OVA-induced allergic airway inflammation with reduced numbers of eosinophils (in local airways, peripheral blood, and bone marrow)(109), application of the mTORC1/2 inhibitor torin 1 resulted in accelerated eosinophil differentiation and elevated levels of eosinophil infiltration in an experimental mouse model of allergy(110).

Additional studies suggest, that autophagy levels in eosinophils may regulate the severity of allergic inflammation(111). Higher levels of autophagy were reported in human sputum and peripheral blood eosinophils from patients with severe asthma compared to non-severe asthma and healthy controls which were further increased by stimulation with IL-5 *in vitro*(112), suggesting, that autophagy could play a role in eosinophil activation and pathogenesis of severe asthma.

Using genetically modified mice that lack Atg5, a protein essential for autophagosome formation (specifically in eosinophils or neutrophils), Germic and colleagues reported*in vitro,* both ROS production and extracellular trap formation in eosinophils and neutrophils to be independent of autophagy(113). In contrast, Silveira *et al.* reported the autophagy inhibitor, 3-methyladenine to reduce extracellular trap formation in the airway, decrease the number of eosinophils, eosinophil peroxidase activity, goblet cells hyperplasia, pro-inflammatory cytokines, and NFκB p65 content while at the same time improving oxidative stress, mitochondrial energy metabolism, and Na^+, K^+-ATPase activity in a mouse experimental asthma model(114). However, in this model indirect effects of 3-methyladenine on eosinophil activation cannot be excluded.

This article is protected by copyright. All rights reserved

Indeed, Choi *et al.* suggested impaired autophagy to indirectly promote eosinophilic inflammation and pathogenesis of eosinophilic chronic rhinosinusitis (ECRS): in their model,myeloid cell-specific deletion of Atg7 aggravated eosinophilia, epithelial hyperplasia, mucosal thickening, and increased production of PG D2 in mice with ECRS via macrophage-derived IL-1β(115). Furthermore, myeloid deficiency for autophagy was associated with gene expression profiles favoring eosinophilic inflammation, Th2 responses, PG D2 dysregulation, and mast cell infiltration *in vivo*(115).

In summary, the observed metabolic flexibility and shifting towards glycolysis may allow eosinophils to both efficiently fulfill their function in inflammatory environments with limited availability of oxygen and quickly adapt to different microenvironments. This speculation is in line with their function in both immune effector responses as well as establishment and maintenance of immune homeostasis(104).

3.5 Basophils

As for eosinophils, data describing the immune metabolic phenotype of basophils are limited, also the role of autophagy in basophil function is currently unknown.Sumhayev and colleagues showed the IgE-mediated activation of primary human basophils to result in an extracellular signal-regulated kinase (ERK)- and p38 MAPK-dependent accumulation of HIF-1a *in vitro*(116) (Fig. 4C). HIF-1a mediates cellular adaptation to hypoxic conditions by regulating glycolysis, angiogenesis, and cell adhesion(117–119). In the study of Sumhayev*et al.,*HIF-1aactivation and the induced glycolysis supported IgE-mediated inflammatory basophil responses by providing the ATP necessary for IL-4- and VEGF-production(116) (Fig. 4C).

In a follow-up study, Sumbayev and colleagues examined the contribution of HIF-1a to the synthesis of angiogenic and inflammatory cytokines from either primary human basophils, LAD2 human mast cells, or THP-1 human myeloid cells *in vitro*(120). As was already observed for IgE-stimulated human basophils, TLR-ligand induced cytokine secretion from HIF-1a knockdown LAD2 mast cells was markedly reduced while degranulation was unaffected(120). Moreover, either LPS-, PGN- or IgE-stimulation of THP-1 cells resulted in HIF-1a accumulation and release of TNF-α, VEGF, and IL-6(120).

Taken together, these results suggest, that HIF-1a and the induced immunologic and metabolic changes (increased glycolysis) are important determinants of basophil effector function(120).

3.6 Mast cells

While information about the immune metabolism in eosinophils and basophils is limited (see above), more dataare available for mast cells. Several studies suggest that ATP-generation via glucose metabolism plays an important role in acute, FcεRI-mediated mast cell activation (Fig. 4).

This article is protected by copyright. All rights reserved

Already 55 years ago Chakravarty and colleagues reported increased glycolytic rates in*ex vivo*-isolated rat mast cells(121). Inhibition of glucose metabolism by 2-DG had no effect on compound 48/80-induced histamine release from rat mast cells while anaphylactic (IgE-mediated) mediator release was inhibited by high concentrations of 2-DG (20-80 nM resulting in 30 to 55% inhibition)(122) (Fig. 4D). These early results suggested, both glucose-transport and -utilization via glycolysis to supporthistamine release from mast cells. In line with an increased glucose metabolism, the concentrations of both pyruvate(123) and ATP(124) were lower in mast cells after antigen challenge.

Concordantly, Takei and colleagues reported depletion of glucose from the culture medium of rat peritoneal mast cells to time-dependently decrease anti-IgE-induced histamine release*in vitro*(125). This reduction of mediator release was suggested to be due to the inhibition of ATP-dependent Ca^{2+}-release from intracellular calcium stores(125). Moreover, mast cells isolated from rats infected with the parasite *Nippostrongylusbrasiliensis* as well as rat peritoneal mast cells *ex vivo*-stimulated with either compound 48/80, polymyxin B, or melittin were reported to both secrete histamine and produce lactate(126).

In accordance with the mainly glycolysis-driven activation of mast cells, inhibiting glycolytic ATP-production in bone marrow-derived mast cells (BMMCs) *in vitro*was shown to attenuate IL-33-mediated mast cell function while inhibiting OxPhoshad little effect on cytokine production(37). Moreover, inhibition of mast cell glycolysis also inhibited IL-33-induced neutrophil recruitment and cytokine production *in vivo*(37).

FcεRI-meditated activation of rat basophil leukemia cells (RBL-2H3) *in vitro*resulted in reduced activity of the glycolytic enzyme M2-type pyruvate kinase (M2PK) which regulates the terminal step of glycolysis(127). Interestingly, FcεRI-meditated inactivation of M2PK, likely resulting in the accumulation of glycolytic intermediates, was shown to be required for mast cell degranulation *in vitro*, suggesting a link between mast cellmetabolism and effector function(127) (Fig. 4D). Moreover, these glycolytic intermediates are also used for the synthesis of e.g. phospholipids or diacylglycerols, molecules that play important roles in mast cell degranulation(128,129).

Concordant with the above-described glucose-dependency of mast cell degranulation, long-term *in vitro*culture of BMMCs with 33 mM glucose increased the FcεRI-dependent release of β-hexosaminidase and leukotriene C4 while having no effect on mast cell viability, surface expression levels of FcεRI, intracellular ATP levels, calcium signaling, or IL-6 secretion(130). These results suggest glucose availability to act as a regulating factor for FcεRI-mediated mast cell responses. Mechanistically, long-term culture with glucose was shown to induce a FcεRI-dependent phosphorylation of cytosolic phospholipase A2 at residue Ser505*in vitro*(130). Since mast cells cultured with glucose also showed increased degranulation upon stimulation with

This article is protected by copyright. All rights reserved

suboptimal antigen concentrations, prolonged high-glucose culture may increase mast cell sensitivity to low antigen doses and therefore have profound effects on the threshold with which allergic reactions are induced(130).

In accordance with the results by Kitahata, culture of the human mast cell lines HMC-1 and LAD2 in high glucose medium (25 mM) increased the levels of intracellular ROS and phosphorylation of ERK-, c-Jun N-terminal kinase- (JNK), and p38-MAPK, which in turn promoted production of pro-inflammatory (TNF-α, IL-1β, IL-6) and pro-allergic cytokines (IL-13)(131). Of note, β-hexosaminindaseproduction was increased in LAD2 cells, while its release was independent of glucose concentration(131). These findings indicate, that ROS induced by *in vitro*culture in high-glucose medium activate pro-inflammatory and pro-allergic cytokine production in mast cells via MAPK-signaling (Fig. 4D).

Erlich and co-authors described mitochondrial-, MAPK-dependent-, and STAT3-driven OxPhosto contribute to IgE-antigen-mediated degranulation of human and mouse mast cells*in vitro*(132) (Fig. 4D) suggesting, that not only glycolysis but also ATP-production by OxPhosin the mitochondrion is involved in fulfilling the energy demands of activated mast cells. In contrast to the before-mentioned studies, *in vitro*mast cell degranulation occurred even in glucose-free medium(132). However, in accordance with the above-mentioned studies, Erlich and colleagues also found MAPK-signaling to be a pivotal pathway connecting mast cell function and metabolism(132) (Fig. 4D).

Phong*et al.*also reported FcεRI-mediated activation of mouse BMMCs*in vitro*to result in a rapid, T cell immunoglobulin and mucin-domain containing protein 3 (TIM3)-dependent induction of glycolysis, while OxPhoswas mostly unchanged(133) (Fig. 4D). These changes are reminiscent of immediate reprogramming observed upon lymphocyte activation(133). Interestingly, at later time points, IgE/Ag-stimulated mast cells displayed a broader metabolic potential *in vitro* with increases in both mast cell capacity for glycolysis and OxPhos, determined by increased OCR and spare respiratory capacity, and both higher ECARand glycolytic reserves, respectively(133) (Fig. 4D). In this experimental setting, inhibition of OxPhosalso decreased both degranulation and cytokine production(133). The observed time-dependent differences in mast cell mitochondrial respiration were suggested to result from more complex, transcriptional reprogramming events which have longer lead times and are therefore not observed in „acute“mast cell stimulations(133). Of note, FAO was found to be dispensable for mast cell activation *in vitro* since the FAO inhibitor etomoxir neither inhibited IgE/Ag-induced mast cell activation, IL-6 production, nor degranulation(133).

To investigate the role of autophagy in mast cell development and effector function, Ushio *et al.* generated BMMCs from mice lacking the essential autophagy-inducing protein Atg7(134). While autophagy was constitutively induced in BMMCs, even under full nutrient conditions, deletion of Atg7 neither impaired BMMC-development, Ca^{2+} mobilization, induction of early signaling

This article is protected by copyright. All rights reserved

molecules, activation of MAPK and NFκB, nor cytokine production upon FcεRI crosslinking(134). However, Atg7-deficent BMMCs showed a strongly impaired ability to degranulate *in vitro*(134). Moreover, cellular ATP content of Atg7-deficient BMMCs was increased compared to controls, suggesting that the observed disruption of degranulation was not caused by energy limitations(134). *In vivo*, reconstitution of mast cell-deficient mice with Atg7-deficient BMMCs strongly impaired passive cutaneous anaphylaxis reactions, demonstrating the *in vivo* relevance of autophagy for mast cell function(134).

In contrast to this,Nian*et al.* reported the inhibition of IL-33-receptor (ST2)/PI3K/mTOR-mediated autophagy in mast cells to induce degranulation and release of inflammatory mediators *in vitro*(135).

In summary, the available data suggest short-term mast cell activation to mainly rely on increased glycolysis while OxPhosalso contributes to mast cell metabolism upon longer stimulation (Fig. 4A).

