Review

The pathogenic role of metabolism in Sjögren's syndrome

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ABSTRACT

The link between immune cell function and cell metabolic reprogramming is currently known under the term "immunometabolism". Similarly to the Warburg's effect described in cancer cells, in activated immune cells an upregulation of specific metabolic pathways has been described and seems to be pathogenic in different inflammatory conditions.

Sjögren's syndrome (SS) is a systemic autoimmune disease that affects the exocrine glands and is characterised by a progressive loss of secretory function. Despite the increasing amount of evidence on the ability of metabolism in regulating cell behaviour in inflammatory or tumoral conditions, the field of metabolism in SS is still for the most part unexplored.

The aim of this review is to summarise currently available studies evaluating cell metabolism in SS with a particular focus on the possible pathogenic role of metabolic changes in immune and non-immune cells in this condition.

Introduction

Cell metabolism has become one of the most exciting areas of investigation in the field of immuno-rheumatology and the link between immune cell function and metabolic reprogramming is currently known under the term "immunometabolism" (1). Both in innate and adaptive immune cells, metabolism is fundamental in determining survival, function, and differentiation. Likewise the described *Warburg's Effect* in cancer cells, which consists in a rewiring of cell metabolism (*i.e.* increased glycolysis) to promote growth, survival, and proliferation (2), in activated immune cells an upregulation of specific metabolic pathways has been described (1). In addition to cancer cells and immune cells, the role of metabolic reprogramming has been also described in the stromal cells composing the microenvironment of tumours and inflammatory conditions (3-5). In this regard, the metabolic co-dependency of cancer and stromal cells is an emerging area of interest and strongly supports the use of novel therapeutics which, by targeting metabolism, can potentially exert a double effect on both the immune cell component and the local microenvironment (6). Such an increasing amount of data provided the rationale to test similar drugs also in inflammatory and autoimmune conditions (7).

Sjögren's syndrome (SS) is a systemic autoimmune disease characterised by inflammation of lacrimal and salivary glands (SG), with a progressive loss of secretory function (8). Despite the improving interest in investigating the role of cell metabolism in the pathogenesis of different autoimmune diseases, this field in SS is still largely unexplored.

The aim of this review is to summarise the currently available studies evaluating cell metabolism in SS and to point out the potential pathogenic role of intracellular metabolic reprogramming in this condition.

General concepts on cell metabolism and its role in autoimmune diseases

The metabolic modifications occurring in cells are finely orchestrated by intracellular complexes sensing the fluctuations of a wide range of nutrients and metabolites including the mammalian target of rapamycin (mTOR) and the adenosine monophosphate-activated protein kinase (AMPK) (9). Such modifications may differ according to the cell type and are strictly dependent on the environmental conditions. For instance, although glycolysis yields less ATP compared to oxidative phosphorylation, activated cells prefer using glycolysis rather than the mitochondrial tricarboxylic acid (TCA) cycle, to

provide the high energy for rapid cell differentiation and immune responses (10). Accordingly, a switch towards aerobic glycolysis has been illustrated in activated M1 macrophages (11), NK cells (12), activated B cells (13) and effector T cells, including T-helper (Th) 1 and Th17 (14). On the other hand, oxidative phosphorylation prevails in resting conditions with evidence of a capacity to favour the acquisition of an anti-inflammatory phenotype as observed in activated M2 macrophages (15) and regulatory T cells (Treg) (16). Activation of glycolysis is therefore largely observed in inflammatory conditions where immune cells, via upregulation of glucose transporters (GLUT), increase the uptake of glucose with the subsequent rise of pyruvate which may either enter the Krebs cycle or be converted in lactate. As a prove of a high glycolysis activation, lactate has been found systemically or locally overproduced in many different inflammatory conditions (17) with lactate levels able to regulate T cell phenotypic changes, as discussed later.

Given these findings, it is not surprising how several studies have linked metabolic reprogramming with a dysregulated immune response contributing to the development of autoimmunity (18). In this regard, a dysregulated cell metabolism has been described in several autoimmune diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (18).

In RA, high intra-articular levels of lactate, possibly due to a rapid synovial fibroblast turnover and proliferation, have been detected along with a potential effect of this molecule in shaping T cell function, including favouring a Th17 phenotype in CD4+ T cells (19). It is interesting to note how in inflammatory conditions, such overexpression of lactate may also act as a negative feedback regulator with consequent immunosuppressive effects (17). In SLE patients and lupus-prone mice, an activated metabolism of CD4+ T cells has been demonstrated too, and the use of metabolic inhibitors seems to provide beneficial effects (20). For instance, in lupus-prone mice, pharmacologic inhibition of glycolysis by 2-deoxyglucose

(2DG) eliminates the expansion of spontaneous germinal-centre (GC) B cells and T follicular helper (Tfh) cells, as well as the production of autoantibodies (21). Furthermore, mitochondrial dysfunction and oxidative stress are key players in SLE, promoting the pathogenic production of reactive oxygens species (ROS) (22).

Despite the increasing evidence on the role of metabolism in the development and progression of autoimmune diseases, data on SS are very limited.

Metabolomic studies in SS: focus on biological fluids

As secreted metabolites are the final products of different intracellular pathways, the metabolomic analysis of biological fluids is an excellent methodology to get information on cell function and activation. Hence, to shed new light on the pathophysiological mechanisms and to identify new potential disease biomarkers, different metabolomics studies have been conducted on serum, saliva and tears of patients with SS.

Metabolomic of saliva

Saliva is a complex fluid produced by salivary glands with a key role in maintaining oral health. It is composed of a variety of substances and its chemical-physical properties can be influenced by physiological (ex: genetic, age, sex, oral hygiene, diet, stress) and pathological conditions (23). Over the years, the metabolomic analysis of saliva has emerged as a tool to identify new biomarkers and understand the pathogenesis of various diseases. Only a few studies investigated the metabolic profile of saliva in SS patients with mixed but rather interesting results (24-26).

In SS saliva, several pathways involving amino acid metabolism seem to be altered. For instance, decreased levels of tyrosine and increased levels of phenylalanine have been demonstrated (24-26). Regarding tyrosine, it is interesting to note how in SS a dysfunction of its kinases receptor (RTKs) has been linked to autoantibodies production, lymphocytic infiltration, and salivary gland dysfunction (27). Proteomic analysis also suggested the role of the enzyme PTPN6, a tyrosine-specific

protein phosphatases, in promoting cellular adhesion in salivary glands (28) and in regulating INFγ immune response in SS murine models (29).

In SS saliva, a significantly higher proportion of choline and taurine has been also observed with evidence of an inverse correlation with the salivary flow rate (30). Considering the role of taurine in the cellular response to osmotic stress and in the regulation of saliva composition through the sodium/taurine cotransporter channel (31), the detected variations in SS look quite interesting and are worth of future investigations. Regarding choline, it is important to remind how in cancer cells a correlation between this amino acid and cell proliferation has been observed (32); due to the metabolic similarities between cancer cells and activated immune cells (33), the higher levels of choline in SS saliva might be a reflection of the local state of inflammation.

Additional metabolic pathways, including tryptophan metabolism, tyrosine metabolism, carbon fixation, and aspartate and asparagine metabolism, were found to be upregulated in SS saliva and related to the inflammatory injury (24). The evidence on tryptophan metabolism is particularly intriguing if we consider both its already described role in immune cell activation in SS (34) and its known association with SS-related neurologic manifestations (35).

Metabolomic of serum

Since serum is the most accessible biological fluid, its metabolomic analysis has been widely used to explore the metabolic dysregulation occurring in various rheumatic diseases (36, 37). In line with data on saliva, an altered amino acid metabolism has been also confirmed in sera from patients with SS (38-40). For instance, the presence of an upregulation of the biosynthesis of different amino acids, including valine, leucine, isoleucine and proline is well described in SS (37, 40). However, tryptophan metabolism, from which derive kynurenine and kynurenic acid via activation of indoleamine 2,3-dioxygenase (IDO), seems one of the most affected pathways. Specifically, higher levels of kynurenine and kynurenic