3.7 ILC2s

In contrast to either basophils, eosinophils, or mast cells, ILC2s were shown to predominantly depend on FAO for their effector function in the context of either helminth infection or malnutrition(136) (Fig. 4E). Here, *ex vivo*-isolated ILC2s displayed constitutive fatty acid uptake(136), and both ILC2 accumulation and ILC2-derived IL-13 production in micewere shown to depend on fatty acid uptake and oxidation while being independent of glucose(136) (Fig. 4E). *In vivo*, blockade of FAO in a model of *Trichurismuris* infection significantly reduced ILC2 accumulation, IL-5-, and IL-13production(136). This dependency of ILC2 function on fatty acids rather than glucose may be the evolutionary result of their need to function in the locally nutrient-(and glucose)-deprived environments created by parasites at sites of infection(137).

In addition to the subordinate role of glycolysis in ILC2 function, Wagner *et al.* reported tumor-derived lactate to suppress local ILC2-proliferation, -survival, and -effector function*in vivo*(138). This suppression of the IL-33/ILC2/eosinophil tumor surveillance axis resulted in enhanced tumor growth(138).

Galle-Trager *et al.* also showed FAO to be critical for ILC2 function(139). Lack of autophagy, which has been described as a major pathway for lipid breakdown by targeting lipids to autophagosomes(140) in Atg5-deficient mice,impaired FAO resulting in increased levels of glycolysis as well as apoptosis in activated ILC2s(139). This shift towards glycolytic metabolism disrupted ILC2 homeostasis and Th2 cytokine production, resulting in reduced ILC2-mediated airway hyperreactivity*in vivo*(139). Moreover, induction of glycolysis was paralleled by reduced cellular ATP-concentrations and accumulation of dysfunctional mitochondria producing excessive amounts of ROS in *ex vivo*-isolated lung ILC2s(139) (Fig. 4E). Taken together, these results

This article is protected by copyright. All rights reserved

confirm the importance of FAO for ILC2 function while also showing autophagy to provide free fatty acids through lipid droplet degradation in order to maintain ILC2 energy balance (Fig. 4E).

In line with these results, Li *et al.* reported a critical role for the E3 ubiquitin ligase von-Hippel-Lindau (VHL) in ILC2 maturation by preventing HIF-1a-mediated induction of glycolysis and inducing IL-33 receptor (ST2) expression(141) (Fig. 4E). Here, in *ex vivo*-isolated ILC2s, reduced ST2 expression was caused by HIF-1a-driven expression of the glycolytic enzyme M2PK, resulting in epigenetic modification of the ST2(141) (Fig. 4E). Based on their results, Li *et al.* proposed a model in which imbalances in the equilibrium between glycolysis and OxPhosalter epigenetic modifications of genes important for ILC2 function (e.g. ST2)(141).

Contradictory to the above mentioned studies, ILC2s were also shown to partly depend on aerobic glycolysis driven by the urea cycle enzyme arginase 1 (Arg1), which metabolizes L-arginine(142). Monticelli showed both mature human and mouse ILC2s to constitutively express Arg1, generating the downstream metabolites proline and polyamines, which in turn drive collagen-synthesis and other bioenergetic pathways critical for cell proliferation(142–144). Genetic deletion of Arg1 in ILC2s resulted in reduced ILC2 function with abrogated papain-induced type 2 lung inflammation*in vivo* due to disrupted ILC2 proliferation and strongly reduced Th2 cytokine production(142). Arg1 inhibition was shown to disrupt amino acid metabolism in *ex vivo*-isolated ILC2s, thereby preventing the generation of L-arginine-derived polyamines which reduced rates of aerobic glycolysis(142).

In summary, the currently available studies suggest that ILC2s (in contrast to e.g. basophils, eosinophils, and mast cells) mainly require FAO for proper development and effector function, while a metabolic shift towards glycolysis may impair ILC2 function (Fig. 4A&E).

4. Conclusion

Type I allergies are the result of complex interactions between different immune cells. In order to fulfill their effector function, the key cell types of allergic inflammation were reported to change their metabolic phenotype during activation, shifting to either glycolysis (epithelial cells, M1 macrophages, DCs, eosinophils, basophils, acutely activated mast cells), OxPhos (M2 macrophages, longer term activated mast cells), or FAO (ILC2s).

In addition to these cell intrinsic changes in metabolic phenotypes, the local microenvironment in which immune responses are initiated and maintained can change the metabolism and therefore the functional properties of the infiltrating immune cells. For example, barrier surfaces are metabolically distinct from the rest of the body with both decreased oxygen tension and lower nutrient levels, especially at sites of inflammation(133).

Therefore, immunometabolism clearly is of importance in allergic diseases.Understanding the

This article is protected by copyright. All rights reserved

metabolic changes associated with the activation of the key immune cell types involved in the induction and maintenance of allergic responses will likely result in a more complete understanding of the disease pathology as well as the identification of novel target molecules and strategies for the treatment of allergic diseases. Indeed, immune metabolic analyses have already identified glycolytic ATP-production(37) and STAT3(132) in mast cells, as well as Arg1(142), VHL(141), and autophagy(139)in ILC2s as potential targets to modulate allergic responses (see above). Further studies can only improve our understanding of the complex and fascinating connections between immune cell metabolism and effector function.

Accepted Article

This article is protected by copyright. All rights reserved

References

1. Flöistrup H, Swartz J, Bergström A, Alm JS, Scheynius A, van Hage M et al. Allergic disease and sensitization in Steiner school children. *J Allergy Clin Immunol* 2006;**117**:59–66.
2. Larché M, Akdis C a, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006;**6**:761–771.
3. Samuchiwal SK, Boyce JA. Role of lipid mediators and control of lymphocyte responses in type 2 immunopathology. *J Allergy Clin Immunol* 2018;**141**:1182–1190.
4. Sokolowska M, Rovati GE, Diamant Z, Untersmayr E, Schwarze J, Lukasik Z et al. Current perspective on eicosanoids in asthma and allergic diseases: EAACI Task Force consensus report, part I. *Allergy* 2021;**76**:114–130.
5. Nicosia S, Capra V, Rovati GE. Leukotrienes as mediators of asthma. *Pulm Pharmacol Ther* 2001;**14**:3–19.
6. Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. *J Exp Med* 2015;**212**:1345–1360.
7. Van den Bossche J, O'Neill LA, Menon D. Macrophage Immunometabolism: Where Are We (Going)? *Trends Immunol* 2017;**38**:395–406.
8. Borregaard N, Herlin T. Energy metabolism of human neutrophils during phagocytosis. *J Clin Invest* 1982;**70**:550–557.
9. Azevedo EP, Rochael NC, Guimarães-Costa AB, de Souza-Vieira TS, Ganilho J, Saraiva EM et al. A Metabolic Shift toward Pentose Phosphate Pathway Is Necessary for Amyloid Fibril- and Phorbol 12-Myristate 13-Acetate-induced Neutrophil Extracellular Trap (NET) Formation. *J Biol Chem* 2015;**290**:22174–22183.
10. Michaeloudes C, Bhavsar PK, Mumby S, Xu B, Hui CKM, Chung KF et al. Role of Metabolic Reprogramming in Pulmonary Innate Immunity and Its Impact on Lung Diseases. *J Innate Immun* 2020;**12**:31–46.
11. Adeva-Andany M, López-Ojén M, Funcasta-Calderón R, Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M et al. Comprehensive review on lactate metabolism in human health. *Mitochondrion* 2014;**17**:76–100.
12. van der Bliek AM, Sedensky MM, Morgan PG. Cell Biology of the Mitochondrion. *Genetics* 2017;**207**:843–871.
13. Corbet C, Feron O. Metabolic and mind shifts: from glucose to glutamine and acetate addictions in cancer. *Curr Opin Clin Nutr Metab Care* 2015;**18**:346–353.
14. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* 2016;**354**:481–484.
15. Tyrakis PA, Palazon A, Macias D, Lee KL, Phan AT, Veliça P et al. S-2-hydroxyglutarate regulates CD8+ T-lymphocyte fate. *Nature* 2016;**540**:236–241.
16. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 2009;**324**:1076–1080.
17. Kovács I, Horváth M, Kovács T, Somogyi K, Tretter L, Geiszt M et al. Comparison of proton channel, phagocyte oxidase, and respiratory burst levels between human eosinophil and neutrophil granulocytes. *Free Radic Res* 2014;**48**:1190–1199.
18. Gaurav R, Bewtra AK, Agrawal DK. Chloride Channel 3 Channels in the Activation and Migration of Human Blood Eosinophils in Allergic Asthma. *Am J Respir Cell Mol Biol* 2015;**53**:235–245.
19. Jones N, Vincent EE, Felix LC, Cronin JG, Scott LM, Hole PS et al. Interleukin-5 drives glycolysis and

This article is protected by copyright. All rights reserved

reactive oxygen species-dependent citric acid cycling by eosinophils. *Allergy* 2020;**75**:1361–1370.

20. Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol* 2011;**27**:441–464.

21. Kim J, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006;**3**:177–185.

22. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* 2004;**4**:891–899.

23. Jones W, Bianchi K. Aerobic glycolysis: beyond proliferation. *Front Immunol* 2015;**6**:227.

24. Warburg O. On the origin of cancer cells. *Science* 1956;**123**:309–314.

25. Ros S, Schulze A. Balancing glycolytic flux: the role of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatases in cancer metabolism. *Cancer Metab* 2013;**1**:8.

26. Vazquez A, Liu J, Zhou Y, Oltvai ZN. Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. *BMC Syst Biol* 2010;**4**:58.

27. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nat Rev Immunol* 2014;**14**:435–446.

28. Yagi Y, Kuwahara M, Suzuki J, Imai Y, Yamashita M. Glycolysis and subsequent mevalonate biosynthesis play an important role in Th2 cell differentiation. *Biochem Biophys Res Commun* 2020;**530**:355–361.

29. Pfeiffer T, Schuster S, Bonhoeffer S. Cooperation and competition in the evolution of ATP-producing pathways. *Science* 2001;**292**:504–507.

30. Linke M, Fritsch SD, Sukhbaatar N, Hengstschläger M, Weichhart T. mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett* 2017;**591**:3089–3103.

31. Ostroukhova M, Goplen N, Karim MZ, Michalec L, Guo L, Liang Q et al. The role of low-level lactate production in airway inflammation in asthma. *Am J Physiol Lung Cell Mol Physiol* 2012;**302**:L300-307.

32. Appel D, Rubenstein R, Schrager K, Williams MH. Lactic acidosis in severe asthma. *Am J Med* 1983;**75**:580–584.

33. Alberts WM, Williams JH, Ramsdell JW. Metabolic acidosis as a presenting feature in acute asthma. *Ann Allergy* 1986;**57**:107–109.

34. Mountain RD, Heffner JE, Brackett NC, Sahn SA. Acid-base disturbances in acute asthma. *Chest* 1990;**98**:651–655.

35. Kelly B, O'Neill LAJ. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* 2015;**25**:771–784.

36. Loftus RM, Finlay DK. Immunometabolism: Cellular Metabolism Turns Immune Regulator. *J Biol Chem* 2016;**291**:1–10.

37. Caslin HL, Taruselli MT, Haque T, Pondicherry N, Baldwin EA, Barnstein BO et al. Inhibiting Glycolysis and ATP Production Attenuates IL-33-Mediated Mast Cell Function and Peritonitis. *Front Immunol* 2018;**9**:3026.