acid, coupled with lower levels of tryptophan, were described in sera from SS patients (38-40). Such results are noteworthy, especially considering the role of the kynurenine pathway in regulating the immune response (34). Specifically, following a cytokine (mainly IFNγ and TNF-α) mediated activation of IDO, the kynurenine pathway displays immunosuppressive properties via regulation of Treg cell functions (34). In line with this evidence, SS patients display an hyperactivation of IDO associated with increased expression of inflammatory and immunological markers (34). Of note, the kynurenine pathway seems also to disrupt the serotoninergic and glutamatergic transmission of the central nervous system, possibly contributing to the appearance of neurological manifestations in SS (35).

Another metabolic pathway that seems to be altered in SS is fatty acid metabolism. Higher serum levels of both stearic acid, a precursor of inflammatory mediators, and linoleic acid, a polyunsaturated fatty acid involved in the formation of cell membranes and beta-oxidation, seem to discriminate SS patients from healthy subjects (40, 41). Additionally, lower levels of L-carnitine, a fatty acids transporter involved in beta-oxidation, were detected suggesting a pathogenic reduction of beta-oxidation and accumulation of fatty acids (42).

Finally, a combined approach of machine learning and serum metabolomics seems to accurately discriminate SS patients from healthy controls. By this approach, three potentially specific biomarkers for SS were identified: L-carnitine and cyclic AMP which are both downregulated and 2-hydroxypalmitic acid which is upregulated (42). Of note, the activation of cAMP/protein kinase A is involved in the expression of AQP5 and muscarinic receptor 3 on the salivary gland acinar cells with consequent potential impact on the salivary flow rate (43, 44).

Metabolomic of tears
In recent years, metabolomic analysis of tears has been used to shed light on the pathogenetic mechanisms underlying dry eye disease and to identify new potential biomarkers (45). However, me-

tabolomic data deriving from the tears of patients with SS are very scant.
To date, only one study sought to investigate the tear metabolic profile of SS (46). A group of nine metabolites potentially useful to differentiate SS patients from healthy controls has been identified (46). Such metabolites signature was characterised by a decrease in two amino acids (serine and aspartate) and one amine (dopamine) and an increase in six phospholipids (three lysophosphatidylcholines, two sphingomyelins, and phosphatidylcholine diacyl). Regarding phospholipids, it is interesting to note how, similarly to different conditions of dry eye disease (47), in SS an upregulation of the phospholipase A2 (PL-A2) has been also detected. Such a finding is particularly remarkable as this enzyme is involved in the generation of precursors of inflammatory mediators (PGE and leukotrienes) and seems to cooperate with TNF-α and IL1β in inducing the inflammatory response in conjunctival epithelial cells (47). Of note, increased levels of PL-A2 have been specifically detected both in the serum and in the salivary glands of patients with SS and have been found associated with MALT lymphoma (48). Finally, in conjunctival epithelial cells of SS there is also evidence of an increase in lipid oxidative stress (49) and a decrease in the activity of antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) (50). This finding, along with the evidence of reduced tears levels of serine (50), an amino acid regulating antioxidant mechanisms, possibly suggest a pathogenic alteration of oxidative stress response in SS ocular surface.

Metabolomic studies at cellular level in SS
Data on the role of cell metabolism in the pathogenesis of SS are limited. Most of the currently available evidence comes from studies investigating SS immune metabolism; specifically, on the metabolism of lymphocytes infiltrating patients' salivary glands. However, additional immune and resident cell types are involved in SS and the cross-talk between epithelial cells, stromal cells (mainly fibroblasts), and infil-

trating lymphocytes has emerged as a key pathogenic player regulating the local microenvironment in this condition (51). The role of salivary gland epithelial cells (SGECs) has been extensively addressed in SS leading to the description of the disease as an "*autoimmune epithelitis*" (52). A concise review of currently available studies investigating the pathogenic role of cell metabolism in SS is reported below with a focus on lymphocytes, SGECs and stromal cells. A summary of the most common intracellular metabolic changes occurring in these three different cell types in SS is reported in Figure 1.

Immune metabolism in SS
A critical role for infiltrating T cells in SS has been described (53). At early stages of the disease, T cells represent the dominant population at tissue level and, according to the severity of infiltration, different infiltrating CD4+ subsets including IFN-γ-producing Th1 cells (54), IL-17-producing Th17 cells (55) and IL-21-producing Tfh and T-peripheral helper (Tph) cells (56) have been described. Due to the importance of CD4+ cells in the pathogenesis of SS, most of the currently available studies on SS cells metabolism are focused on this specific subset.
To fulfil the energy demands upon activation, T cells usually undergo profound metabolic reprogramming (57). One of the metabolic mechanisms sustaining CD4+ T cell proliferation is represented by glutaminolysis, a process converting the glutamine into glutaminate and alpha-ketoglutarate (α-KG) (57). Fu *et al*. recently described how glutaminase 1 (Gls1), a major enzyme responsible for glutaminolysis, might be involved in the pathogenesis of SS due to its documented upregulation in both infiltrating and circulating CD4+ T cells (58). Treatment with an inhibitor of glutaminolysis, BPTES (5-phenylacetamido-1,3,4-thiadiazol-2-yl), significantly abolishes the proliferation of T cells *in vitro* and restores the salivary flow rate of SS mouse models *in vivo*; notably, following treatment, a significant reduction of IFNγ and IL-17A-producing cells in mice salivary glands was also observed (58).
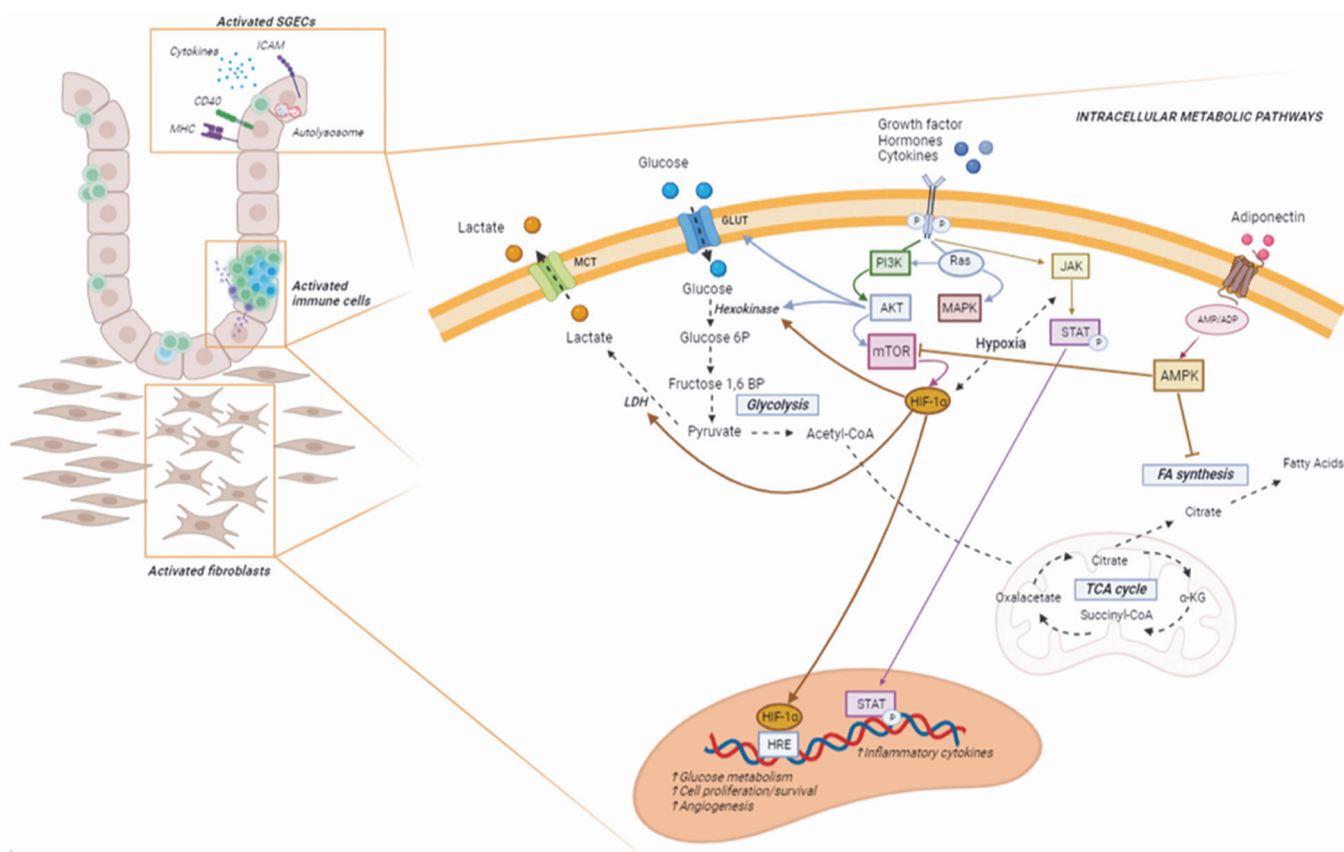
Fig. 1. Interconnection between intracellular pro-inflammatory signals and activated metabolic pathways in the main cell types involved in SS pathogenesis: immune cells, salivary gland epithelial cells (SGECs) and fibroblasts.
αKG: α-Ketoglutaric acid; AKT: serine/threonine protein kinase; AMP/ADP: adenosine monophosphate/adenosine diphosphate; AMPK: AMP-activated protein kinase; FA: fatty acids; GLUT: glucose transporter; HIF-1: hypoxia-inducible factor; HRE: hypoxia-response element; ICAM: intercellular adhesion molecule; JAK: Janus kinase; LDH: lactate dehydrogenase; MAPK: mitogen-activated protein kinases; MCT: monocarboxylate transporters; mTOR: mammalian target of rapamycin; PI3K: phosphoinositide 3-kinases; RAS: rat sarcoma; STAT: signal transducer and activator of transcription.