38. Jha AK, Huang SC-C, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 2015;**42**:419–430.

39. O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol*

This article is protected by copyright. All rights reserved

2016;**16**:553–565.

40. Ryan DG, O'Neill LAJ. Krebs cycle rewired for macrophage and dendritic cell effector functions. *FEBS Lett* 2017;**591**:2992–3006.

41. Arts RJW, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E et al. Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. *Cell Metab* 2016;**24**:807–819.

42. Dominguez-Andres J, Netea MG. Long-term reprogramming of the innate immune system. *J Leukoc Biol* 2019;**105**:329–338.

43. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 2020;**20**:375–388.

44. Mitroulis I, Ruppova K, Wang B, Chen L-S, Grzybek M, Grinenko T et al. Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. *Cell* 2018;**172**:147-161.e12.

45. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* 2009;**457**:557–561.

46. Fanucchi S, Domínguez-Andrés J, Joosten LAB, Netea MG, Mhlanga MM. The Intersection of Epigenetics and Metabolism in Trained Immunity. *Immunity* 2021;**54**:32–43.

47. Domínguez-Andrés J, Joosten LA, Netea MG. Induction of innate immune memory: the role of cellular metabolism. *Curr Opin Immunol* 2019;**56**:10–16.

48. Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG. Therapeutic targeting of trained immunity. *Nat Rev Drug Discov* 2019;**18**:553–566.

49. Eljaszewicz A, Ruchti F, Radzikowska U, Globinska A, Boonpiyathad T, Gschwend A et al. Trained immunity and tolerance in innate lymphoid cells, monocytes, and dendritic cells during allergen-specific immunotherapy. *J Allergy Clin Immunol* Published Online First: 9 October 2020. doi:10.1016/j.jaci.2020.08.042

50. Quintin J, Saeed S, Martens JHA, Giamarellos-Bourboulis EJ, Ifrim DC, Logie C et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 2012;**12**:223–232.

51. Bekkering S, Quintin J, Joosten LAB, van der Meer JWM, Netea MG, Riksen NP. Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arterioscler Thromb Vasc Biol* 2014;**34**:1731–1738.

52. Crişan TO, Cleophas MCP, Novakovic B, Erler K, van de Veerdonk FL, Stunnenberg HG et al. Uric acid priming in human monocytes is driven by the AKT-PRAS40 autophagy pathway. *Proc Natl Acad Sci U S A* 2017;**114**:5485–5490.

53. Chen F, Wu W, Millman A, Craft JF, Chen E, Patel N et al. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. *Nat Immunol* 2014;**15**:938–946.

54. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS et al. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 2007;**447**:326–329.

55. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K et al. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell* 2018;**172**:162-175.e14.

56. Saeed S, Quintin J, Kerstens HHD, Rao NA, Aghajanirefah A, Matarese F et al. Epigenetic programming of

This article is protected by copyright. All rights reserved

monocyte-to-macrophage differentiation and trained innate immunity. *Science* 2014;**345**:1251086.

57. Netea MG, Quintin J, van der Meer JWM. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 2011;**9**:355–361.

58. Netea MG, Joosten LAB, Latz E, Mills KHG, Natoli G, Stunnenberg HG et al. Trained immunity: A program of innate immune memory in health and disease. *Science* 2016;**352**:aaf1098.

59. Benito-Villalvilla C, Soria I, Pérez-Diego M, Fernández-Caldas E, Subiza JL, Palomares O. Alum impairs tolerogenic properties induced by allergoid-mannan conjugates inhibiting mTOR and metabolic reprogramming in human DCs. *Allergy* 2020;**75**:648–659.

60. Stark JM, Tibbitt CA, Coquet JM. The Metabolic Requirements of Th2 Cell Differentiation. *Front Immunol* 2019;**10**:2318.

61. Pelgrom LR, Everts B. Metabolic control of type 2 immunity. *Eur J Immunol* 2017;**47**:1266–1275.

62. Boothby M, Rickert RC. Metabolic Regulation of the Immune Humoral Response. *Immunity* 2017;**46**:743–755.

63. Masterson JC, Biette KA, Hammer JA, Nguyen N, Capocelli KE, Saeedi BJ et al. Epithelial HIF-1α/claudin-1 axis regulates barrier dysfunction in eosinophilic esophagitis. *J Clin Invest* 2019;**129**:3224–3235.

64. van de Wetering C, Aboushousha R, Manuel AM, Chia SB, Erickson C, MacPherson MB et al. Pyruvate Kinase M2 Promotes Expression of Proinflammatory Mediators in House Dust Mite-Induced Allergic Airways Disease. *J Immunol* 2020;**204**:763–774.

65. Bueno M, Calyeca J, Rojas M, Mora AL. Mitochondria dysfunction and metabolic reprogramming as drivers of idiopathic pulmonary fibrosis. *Redox Biol* 2020;**33**:101509.

66. Xu W, Ghosh S, Comhair SAA, Asosingh K, Janocha AJ, Mavrakis DA et al. Increased mitochondrial arginine metabolism supports bioenergetics in asthma. *J Clin Invest* 2016;**126**:2465–2481.

67. Mabalirajan U, Dinda AK, Kumar S, Roshan R, Gupta P, Sharma SK et al. Mitochondrial structural changes and dysfunction are associated with experimental allergic asthma. *J Immunol* 2008;**181**:3540–3548.

68. Aguilera-Aguirre L, Bacsi A, Saavedra-Molina A, Kurosky A, Sur S, Boldogh I. Mitochondrial dysfunction increases allergic airway inflammation. *J Immunol* 2009;**183**:5379–5387.

69. Rodriguez-Perez N, Schiavi E, Frei R, Ferstl R, Wawrzyniak P, Smolinska S et al. Altered fatty acid metabolism and reduced stearoyl-coenzyme a desaturase activity in asthma. *Allergy* 2017;**72**:1744–1752.

70. Qian X, Aboushousha R, van de Wetering C, Chia SB, Amiel E, Schneider RW et al. IL-1/inhibitory κB kinase ε-induced glycolysis augment epithelial effector function and promote allergic airways disease. *J Allergy Clin Immunol* 2018;**142**:435-450.e10.

71. Unno N, Menconi MJ, Salzman AL, Smith M, Hagen S, Ge Y et al. Hyperpermeability and ATP depletion induced by chronic hypoxia or glycolytic inhibition in Caco-2BBe monolayers. *Am J Physiol* 1996;**270**:G1010-1021.

72. Obeso D, Mera-Berriatua L, Rodríguez-Coira J, Rosace D, Fernández P, Martín-Antoniano IA et al. Multi-omics analysis points to altered platelet functions in severe food-associated respiratory allergy. *Allergy* 2018;**73**:2137–2149.

73. Biswas SK, Chittezhath M, Shalova IN, Lim J-Y. Macrophage polarization and plasticity in health and disease. *Immunol Res* 2012;**53**:11–24.

This article is protected by copyright. All rights reserved

74. Murray PJ. Macrophage Polarization. *Annu Rev Physiol* 2017;**79**:541–566.
75. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;**3**:23–35.
76. Feingold KR, Shigenaga JK, Kazemi MR, McDonald CM, Patzek SM, Cross AS et al. Mechanisms of triglyceride accumulation in activated macrophages. *J Leukoc Biol* 2012;**92**:829–839.
77. Yu Q, Wang Y, Dong L, He Y, Liu R, Yang Q et al. Regulations of Glycolytic Activities on Macrophages Functions in Tumor and Infectious Inflammation. *Front Cell Infect Microbiol* 2020;**10**:287.
78. Suzuki H, Hisamatsu T, Chiba S, Mori K, Kitazume MT, Shimamura K et al. Glycolytic pathway affects differentiation of human monocytes to regulatory macrophages. *Immunol Lett* 2016;**176**:18–27.
79. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 2010;**115**:4742–4749.
80. Everts B, Amiel E, van der Windt GJW, Freitas TC, Chott R, Yarasheski KE et al. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 2012;**120**:1422–1431.
81. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 2013;**496**:238–242.
82. Michl J, Ohlbaum DJ, Silverstein SC. 2-Deoxyglucose selectively inhibits Fc and complement receptor-mediated phagocytosis in mouse peritoneal macrophages II. Dissociation of the inhibitory effects of 2-deoxyglucose on phagocytosis and ATP generation. *J Exp Med* 1976;**144**:1484–1493.
83. Guak H, Al Habyan S, Ma EH, Aldossary H, Al-Masri M, Won SY et al. Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. *Nat Commun* 2018;**9**:2463.
84. Everts B, Amiel E, Huang SC-C, Smith AM, Chang C-H, Lam WY et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat Immunol* 2014;**15**:323–332.
85. Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N et al. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* 2003;**112**:645–657.
86. Li C, Wang Y, Li Y, Yu Q, Jin X, Wang X et al. HIF1α-dependent glycolysis promotes macrophage functional activities in protecting against bacterial and fungal infection. *Sci Rep* 2018;**8**:3603.
87. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG et al. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. *J Immunol* 2008;**180**:4697–4705.
88. Bhandari T, Olson J, Johnson RS, Nizet V. HIF-1α influences myeloid cell antigen presentation and response to subcutaneous OVA vaccination. *J Mol Med (Berl)* 2013;**91**:1199–1205.
89. Ryan DG, O'Neill LAJ. Krebs Cycle Reborn in Macrophage Immunometabolism. *Annu Rev Immunol* 2020;**38**:289–313.
90. Sukhbaatar N, Hengstschläger M, Weichhart T. mTOR-Mediated Regulation of Dendritic Cell Differentiation and Function. *Trends Immunol* 2016;**37**:778–789.
91. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* 2006;**4**:13–24.
92. Liu P-S, Wang H, Li X, Chao T, Teav T, Christen S et al. α-ketoglutarate orchestrates macrophage activation

This article is protected by copyright. All rights reserved

through metabolic and epigenetic reprogramming. *Nat Immunol* 2017;**18**:985–994.

93. Melgert BN, ten Hacken NH, Rutgers B, Timens W, Postma DS, Hylkema MN. More alternative activation of macrophages in lungs of asthmatic patients. *J Allergy Clin Immunol* 2011;**127**:831–833.

94. Girodet P-O, Nguyen D, Mancini JD, Hundal M, Zhou X, Israel E et al. Alternative Macrophage Activation Is Increased in Asthma. *Am J Respir Cell Mol Biol* 2016;**55**:467–475.

95. Jin Q-W, Zhang N-Z, Li W-H, Qin H-T, Liu Y-J, Ohiolei JA et al. Trichinella spiralis Thioredoxin Peroxidase 2 Regulates Protective Th2 Immune Response in Mice by Directly Inducing Alternatively Activated Macrophages. *Front Immunol* 2020;**11**:2015.

96. Kim EG, Kim MN, Hong JY, Lee JW, Kim SY, Kim KW et al. Chitinase 3-Like 1 Contributes to Food Allergy via M2 Macrophage Polarization. *Allergy Asthma Immunol Res* 2020;**12**:1012–1028.