As previously mentioned, also glycolysis is a crucial metabolic pathway upregulated in proliferating T cells. Following TCR engagement and concomitant co-stimulation, glucose transporters and enzymes associated with glycolysis are upregulated in T cells (59). Although data in SS are still scant, the role of glycolysis in CD4+ T cells from SS-like NOD/Ltj mice has been demonstrated (60). Specifically, inhibition of glycolysis via administration of 2DG, seems to significantly decrease the extent of CD4+ infiltrates and attenuates the degree of salivary flow impairment. Such reprogramming of glycolysis was found to be driven by an increased expression of a long noncoding RNAs (lncRNA PVT1) which maintains the expression of Myc, a transcription factor regulating glucose metabolism (60). Finally, a T cell-specific reduction of a miRNA regulating glucose metabolism (miR-31-5p), has been observed in SS patients (61).

As mentioned above, salivary gland infiltration of B cells normally occurs at a later stage. B cell recruitment and proliferation potentially result in the formation of fully formed GC-like structures where a continuous stimulation of B cells leads to the perpetuation of autoimmunity and has been linked to malignant transformation (62). From a metabolic point of view, upon activation, also B cells in SS exhibit higher glycolysis capacity and maximal oxidative respiration (OXPHOS) (63). Additionally, the expression of GLUT1 in B cells from SS patients is higher than in healthy controls and inhibition by 2DG results in a reduction of proliferation, formation of plasma/plasmablasts and production of immunoglobulins (63).

Salivary gland epithelial cell (SGEC) metabolism
Tissue inflammation in SS has long been postulated to be driven by the lo-

cal aberrantly activated SGECs (52) which contribute to immune cell activation via the expressions of a range of immune-related proteins, including major histocompatibility complex class I and II, costimulatory molecules, chemokines, and cytokines (64, 65). In turn, inflammatory mediators produced by infiltrating lymphocytes subvert the homeostatic regulation of SGECs, which results in impaired secretory function. The crosstalk between SGECs and infiltrating lymphocytes is extensively documented (66) but the cellular, molecular and metabolic mechanisms sustaining the aberrant activation of SGECs in SS are still undetermined.
The endoplasmic reticulum (ER) and mitochondria are central intracellular regulators of cell metabolism, and the ER stress seems to exert profound effects on cell function (67). In this regard, the effect of ER stress on the metabolic behaviour and viability of

SS SGECs has been investigated with fascinating data providing preliminary evidence of a link between metabolic rewiring and the pathogenic behaviour of these cells in SS (68-72).

One of the first proofs comes from a study from Katsiougiannis et al. which demonstrated how aberrantly activated SGECs in SS produce a high amount of adiponectin (71), an adipocytokine regulating metabolism (glucose levels, lipid metabolism, and insulin sensitivity) with additional immunoregulatory properties (73). Later, it was demonstrated how adiponectin is not only highly produced in SS but seems to both exert antiproliferative effects on SGECs and to protect these cells from spontaneous and induced (IFNγ mediated) apoptosis through an AMPK-dependent pathway (68). As above stated, AMPK is a crucial sensor for energy and nutrient availability and is a key regulator of cell metabolism and autophagy activation (74). When ATP synthesis is unable to meet the demands of ATP consumption, AMP and ADP accumulate and activate AMPK, resulting in a reduction of the anabolic process and induction of catabolic processes, such as glycolysis, fatty acid oxidation and autophagy induction (via inhibition of mTOR) (74). Such a link with autophagy is not surprising as alterations in cellular metabolism are one of the fundamental mechanisms by which cells maintain homeostasis (74). Of note, autophagy does not only provide survival but plays a key role in modulating immune cell function and shaping immune responses (75).

In this regard, SGEC of SS display a stressed ER which is extensively dilated and linked to the activation of autophagic and apoptotic mechanisms which are strictly connected with the redistribution of autoantigens on the cell membrane and the induction of autoimmunity (70). In line with this evidence, we recently demonstrated how besides apoptosis, which is likely the consequence of aberrant chronic stress no longer counteracted by pro-survival signals in cells, in SGECs from SS there is evidence of a maladaptive activation of autophagic mechanisms (72). Specifically, autophagy is not only ab-

errantly upregulated because of the local inflammatory assault but it also sustains SGECs activation and is associated with SS histological severity (72). Taken together, currently available evidence strongly points out to a profound metabolic rewiring taking place in SGECs of patients with SS linked to the acquisition of a pro-inflammatory phenotype which supports their pathogenic role in SS.

Stromal cell metabolism in the salivary glands
Fibroblasts are a very heterogeneous group of cells involved in different physiological functions including wound healing, extracellular matrix production and stem cell compartments support. In recent years, single-cell profiling technologies allowed a greater understanding of their heterogeneity and functions, shedding new light on their pro-inflammatory and immune-modulatory role in inflammatory conditions (76).

Following activation by a series of proinflammatory stimuli, fibroblasts can indeed produce different immune-modulating cytokines as well as chemotactic and growth factors. Increasing evidence suggests how in inflammatory conditions these cells can display a pathogenic phenotype, acting as a bridge between acute and chronic inflammatory responses and being responsible for organising inflammatory infiltrates and eventually contributing to the formation of tertiary lymphoid structures (77). In this regard, it has already been shown how activated fibroblasts, both in RA and in SS, can produce chemokines (such as CXCCL13 and CCL19) responsible for the chemotactic attraction of B and T lymphocytes at the tissue level (51, 77). Additionally, two shared pro-inflammatory clusters (CXCL10+CCL19+ immune-interacting and SPARC+COL3A1+ vascular-interacting fibroblasts) have been recently identified in four different inflammatory diseases, including SS (78). Despite these data, fibroblast metabolic profiling in rheumatic diseases is still an almost unexplored field. There are no current studies in SS focusing on metabolism in stromal cells

from the inflamed salivary glands, thus we can only infer potential metabolic changes from the few available studies showing dysregulation of metabolic pathways in fibroblasts from the RA joints. Specifically, fibroblast-like synoviocytes (FLS) from patients with RA seem to display higher levels of glucose metabolism (79). More in detail, in FLS from RA patients, an upregulation of the glycolysis marker GLUT-1 has been described along with a decreased rate in proliferation and secretion of both IL-6 and MMP3 following treatment with the glycolysis inhibitor 2DG (79). In line with these findings, higher FLS expression of the glycolytic enzyme hexokinase-2 (HK-2) has been detected and seems to delineate a more invasive and aggressive RA FLS phenotype (80). Interestingly, also in lung fibroblasts from patients with pulmonary fibrosis a metabolic reprogramming, mainly consisting of an upregulated aerobic glycolysis, has been described (81).