97. Otobe S, Hisamoto T, Miyagaki T, Morimura S, Suga H, Sugaya M et al. CX3CR1 Deficiency Attenuates DNFB-Induced Contact Hypersensitivity Through Skewed Polarization Towards M2 Phenotype in Macrophages. *Int J Mol Sci* 2020;**21**. doi:10.3390/ijms21197401

98. Tiotiu A, Zounemat Kermani N, Badi Y, Pavlidis S, Hansbro PM, Guo Y-K et al. Sputum macrophage diversity and activation in asthma: Role of severity and inflammatory phenotype. *Allergy* Published Online First: 1 August 2020. doi:10.1111/all.14535

99. Cheng G, Swaidani S, Sharma M, Lauer ME, Hascall VC, Aronica MA. Correlation of hyaluronan deposition with infiltration of eosinophils and lymphocytes in a cockroach-induced murine model of asthma. *Glycobiology* 2013;**23**:43–58.

100. Sokolowska M, Chen L-Y, Eberlein M, Martinez-Anton A, Liu Y, Alsaaty S et al. Low molecular weight hyaluronan activates cytosolic phospholipase A2α and eicosanoid production in monocytes and macrophages. *J Biol Chem* 2014;**289**:4470–4488.

101. Elesela S, Morris SB, Narayanan S, Kumar S, Lombard DB, Lukacs NW. Sirtuin 1 regulates mitochondrial function and immune homeostasis in respiratory syncytial virus infected dendritic cells. *PLoS Pathog* 2020;**16**:e1008319.

102. Sinclair C, Bommakanti G, Gardinassi L, Loebbermann J, Johnson MJ, Hakimpour P et al. mTOR regulates metabolic adaptation of APCs in the lung and controls the outcome of allergic inflammation. *Science* 2017;**357**:1014–1021.

103. Sher R, Wadee A, Joffe M. The enhancement of eosinophil function by lymphocyte supernatants. *Clin Exp Immunol* 1983;**51**:525–534.

104. Porter L, Toepfner N, Bashant KR, Guck J, Ashcroft M, Farahi N et al. Metabolic Profiling of Human Eosinophils. *Front Immunol* 2018;**9**:1404.

105. Peachman KK, Lyles DS, Bass DA. Mitochondria in eosinophils: functional role in apoptosis but not respiration. *Proc Natl Acad Sci USA* 2001;**98**:1717–1722.

106. Kottyan LC, Collier AR, Cao KH, Niese KA, Hedgebeth M, Radu CG et al. Eosinophil viability is increased by acidic pH in a cAMP- and GPR65-dependent manner. *Blood* 2009;**114**:2774–2782.

107. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F et al. Lactate Regulates Metabolic and Pro-inflammatory Circuits in Control of T Cell Migration and Effector Functions. *PLoS Biol*

This article is protected by copyright. All rights reserved

2015;**13**:e1002202.
108. Germic N, Frangez Z, Yousefi S, Simon H-U. Regulation of the innate immune system by autophagy: neutrophils, eosinophils, mast cells, NK cells. *Cell Death Differ* 2019;**26**:703–714.
109. Hua W, Liu H, Xia L-X, Tian B-P, Huang H-Q, Chen Z-Y et al. Rapamycin inhibition of eosinophil differentiation attenuates allergic airway inflammation in mice. *Respirology* 2015;**20**:1055–1065.
110. Zhu C, Xia L, Li F, Zhou L, Weng Q, Li Z et al. mTOR complexes differentially orchestrates eosinophil development in allergy. *Sci Rep* 2018;**8**:6883.
111. Liu J-N, Suh D-H, Trinh HKT, Chwae Y-J, Park H-S, Shin YS. The role of autophagy in allergic inflammation: a new target for severe asthma. *Exp Mol Med* 2016;**48**:e243.
112. Ban G-Y, Pham DL, Trinh THK, Lee S-I, Suh D-H, Yang E-M et al. Autophagy mechanisms in sputum and peripheral blood cells of patients with severe asthma: a new therapeutic target. *Clin Exp Allergy* 2016;**46**:48–59.
113. Germic N, Stojkov D, Oberson K, Yousefi S, Simon H-U. Neither eosinophils nor neutrophils require ATG5-dependent autophagy for extracellular DNA trap formation. *Immunology* 2017;**152**:517–525.
114. Silveira JS, Antunes GL, Kaiber DB, da Costa MS, Ferreira FS, Marques EP et al. Autophagy induces eosinophil extracellular traps formation and allergic airway inflammation in a murine asthma model. *J Cell Physiol* 2020;**235**:267–280.
115. Choi GE, Yoon S-Y, Kim J-Y, Kang D-Y, Jang YJ, Kim HS. Autophagy deficiency in myeloid cells exacerbates eosinophilic inflammation in chronic rhinosinusitis. *J Allergy Clin Immunol* 2018;**141**:938-950.e12.
116. Sumbayev VV, Nicholas SA, Streatfield CL, Gibbs BF. Involvement of hypoxia-inducible factor-1 HiF(1alpha) in IgE-mediated primary human basophil responses. *Eur J Immunol* 2009;**39**:3511–3519.
117. Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol Med* 2002;**8**:S62-67.
118. Oda T, Hirota K, Nishi K, Takabuchi S, Oda S, Yamada H et al. Activation of hypoxia-inducible factor 1 during macrophage differentiation. *Am J Physiol, Cell Physiol* 2006;**291**:C104-113.
119. Walmsley SR, Cadwallader KA, Chilvers ER. The role of HIF-1alpha in myeloid cell inflammation. *Trends Immunol* 2005;**26**:434–439.
120. Sumbayev VV, Yasinska I, Oniku AE, Streatfield CL, Gibbs BF. Involvement of hypoxia-inducible factor-1 in the inflammatory responses of human LAD2 mast cells and basophils. *PLoS ONE* 2012;**7**:e34259.
121. Chakravarty N. GLYCOLYSIS IN RAT PERITONEAL MAST CELLS. *J Cell Biol* 1965;**25**:123–128.
122. Chakravarty N. Further observations on the inhibition of histamine release by 2-deoxyglucose. *Acta Physiol Scand* 1968;**72**:425–432.
123. Pleshkova SM, Tsvetkova TV. [Redox processes in allergic reactions of the delayed type to microbial antigens]. *Biull Eksp Biol Med* 1978;**86**:347–349.
124. Norn S, Elmgreen J, Stahl Skov P, Holme Jorgensen P, Ankjaergaard N, Hagen Petersen S. Influence of hyposensitization of ATP level and CO2 production of mast cells in anaphylaxis. *Clin Exp Immunol* 1976;**26**:162–168.
125. Takei M, Endo K. Histamine release and calcium concentrations in rat mast cells are dependent on intracellular ATP: effects of prostaglandin D2. *Prostaglandins Leukot Essent Fatty Acids* 1994;**50**:357–362.
126. Yoshizaki K, Arizono N, Hayano T, Watari H. Allergen-induced histamine secretion associated with lactate

This article is protected by copyright. All rights reserved

production in mast cells detected by 1H NMR. *Magn Reson Med* 1993;**29**:732–736.

127. Ryu H, Walker JKL, Kim S, Koo N, Barak LS, Noguchi T et al. Regulation of M2-type pyruvate kinase mediated by the high-affinity IgE receptors is required for mast cell degranulation. *Br J Pharmacol* 2008;**154**:1035–1046.

128. Bell RM, Coleman RA. Enzymes of glycerolipid synthesis in eukaryotes. *Annu Rev Biochem* 1980;**49**:459–487.

129. Cohen JS, Brown HA. Phospholipases stimulate secretion in RBL mast cells. *Biochemistry* 2001;**40**:6589–6597.

130. Kitahata Y, Nunomura S, Terui T, Ra C. Prolonged culture of mast cells with high-glucose medium enhances the Fc epsilon RI-mediated degranulation response and leukotriene C4 production. *Int Arch Allergy Immunol* 2010;**152 Suppl 1**:22–31.

131. Nagai K, Fukushima T, Oike H, Kobori M. High glucose increases the expression of proinflammatory cytokines and secretion of TNFα and β-hexosaminidase in human mast cells. *Eur J Pharmacol* 2012;**687**:39–45.

132. Erlich TH, Yagil Z, Kay G, Peretz A, Migalovich-Sheikhet H, Tshori S et al. Mitochondrial STAT3 plays a major role in IgE-antigen-mediated mast cell exocytosis. *J Allergy Clin Immunol* 2014;**134**:460–469.

133. Phong B, Avery L, Menk AV, Delgoffe GM, Kane LP. Cutting Edge: Murine Mast Cells Rapidly Modulate Metabolic Pathways Essential for Distinct Effector Functions. *J Immunol* 2017;**198**:640–644.

134. Ushio H, Ueno T, Kojima Y, Komatsu M, Tanaka S, Yamamoto A et al. Crucial role for autophagy in degranulation of mast cells. *J Allergy Clin Immunol* 2011;**127**:1267-1276.e6.

135. Nian J-B, Zeng M, Zheng J, Zeng L-Y, Fu Z, Huang Q-J et al. Epithelial cells expressed IL-33 to promote degranulation of mast cells through inhibition on ST2/PI3K/mTOR-mediated autophagy in allergic rhinitis. *Cell Cycle* 2020;**19**:1132–1142.

136. Wilhelm C, Harrison OJ, Schmitt V, Pelletier M, Spencer SP, Urban JF et al. Critical role of fatty acid metabolism in ILC2-mediated barrier protection during malnutrition and helminth infection. *J Exp Med* 2016;**213**:1409–1418.

137. Budischak SA, Sakamoto K, Megow LC, Cummings KR, Urban JF, Ezenwa VO. Resource limitation alters the consequences of co-infection for both hosts and parasites. *Int J Parasitol* 2015;**45**:455–463.

138. Wagner M, Ealey KN, Tetsu H, Kiniwa T, Motomura Y, Moro K et al. Tumor-Derived Lactic Acid Contributes to the Paucity of Intratumoral ILC2s. *Cell Rep* 2020;**30**:2743-2757.e5.

139. Galle-Treger L, Hurrell BP, Lewis G, Howard E, Jahani PS, Banie H et al. Autophagy is critical for group 2 innate lymphoid cell metabolic homeostasis and effector function. *J Allergy Clin Immunol* 2020;**145**:502-517.e5.

140. Ouimet M, Franklin V, Mak E, Liao X, Tabas I, Marcel YL. Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase. *Cell Metab* 2011;**13**:655–667.

141. Li Q, Li D, Zhang X, Wan Q, Zhang W, Zheng M et al. E3 Ligase VHL Promotes Group 2 Innate Lymphoid Cell Maturation and Function via Glycolysis Inhibition and Induction of Interleukin-33 Receptor. *Immunity* 2018;**48**:258-270.e5.

142. Monticelli LA, Buck MD, Flamar A-L, Saenz SA, Tait Wojno ED, Yudanin NA et al. Arginase 1 is an innate lymphoid-cell-intrinsic metabolic checkpoint controlling type 2 inflammation. *Nat Immunol* 2016;**17**:656–665.

This article is protected by copyright. All rights reserved

143. Rath M, Müller I, Kropf P, Closs EI, Munder M. Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages. *Front Immunol* 2014;**5**:532.
144. Murray PJ. Amino acid auxotrophy as a system of immunological control nodes. *Nat Immunol* 2016;**17**:132–139.
145. Barber D, Villaseñor A, Escribese MM. Metabolomics strategies to discover new biomarkers associated to severe allergic phenotypes. *Asia Pac Allergy* 2019;**9**:e37.
146. Gordon BR, Leggat W, Motti CA. Extraction protocol for nontargeted NMR and LC-MS metabolomics-based analysis of hard coral and their algal symbionts. *Methods Mol Biol* 2013;**1055**:129–147.
147. Menk AV, Scharping NE, Moreci RS, Zeng X, Guy C, Salvatore S et al. Early TCR Signaling Induces Rapid Aerobic Glycolysis Enabling Distinct Acute T Cell Effector Functions. *Cell Rep* 2018;**22**:1509–1521.
148. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. *Cell Metab* 2016;**24**:657–671.
149. Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nat Med* 2009;**15**:1383–1391.
150. Giugliano D, Ceriello A, Esposito K. The effects of diet on inflammation: emphasis on the metabolic syndrome. *J Am Coll Cardiol* 2006;**48**:677–685.
151. Christ A, Lauterbach M, Latz E. Western Diet and the Immune System: An Inflammatory Connection. *Immunity* 2019;**51**:794–811.
152. Samson SL, Garber AJ. Metabolic syndrome. *Endocrinol Metab Clin North Am* 2014;**43**:1–23.
153. Patel S, Custovic A, Smith JA, Simpson A, Kerry G, Murray CS. Cross-sectional association of dietary patterns with asthma and atopic sensitization in childhood - in a cohort study. *Pediatr Allergy Immunol* 2014;**25**:565–571.
154. Vinding RK, Stokholm J, Chawes BLK, Bisgaard H. Blood lipid levels associate with childhood asthma, airway obstruction, bronchial hyperresponsiveness, and aeroallergen sensitization. *J Allergy Clin Immunol* 2016;**137**:68-74.e4.
155. Chen YC, Tung KY, Tsai CH, Su MW, Wang PC, Chen CH et al. Lipid profiles in children with and without asthma: interaction of asthma and obesity on hyperlipidemia. *Diabetes Metab Syndr* 2013;**7**:20–25.
156. Zhao M, Chiriboga D, Olendzki B, Xie B, Li Y, McGonigal LJ et al. Substantial Increase in Compliance with Saturated Fatty Acid Intake Recommendations after One Year Following the American Heart Association Diet. *Nutrients* 2018;**10**. doi:10.3390/nu10101486
157. Harrison S, Brassard D, Lemieux S, Lamarche B. Consumption and Sources of Saturated Fatty Acids According to the 2019 Canada Food Guide: Data from the 2015 Canadian Community Health Survey. *Nutrients* 2019;**11**. doi:10.3390/nu11091964
158. Liu Q, Matthan NR, Manson JE, Howard BV, Tinker LF, Neuhouser ML et al. Plasma Phospholipid Fatty Acids and Coronary Heart Disease Risk: A Matched Case-Control Study within the Women's Health Initiative Observational Study. *Nutrients* 2019;**11**. doi:10.3390/nu11071672
159. Rogero MM, Calder PC. Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. *Nutrients* 2018;**10**. doi:10.3390/nu10040432

This article is protected by copyright. All rights reserved

160. Listenberger LL, Han X, Lewis SE, Cases S, Farese RV, Ory DS et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A* 2003;**100**:3077–3082.

161. Ding Y, Subramanian S, Montes VN, Goodspeed L, Wang S, Han C et al. Toll-like receptor 4 deficiency decreases atherosclerosis but does not protect against inflammation in obese low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2012;**32**:1596–1604.

162. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 2006;**116**:3015–3025.

163. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT-H et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat Immunol* 2011;**12**:408–415.

164. von Schacky C. n-3 fatty acids and the prevention of coronary atherosclerosis. *Am J Clin Nutr* 2000;**71**:224S-7S.

165. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 2010;**142**:687–698.

166. Venter C, Meyer RW, Nwaru BI, Roduit C, Untersmayr E, Adel-Patient K et al. EAACI position paper: Influence of dietary fatty acids on asthma, food allergy, and atopic dermatitis. *Allergy* 2019;**74**:1429–1444.

167. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest* 2018;**128**:2657–2669.

168. Radzikowska U, Rinaldi AO, Çelebi Sözener Z, Karaguzel D, Wojcik M, Cypryk K et al. The Influence of Dietary Fatty Acids on Immune Responses. *Nutrients* 2019;**11**. doi:10.3390/nu11122990

169. de Matos OG, Amaral SS, Pereira da Silva PEM, Perez DA, Alvarenga DM, Ferreira AVM et al. Dietary supplementation with omega-3-PUFA-rich fish oil reduces signs of food allergy in ovalbumin-sensitized mice. *Clin Dev Immunol* 2012;**2012**:236564.

170. van den Elsen LWJ, Meulenbroek L a. PM, van Esch BC a. M, Hofman GA, Boon L, Garssen J et al. CD25+ regulatory T cells transfer n-3 long chain polyunsaturated fatty acids-induced tolerance in mice allergic to cow's milk protein. *Allergy* 2013;**68**:1562–1570.

171. Kull I, Bergström A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. *Allergy* 2006;**61**:1009–1015.

172. van Elten TM, van Rossem L, Wijga AH, Brunekreef B, de Jongste JC, Koppelman GH et al. Breast milk fatty acid composition has a long-term effect on the risk of asthma, eczema, and sensitization. *Allergy* 2015;**70**:1468–1476.

173. Shively CA, Appt SE, Vitolins MZ, Uberseder B, Michalson KT, Silverstein-Metzler MG et al. Mediterranean versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates. *Obesity (Silver Spring)* 2019;**27**:777–784.

174. Schatz M, Zeiger RS, Zhang F, Chen W, Yang S-J, Camargo CA. Overweight/obesity and risk of seasonal asthma exacerbations. *J Allergy Clin Immunol Pract* 2013;**1**:618–622.

175. Barros R, Moreira A, Fonseca J, Delgado L, Castel-Branco MG, Haahtela T et al. Dietary intake of α-linolenic acid and low ratio of n-6:n-3 PUFA are associated with decreased exhaled NO and improved asthma control. *Br J Nutr* 2011;**106**:441–450.

176. Burns JS, Dockery DW, Neas LM, Schwartz J, Coull BA, Raizenne M et al. Low dietary nutrient intakes and

This article is protected by copyright. All rights reserved

respiratory health in adolescents. *Chest* 2007;**132**:238–245.

177. Hemmelgarn B, Ernst P. Airway function among Inuit primary school children in far northern Quebec. *Am J Respir Crit Care Med* 1997;**156**:1870–1875.

178. Backer V, Nepper-Christensen S, Porsbjerg C, von Linstow M-L, Reersted P, Greenlandic Population Study. Respiratory symptoms in greenlanders living in Greenland and Denmark: a population-based study. *Ann Allergy Asthma Immunol* 2004;**93**:76–82.

179. Bruce SG, Riediger ND, Lix LM. Chronic disease and chronic disease risk factors among First Nations, Inuit and Métis populations of northern Canada. *Chronic Dis Inj Can* 2014;**34**:210–217.

180. Lauer ME, Majors AK, Comhair S, Ruple LM, Matuska B, Subramanian A et al. Hyaluronan and Its Heavy Chain Modification in Asthma Severity and Experimental Asthma Exacerbation. *J Biol Chem* 2015;**290**:23124–23134.

181. Sokolowska M, Chen L-Y, Liu Y, Martinez-Anton A, Logun C, Alsaaty S et al. Dysregulation of lipidomic profile and antiviral immunity in response to hyaluronan in patients with severe asthma. *J Allergy Clin Immunol* 2017;**139**:1379–1383.

Accepted Article

This article is protected by copyright. All rights reserved

Figure legends

Figure 1: Major pathways contributing to cellular energy production and metabolic shift associated with the Warburg effect. Under normal conditions (A), cells take up glucose from their medium that degrade it in the cytoplasm into pyruvate. Pyruvate is subsequently imported into the mitochondrion where it is used in the Krebs cycle to generate reduction equivalents (NADH). These reduction equivalents are then used employing oxygen as terminal electron acceptor to generate a proton gradient over the inner mitochondrial membrane (measured as oxygen consumption rate (OCR)). This protein gradient drives the generation of ATP via the ATP-synthase complex. Alternatively, the Krebs cycle can also be fueled by either fatty acids degraded into acetyl-CoA molecules or glutamate derived from amino acid metabolism.Under certain conditions, cancer cells, strongly proliferating cells, or activated immune cells can switch to predominantly producing lactate from glucose instead of fully oxidizing glucose into CO_2 in the mitochondrion (B, measured as extracellular acidification rate (ECAR)). While being energetically less efficient than OxPhos, glycolysis allows for a faster energy generation. In addition, the „disrupted"Krebs cycle, resulting from the lack of pyruvate, allows for the generation of important biosynthetic intermediates needed for immune cell effector function. Here, directly and indirectly antimicrobial molecules such as prostaglandins, NO, ROS, or itaconate are important immune effector molecules needed by the activated immune cells. Additionally, fumarate and acetyl-CoA (generated for example from citrate via the ATP-citrate lyase, ACL) can be used for epigenetic modification. Abbreviations: OxPhos: oxidative phosphorylation, FAO: fatty acid oxidation, ROS: reactive oxygen species, NO: nitric oxide, FAS: fatty acid synthesis, PDH: pyruvate dehydrogenase, ACL: ATP-citrate lyase, GDH: glutamate dehydrogenase, IDH: isocitrate dehydrogenase, IRG1: immune-responsive gene 1, HIF-1a: hypoxia inducible factor 1a;

Fig. 2: Metabolic phenotype and main signaling pathways associated with the allergen-mediated activation of epithelial cells. Epithelial cells are grouped within the metabolic pathways (glycolysis, oxidative phosphorylation (OxPhos), fatty acid oxidation (FAO), center) according to the published and discussed literature (A). Ovals represent the indicated energy pathway: violet: FAO; red: OxPhos; yellow glycolysis; In epithelial cells, allergen-induced activation results in mitochondrial dysfunction and reduced ATP production likely caused by reactive oxygen species (ROS)- and nitric oxide (NO)-dependent mitochondrial damage (B). This mitochondrial dysfunction was shown to contribute to lung inflammation, airway hyperreactivity, and reduced antiviral responses. Allergen contact also triggers a dimeric pyruvate kinase M2 (PKM2)- and inhibitory κ B kinase-epsilon (IκKε)-dependent production of IL-1ß and increased rates of glycolysis. Increased glycolysis was shown to contribute to the release of the Th2-promoting cytokines thymic stromal lymphopoietin (TSLP) and granulocyte macrophage colony-stimulating factor (GM-CSF), as well as the induction of pro-inflammatory responses. Furthermore, via increased production of citrate, glycolytic metabolism results in increased levels of mevalonate biosynthesis and protein prenyulation, which were shown to be important for Th2 differentiation. Finally, either HIF-1a knockdown, hypoxic culture