Taken together, the above-mentioned data strongly suggest that a fibroblast metabolic rewiring is involved in their acquired immunological functions; this data, linked with the emerging evidence of a pathogenic pro-inflammatory role of fibroblasts in inflamed salivary glands (78), strongly support the need to further explore stromal cell metabolism also in SS.

Modulating metabolism as a potential therapeutic avenue in SS: current evidence and new perspectives
Since hyperactivated or autoreactive immune cells utilise metabolic pathways which differ from cells with a normal homeostatic activity, inflammatory conditions offer a unique opportunity to selectively target cells based on their metabolic demand. This concept is quite crucial as the idea of targeting metabolism could raise doubts on the possibility to affect indiscriminately different cell types with potential drug toxicity. A model of 'cellular selectivity based on demand' has been recently proposed based on the hypothesis that blocking fundamental metabolic pathways will selectively affect the cells with the great-

est demand for those pathways and not alter normal cellular homeostatic function (7). This hypothesis is strengthened by the evidence of a lack of toxicity observed in studies evaluating the effect of drugs targeting metabolisms in cells and, more in general, in humans. For instance, inhibition of glycolysis by 2DG can be used in cells cultures (82) or even in the clinical setting (83) without evidence of detrimental or adverse effects. Even concomitant blocking of glycolysis, glutamine metabolism and complex I (by metformin) seems to prevent allograft rejection with no evidence of toxicity (84). Due to these early but encouraging results, drugs targeting metabolism are currently under investigation in different tumours with the aims of both directly targeting tumour cells and improving the effect of chemotherapy (85-87).

Drugs targeting metabolism in rheumatic diseases
Small molecules modulating metabolism are already in use clinically for inflammatory diseases. Dimethyl fumarate (DMF) for instance, which is used to treat psoriasis, exerts metabolic effects on a wide range of immune cells by balancing intracellular redox and regulating both the pentose phosphate pathway and fatty acid metabolism (88). Metformin, which is used to treat type 2 diabetes, inhibits the mitochondrial electron transport chain at complex I, with consequent regulation of oxidative phosphorylation and induction of AMPK (88). Rapamycin, which is commonly used to prevent organ rejection after transplants, inhibits mTOR and is known to promote tolerance and generation of memory T cells and tissue-resident macrophages (88).
The effect of these treatments in autoimmune diseases has still to be clarified and, once again, most of the evidence comes from SLE and RA. In mouse models of SLE, the combined inhibition of oxidative phosphorylation and glycolysis by metformin and 2DG respectively, reduced IFNγ production by CD4$^+$ T cells, normalising T cell metabolism and reversing disease biomarkers (89). Additionally, the use of metformin proved to be an effective

adjuvant therapy in achieving treat-to-target in SLE patients (90). Finally, direct inhibition of mTORC1 with rapamycin possibly mitigates the disease as demonstrated both in murine models of SLE (91) and in SLE clinical trials (92). In experimental autoimmune arthritis, metformin treatment promoted the balance between Treg and T effector cells and alleviated clinical disease (93). Regarding glycolysis inhibition, treatment of RA mouse models with 3-bromopyruvate, a HK2 inhibitor, alleviated symptoms by enhancing Treg cell generation, suppressing Th17 cell generation and decreasing dendritic cell activation (94). Accordingly, in a different study on mice, 2-DG reduced joint inflammation by decreasing the activation of both innate and adaptive immune responses (95).