This article is protected by copyright. All rights reserved

conditions, or inhibition of glycolysis by 2-deoxyglucose were shown to result in disrupted barrier integrity via a reduced expression of the tight junction protein claudin-1. For more detailed information see text. Abbreviations: OxPhos: oxidative phosphorylation, FAO: fatty acid oxidation, ROS: reactive oxygen species, NO: nitric oxide, PKM2: pyruvate kinase M2,IκKε: inhibitory κ B kinase-epsilon, TSLP: thymic stromal lymphopoietin, GM-CSF: granulocyte macrophage colony-stimulating factor, HIF-1a: hypoxia inducible factor a;

Fig. 3: Metabolic phenotype and main signaling pathways associated with the activation of dendritic cells, M1-, and M2-macrophages. Cell types are grouped within the metabolic pathways (glycolysis, oxidative phosphorylation (OxPhos), fatty acid oxidation (FAO), center) according to the published and discussed literature (A). Ovals represent the indicated energy pathway: violet: FAO; red: OxPhos; yellow glycolysis; Activated DCs and M1-macrophages display a highly glycolytic phenotype. In both cell types, PAMP- or cytokine-mediated activation triggers a HIF-1a- and mTOR-dependent activation of glycolysis, paralleled by a disrupted Krebs cycle (B). Increased levels of glycolysis were shown to contribute to the production of pro-inflammatory cytokines, expression of MHCII- and co-stimulatory molecules, phagocytosis, and cell migration, allowing these cells to activate T cells and fulfil their effector function. Moreover, mTOR-signaling was shown to contribute to increasedFAO in dendritic cells which promoted the induction of Th2 responses. Activation of FAO could be inhibited by Sirtuin 1(B). In M2-macrophages activation via Th2 cytokines triggers a STAT6- andperoxisome proliferator-activated receptor γ co-activator 1 beta (PGC-1β)-dependent activation of both OxPhos and FAO promoting their anti-inflammatory effector function (C). Concurrently, M2-macrophage activation is paralleled by a reduction in glycolytic metabolism. *Trichinella spiralis* thioredoxin peroxidase-2 and chitinase-3-like protein 1 (CHI3L1, via MAPK and PI3K/AKT phosphorylation) were shown to promote M2 polarization and enhance Th2 responses.For more detailed information see text. Consolidated signaling pathways involved in metabolic reprogramming are indicated for each cell type. Abbreviations: OxPhos: oxidative phosphorylation, PI3K: phosphoinositide 3-kinase, Akt: protein kinase B, STAT6: signal transducer and activator of transcription 6, PGC-1β:peroxisome proliferator-activated receptor γ co-activator 1 beta, HIF-1a: hypoxia inducible factor 1a, PAMP: pathogen associated molecular pattern, CHI3L1: chitinase-3-like,mTOR: mammalian target of rapamycin, FAO: fatty acid oxidation, Jmjd3: Jumonji domain-containing protein D3;

Fig. 4: Metabolic phenotype and main signaling pathways associated with the activation of basophils, eosinophils, mast cells, and ILC2s. Cell types are grouped within the metabolic pathways (glycolysis, oxidative phosphorylation (OxPhos), fatty acid oxidation (FAO), center) according to the published and discussed literature (A). Ovals represent the indicated energy pathway: violet: FAO; red: OxPhos; yellow glycolysis; In eosinophils, IL-5-mediated activation results in a phosphoinositide 3-kinase (PI3K)-, Akt-, and signal transducer and activator of transcription 5 (STAT5)-dependent activation of both glycolysis and

This article is protected by copyright. All rights reserved

OxPhos, suggesting metabolic flexibility (B). Ag/IgE-dependent activation of basophils results in a HIF-1a-dependent activation of both ERK and p38 MAPK which mediate production of pro-allergic cytokines (C).In acutely activated mast cells, Ag/IgE-stimulation via FcεRI triggers an inactivation of the M2-type pyruvate kinase (M2PK) which in turn promotes glycolysis via activation of MAPK and T cell immunoglobulin and mucin-domain containing protein 3 (TIM3). Glycolysis-derived ATP can then be used for mast cell degranulation and cytokine production. Upon longer-term activation, mast cells can additionally employ MAPK- and STAT3-dependentOxPhos for energy generation allowing for degranulation and cytokine production also in glucose-free medium (D). In ILC2s activated by either cytokines, proteins, or parasitesincreased energy demand is met by FAO promoting ILC2 accumulation and production of effector cytokines (IL-5 and IL-13). The free fatty acids required for FAO in ILC2s are generated by autophagy. In contrast to this, HIF-1a-dependent glycolysis (which can be inhibited by the E3 ligase von-Hippel-Lindau(VHL)) results in ILC2 inhibition characterized by reduced cellular ATP concentration, mitochondrial dysfunction, and reduced ST-2 expression by epigenetic modification (E).For moredetailed information see text. Consolidated signaling pathways involved in metabolic reprogramming are indicated for each cell type. Abbreviations: OxPhos: oxidative phosphorylation, STAT5: signal transducer and activator of transcription 5, FAO: fatty acid oxidation, VHL: von-Hippel-Lindau, PI3K: phosphoinositide 3-kinase, Akt: protein kinase B, Ag: antigen, HIF-1a: hypoxia inducible factor 1a, ERK: extracellular signal-regulated kinase, MAPK: mitogen activated protein kinase, ROS: reactive oxygen species, ST-2: IL-33 receptor, FcεRI: high affinity IgE receptor, ß-hex: beta hexosaminidase, LTC4: leukotriene C4, TIM3: T cell immunoglobulin and mucin-domain containing protein 3, M2PK: M2-type pyruvate kinase, 2-DG: 2-deoxy-glucose, ILC2: innate like lymphocytes type II cell;

Accepted Article

This article is protected by copyright. All rights reserved

Box 1: Methodological approaches to study immunometabolism

The recent breakthroughs in immune metabolic analyses have been made possible by a combination of both technological advances and established methods. “Omics” or system level approaches such as metabolomics, single cell genomics, transcriptomics, or proteomics have enabled a highly detailed understanding of global cellular responses to defined stimuli.

Hereby, genomics describes the structure, function, evolutionary mapping, and editing of genomes, transcriptomics the sum of all RNA transcripts (messenger RNA, ribosomal RNA, transfer RNA, microRNA, and other non-coding RNAs), and proteomics the structure, function, interaction or modification of proteins (see also(145)). Relatively new in the field of omics, metabolomics characterizes both the intermediates and final molecules produced during the investigated sample’s metabolic activity(145). Metabolomic analyses are mainly based on nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based techniques(145,146).The results obtained from transcriptomics, proteomics, and metabolomics analyses are highly dependent on timepoint, cell type, treatment, and environmental factors and can therefore be used to describe changes associated with disease development and progression(145).

Equally important, recent advances in computational high-throughput analyses have allowed to analyze and integrate the extensive datasets generated by the above-mentioned system level approaches.

Another breakthrough technique, Metabolic Flux Analysis using Agilent Seahorse technology, has enabled researchers to monitor both the extracellular acidification rate (ECAR, as a proxy for lactate production and therefore glycolytic metabolism) and the oxygen consumption rate (OCR, as a measure of mitochondrial respiration) in real-time using relatively low numbers of living cells. Seahorse technology therefore allows studyingboth the overall metabolic state and response to exogenously applied stimuli and inhibitors even in rare cell populations.

These new technologies are complemented by established assays such as colorimetric determination of certain metabolites (e.g. glucose, lactate, or amino acids), mass spectrometry, as well as radioactive carbon tracing studies (to determine the fate of exogenously applied nutrients) which allow for a detailed analysis of specific key molecules.

This combination of recent advances and established methodsenables a comprehensive overall assessment of the metabolome that can be correlated to for example the overall transcriptome or proteome of the respective sample(38,81). The key methods used in immune metabolic analyses are summarized in table 1.

This article is protected by copyright. All rights reserved

Method	Application	Key publication
Single cell genomics, transcriptomics, proteomics	Determination of the genome-wide gene-/RNA-/protein expression-/protein modification-levels to comprehensively characterize the investigated sample, find new target molecules, pathways, and establish novel relationships	(14,15,38,41,44,55,56,81,91)
Metabolomics	Molecular analysis of the overall metabolites (intermediate and final) in a biological system to determine the overall metabolic state and identify key metabolic pathways and interactions	(15,41,44,81,142)
Metabolic Flux Analysis	Determination of overall cellular metabolic state via real-time measurement of extracellular acidification rate (ECAR, glycolysis) and oxygen consumption rate (OCR, mitochondrial respiration)	(14,44,81,83,84,142,147)
Radioactive carbon tracing	Fate determination of exogenously applied nutrients	(41,81,83,84,91,148)
Colorimetric metabolite determination, mass spectrometry, lipidomics	Detection and/or quantification of single key metabolites to confirm findings obtained with the above-mentioned approaches	(15,44,69,72,81,149)

Table 1. Key methods used in immune metabolic analyses

Accepted Article

This article is protected by copyright. All rights reserved

Box 2: Influence of diet on initiation and control of inflammation in allergy

Western lifestyle is associated with the increased consumption of calorie-rich diets high in refined starches, sugar, saturated and trans-fatty acids, while at the same time being low in natural antioxidants and fiber (derived from fruits, vegetables, and whole grains) and omega-3 fatty acids (termed “Western Diet”)(150). Combined with chronic overnutrition, a mostly sedentary lifestyle, higher levels of stress, and increased exposure to air pollution, “Western Diet” results in chronic low-level metabolic inflammation, which was termed “metaflamation” (reviewed in(151)). Risk factors for this chronic inflammatory state are summarized in the “metabolic syndrome“ and include abdominal obesity, hypertension, insulin resistance, abnormalities in fat metabolism (hyperglycemia, hypertriglyceridemia, decreased high-density lipoprotein cholesterol), and aging(150,152).

Mechanistically, consumption of a “Western Diet” was associated with activation of the innate immune system, resulting in both the production of excessive amounts of pro-inflammatory cytokines and formation of free radicals(55,151).

Diet-induced inflammation was also suggested to contribute to allergy development: consumption of a “Western Diet”, obesity, and high levels of low-density lipoprotein cholesterol were positively correlated with the risk of developing childhood asthma(153–155).

In line with this, especially overconsumption of saturated fatty acids (FAs) was repeatedly reported to trigger inflammation and be associated with an increased risk of atherosclerosis, coronary heart disease, obesity, and “metabolic syndrome”(156–159).In this context, mouse studies showed high concentrations of saturated FAs to have direct cytotoxic effects(160), contribute to TLR4-mediated inflammation(161,162), induce endoplasmic reticulum-stress sensed by macrophages(149), and induce mitochondrial ROS-dependent activation of the NLRP3 inflammasome via inhibition of adenosine monophosphate-activated protein kinase (palmitate(163)).

In contrast to this, unsaturated FAs (such as omega-3 FAs, e.g. alpha-linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid) are widely described as being anti-inflammatory(150,164). Mechanistically, the beneficial effects of long-chain omega-3 FAs are likely mediated by (I) regulating hormone secretion and both (II) direct anti-inflammatory effects via GPR120-dependent inhibition of TNFR-, TLR2-, and TLR4-signaling and inhibition of NF-κB-dependent pro-inflammatory cytokine secretion(165,166) and (III) indirect anti-inflammatory effects via acting as substrates for the generation of potent anti-inflammatory molecules (resolvin, maresin, and protectin)(167). Accordingly, anti-inflammatory effects of poly-unsaturated FAs (PUFAs, having more than one double bond in their carbon backbone) were reported on epithelial cells (restoration of barrier function and reduction of pro-inflammatory mediators), macrophages (modulation of TLR-signaling), DCs (reduction of activation status and therefore T cell activatory

This article is protected by copyright. All rights reserved

potential), neutrophils (restoration of balanced immune responses by decreasing pro-resolving functions), T cells (induction of Tregs, reduction of T cell proliferation, -activation, and -differentiation into Th1/Th17 subtypes) (extensively reviewed in(168)).

Supplementation with/ consumption of fish oil rich in unsaturated omega-3 FAs was shown to suppress inflammatory immune responses in mouse food allergy models(169,170) and reduce allergy frequencies in children in the first four years of life(171).

Furthermore, imbalances in the intake of PUFAs (higher intake of n-6 poly-unsaturated FA compared to n-3 PUFA), influencing the balance between Th1 and Th2 responses via inhibition of IL-13 production(172), were shown to be associated with higher risks of obesity-related asthma(173,174) and uncontrolled asthma with severe clinical outcomes and frequent exacerbations(175). Additionally, low intake levels of n-3 PUFAs were reported to result in worsening of asthma and pulmonary function(176), while the prevalence of asthma is significantly reduced for example in Inuit populations(177–179)(which naturally have higher intake of n-3 PUFAs). Clinically, high intake of n-3 PUFA improved the prevalence of asthma symptoms, asthma control, lung function, airway hyperresponsiveness, and severity of exercise-induced bronchoconstriction (reviewed in(168)).

Hyaluronan (HA) is a major component of the extracellular matrix. It was reported to accumulate in both lung and serum of asthmatic patients and correlate with disease severity(180). In this context, Sokolowska*et al.* demonstrated fragmented HA to increase the production of several classes of cPLA2a-dependent lipids (COX metabolites: thromboxane B2, prostaglandin E2, D2, and B2; metabolites from other pathways: 15-hydroxyeicosatetraenoic acid, 11,12-epoxyeicosatrienoic acid, and 14,15-epoxyeicosatrienoic acid; non-AA metabolites: 13-hydroxyoctadecatrienoic acid and 16(17)-epoxydocosapentaenoic acid) in patients with severe asthma(181). In addition, low molecular weight HA treatment resulted in decreases in antiviral gene and protein expression (determined by whole-genome analysis) in patients with severe asthma, possibly caused by a reduced expression of genes involved in interferon-signaling, cell movement of lymphocytes, homing of T cells, and other canonical pathways(181). Taken together, these results suggested a connection between extracellular matrix, global lipid mediator production, decreased antiviral responses and asthma(181).

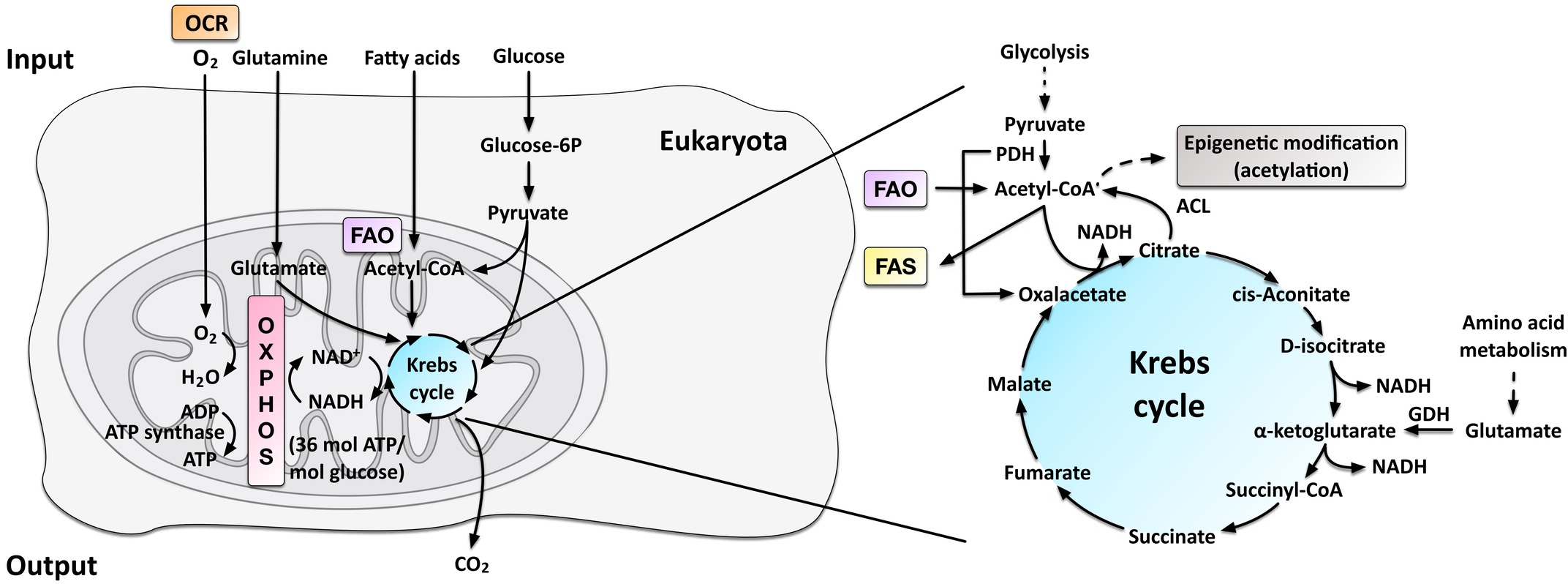
This article is protected by copyright. All rights reserved