Drugs targeting metabolism in SS
Due to the capacity of exerting anti-inflammatory and immunomodulatory effects by activating AMPK and inhibiting mTOR, the therapeutic effect of metformin has been investigated in animal models of SS with encouraging results. In the non-obese diabetic (NOD)/ShiLtJ mice model of SS, metformin administration restored the salivary flow rate and reduced salivary gland inflammation (96). Specifically, metformin provided effects on inflammation by decreasing the salivary gland expression of IL-6, TNF-α, and IL-17, reducing the Th17 and Th1 cell populations and modulating the balance between Tfh and follicular regulatory T cells (Tfr). Of note, a decrease in B cell differentiation in GCs-like structure, along with a reduction in IgG serum levels, was also observed (96). Such immunomodulatory effect of metformin on B cells was further observed in supernatants from SS where metformin seems to inhibit the proliferation and the differentiation of plasmablasts and to decrease both IgG and IgM levels (63). The above-mentioned effect of metformin on hyposalivation has been also confirmed in mouse models of type 2 diabetes (97). Specifically, the combination of artesunate and metformin seems to mitigate hyposalivation by regulating the PI3K/Akt pathway and affecting cell homeo-

stasis (*i.e.* apoptosis and autophagy) in salivary glands (97). In line with this evidence, in a large study conducted on more than 15,000 patients with type 2 diabetes, metformin exposure was found associated with a reduced risk of developing SS (98).
Besides metformin, additional evidence come from the potential utility of mTOR inhibition (by rapamycin which is named Sirolimus) in patients with SS. mTOR, which is composed of two subunits mTORC1 and mTORC2, is one of the main intracellular metabolic sensors having a crucial role in regulating cell survival and proliferation. Recently, an increased mTORC1 activity in salivary gland B and T cells associated with local and systemic B cell hyperactivity was demonstrated in patients with SS (99). The proliferation of B cells and T cells, along with the production of IgG and IFNγ, was effectively halted *in vitro* by rapamycin (99). Intriguing effects were also observed on Tfh and follicular regulatory T cells (Tfr) from patients with SS. As the increased ratio between Tfh (specifically PD-1+ICOS+Tfh) and Tfr (CD45RA-Foxp3high activated Tfr cells) can effectively discriminate SS patients from healthy controls, it is interesting to note how the systemic administration of rapamycin seems to restore such ratio with a parallel capacity of dampening disease activity (100). An additional effect was also demonstrated on circulating SS B cells where rapamycin was able to suppress B cell proliferation, activation, and autoantibody production (63). Despite the evidence on immune cells, most of the currently available data on the potential utility of rapamycin in SS, come from studies evaluating the effect of this treatment on mouse models of dry eye disease. In these models, the administration of rapamycin [delivered by a microspheres system (RPM)] improved tear secretion, decreased corneal endothelial cell injury, and improved histological damage of the cornea (101). Eye drop administration of rapamycin ameliorated lacrimal gland inflammation in SS mouse models while improving ocular surface integrity and tear secretion (102). Potential beneficial effects of its local administration were further con-

firmed in dacryoadenitis with no evidence of systemic toxicity (103). Due to such encouraging data, the therapeutic effect of rapamycin, combined with low-dose of IL-2, is currently under evaluation in one clinical trial on SS patients (NCT05605665). Additionally, the effectiveness and security of sub-conjunctival application of sirolimus in moderate to severe dry eye disease is currently under evaluation in two different clinical studies (NCT04115800, NCT00814944).

Conclusions

This is the first review to overview the currently available knowledge on cell metabolism in SS and its potential pathogenic effect in this condition. Although scant, data from studies conducted on biological fluids (serum, saliva and tears), specific cell types (lymphocytes, SGECs and fibroblasts) and animal models of SS, strongly suggest that the presence of a profound metabolic rewiring in SS is closely linked to the acquisition of a pro-inflammatory phenotype and exert a critical pathogenic role in SS. Due to the increasing amount of evidence on the ability of metabolism in shaping and regulating cell behaviour, aberrant expression of specific metabolic pathways in SS is likely crucial in contributing to both the development and the chronicity of this condition. If so, the use of drugs targeting metabolic pathways aberrantly activated in the inflammatory microenvironment should be looked at with interest as possible novel therapeutics capable of regulating the misbehaviour of different cell types known to be pathogenic in SS.

Acknowledgements

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