all_14843_f1.pdf

Accepted Article

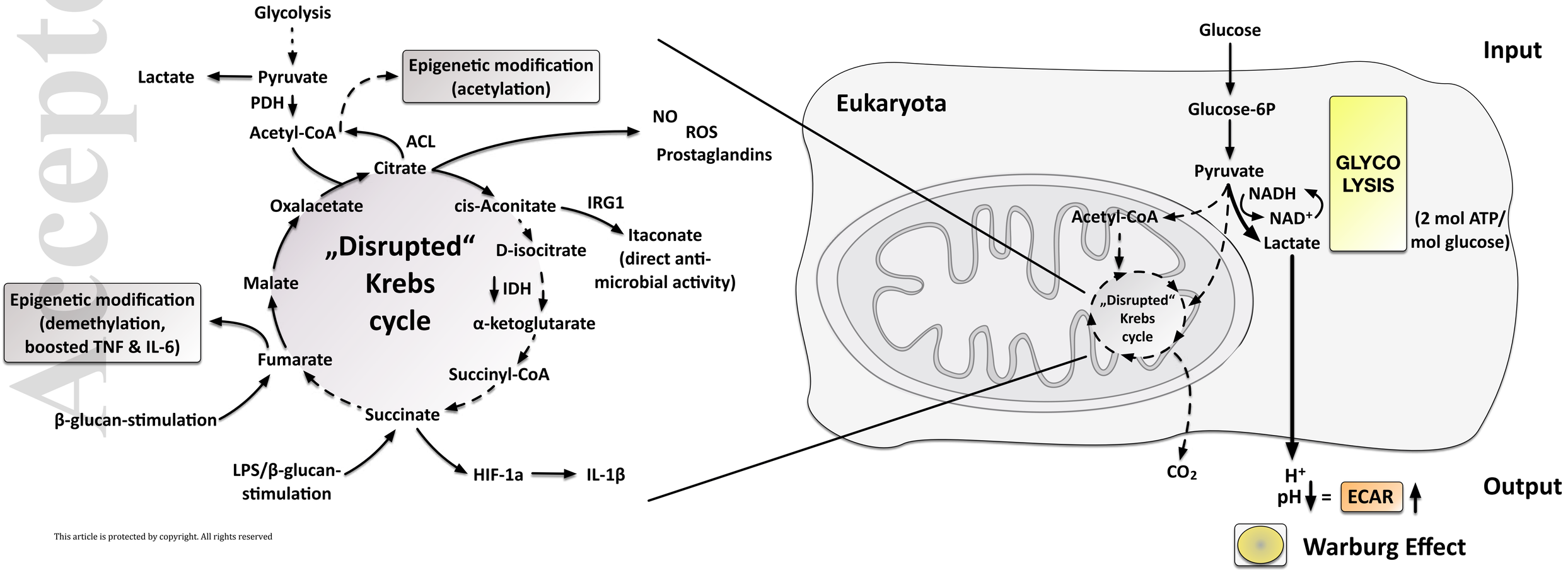
A

Steady-state metabolism



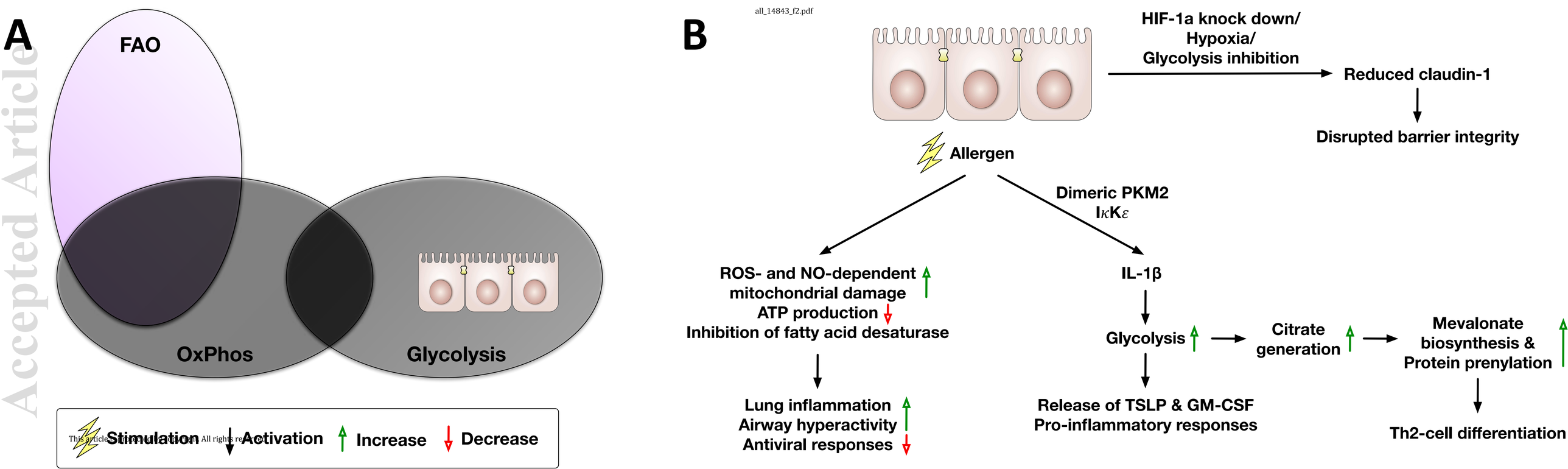
B

Cancer cells, proliferating cells, activated immune cells: „Warburg metabolism“



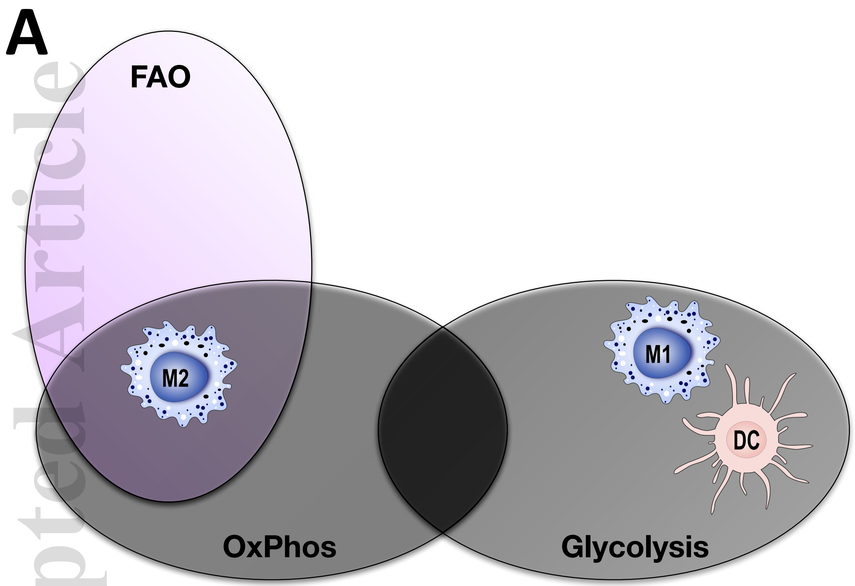
This article is protected by copyright. All rights reserved

Accepted Article

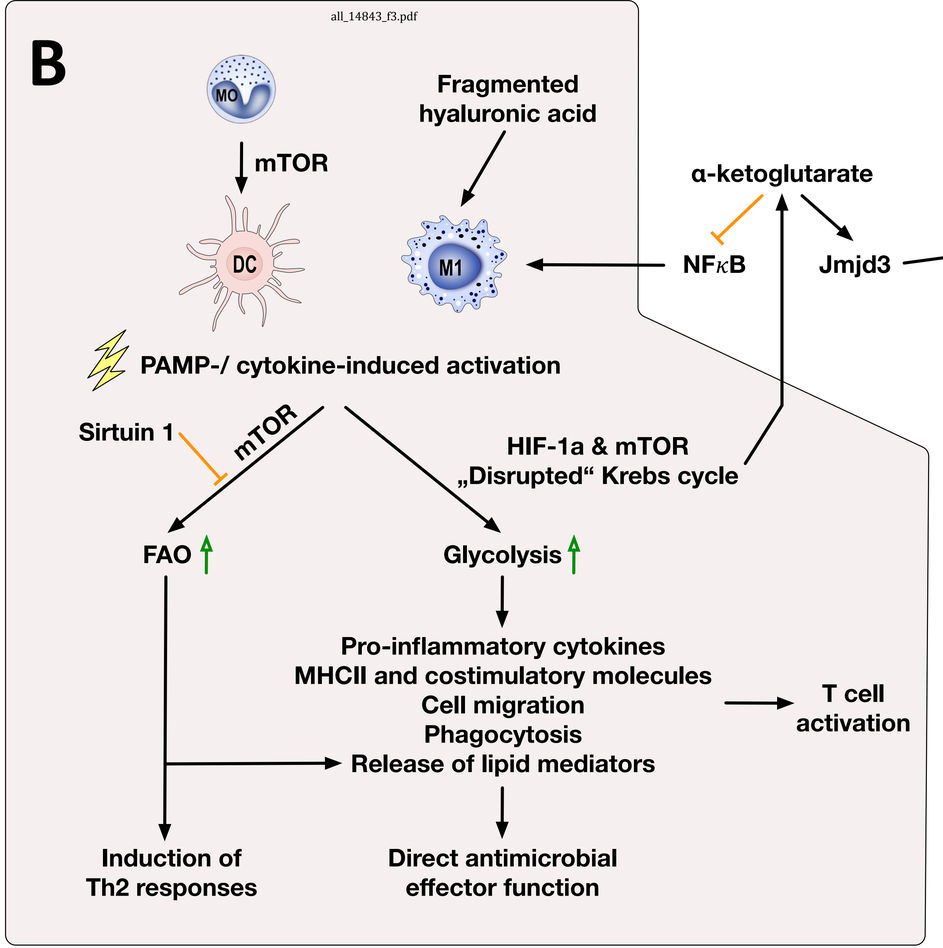


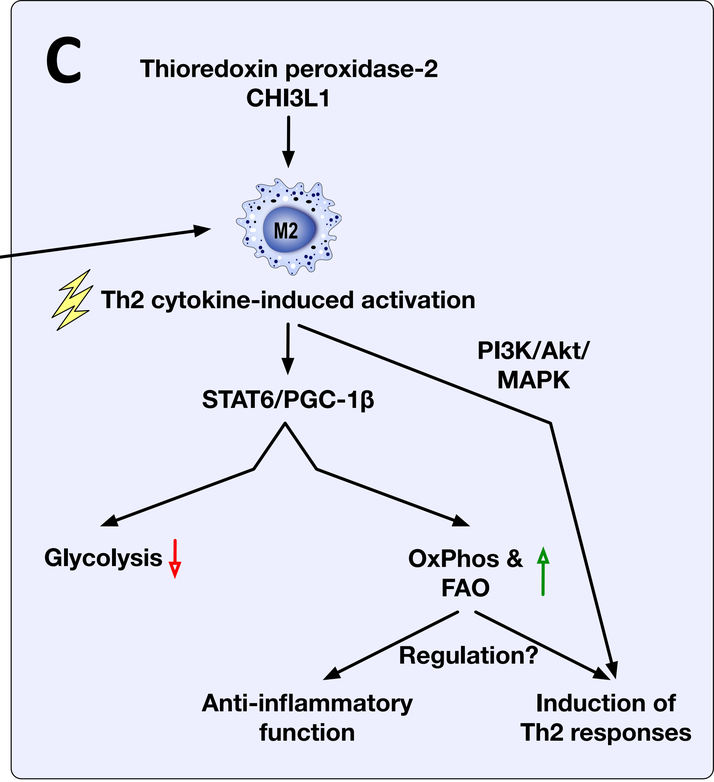
This article is protected by copyright. All rights reserved.

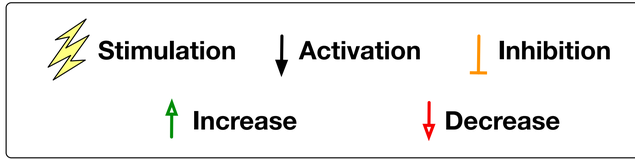
Accepted Article



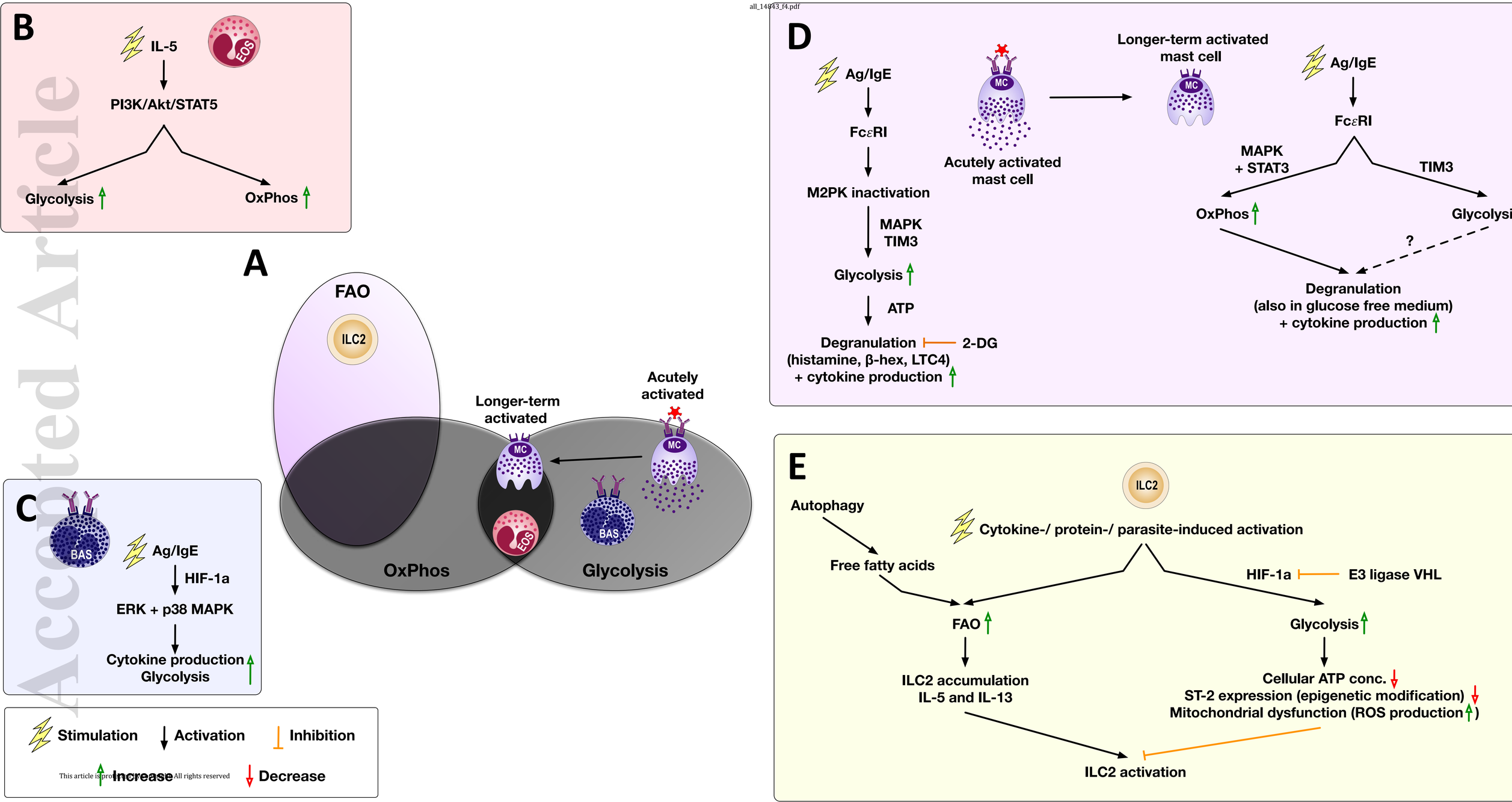
This article is protected by copyright. All rights reserved







all_14843_f4.pdf



This article is protected by copyright. All rights reserved

Accepted Article