

Metabolic pressure and the breach of immunological self-tolerance

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The prevalence of autoimmune disorders in affluent countries has reached epidemic proportions. Over the past 50 years, a reverse trend between the frequency of infectious diseases and the incidence of autoimmune and allergic diseases led to the so-called ‘hygiene hypothesis’. Given the epidemiological evidence and recent experimental data, we propose that this concept should also include metabolic pressure secondary to exposure to excessive daily caloric intake and overnutrition. We discuss how metabolic workload can modulate immunological tolerance and review the molecular mechanisms and the state of the art of the field. We also critically evaluate possibilities for restoring immunological homeostasis under conditions of metabolic pressure.

Many studies and practical evidence have confirmed that undernutrition, in the early stages of life, is tightly associated with impaired immunological function directed against infectious agents^{1,2}. Moreover, malnourished people are commonly targeted by pathogens, with hyponutrition representing the most common cause of secondary immunodeficiency worldwide^{1,2}. The failure of adequate immunological responsiveness under conditions of hyponutrition, particularly in very young people (whose metabolism is higher), could be ascribed to the following: lower energy (stored as fat that is diminished by the lack of food) that can affect immune-cell proliferation and the production of antibodies and cytokines; and a decrease in adipose-tissue-derived adipocytokines, which signal the brain and the periphery that sufficient food is stored as fat to support the immune response³.

In contrast, in affluent countries, the easy availability of food and improved hygienic conditions are associated with a reduced prevalence of infections^{4,5}. However, daily exposure to excess calories (and the consequent increase in body fat) facilitate the hyperactivation of intracellular nutrient-energy-sensing pathways and metabolic overload in peripheral tissues, including immune cells^{6,7}. We propose that such environmental metabolic pressure can act synergistically with the effects of the hygiene hypothesis to cause the breach of

immunological self-tolerance in industrialized countries. We highlight how metabolic pressure can alter the mechanisms that control immunological self-tolerance and the regulatory T cells (T_{reg} cells) that are major sensors of the metabolic state, and delineate the rationale for the use of new therapeutic approaches for the manipulation of immune-system-mediated disorders.

Overnutrition, the hygiene hypothesis and autoimmune disease

Changes in lifestyle have been considered a major contributor to the rise in the frequency of autoimmune disorders and allergies (by 300%) in industrialized and developing countries. This trend started in the 1950s, and its persistence over time has been the object of intense investigation^{6–10} (Fig. 1).

Autoimmune diseases afflict about 5–9% of the US population (affecting women more frequently than men) and often congregate in families, a finding suggestive of a genetic predisposition that has been confirmed by genetic studies¹⁰. The incidence of autoimmune disorders and their prevalence between 1985 and 2015 showed a net percent increase per year of $19.1\% \pm 43.1\%$ (incidence) and of $12.5\% \pm 7.9\%$ (prevalence) (mean $\pm$ s.d.)^{8,9}. A proposed explanation for this rise is the change in environmental factors, including the decrease in infectious diseases due to improved hygienic standards, exposure to environmental pollutants and low intake of vitamin D^{4,5}. The inclusion in this list of an increased daily caloric intake from processed foods has been considered only recently. Since the 1970s, there has been an average increase of about 35% in the availability of calories per capita, in kilocalories per day (which corresponds to about 2,800 kilocalories per day)^{11,12}. Industrialized foods often contain the ‘holy triad’ of high sugar–high fat–high salt, which are known to hyperactivate intracellular nutrient-energy-sensing pathways¹³. Epidemiological studies have shown that an elevated body mass index (BMI) is a causal risk factor for autoimmunity^{14,15}. Obesity in the US population has doubled since 1980, with overweight and/or obese people now representing about 46% of the population¹⁶. The intersection between metabolic overload and the hygiene hypothesis clearly could represent a novel explanation for the continued increase in autoimmunity in overweight people^{14–16} (Fig. 1). That proposal is also supported by the finding that low caloric intake, malnutrition and recurrent infections early in life, which are characteristics typical of underdeveloped countries, are associated with the lowest frequency of autoimmune disorders^{4,5,17}.

Nutritional status and metabolism are major driving forces in longevity. Food-seeking behaviors are evolutionarily selected to permit survival in times of famine^{18,19}. Food signals, through multiple mediators (including adipocytokines), that energy-consuming processes,

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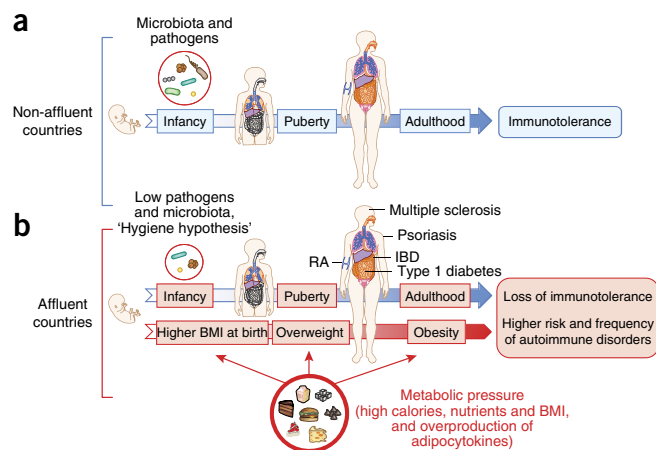


Figure 1 Increased incidence of autoimmunity in affluent countries according to the hygiene hypothesis and the concept of early metabolic pressure and overload. (a) In non-affluent countries, early exposure to a microbial burden supports a balance between protection from infection and immunological self-tolerance. (b) In affluent and industrialized countries, effects of the hygiene hypothesis, together with the increased metabolic workload (metabolic pressure) favored by substantial intake of daily calories and nutrients, are associated with a high BMI that supports the loss of immunotolerance. IBD, inflammatory bowel disease; RA, rheumatoid arthritis.

such as reproduction and immune responses, can be supported^{3,18,19}. Among the adipocytokines, leptin has a unique role not only because it signals that sufficient food is stored as fat to inhibit more food intake at the hypothalamic level but also because it modulates energy metabolism and immune responses^{3,18,19}. Leptin can reverse starvation-induced immunosuppression, and its circulating concentrations positively correlate with the number of naive CD4⁺ T cells; therefore, it represents a key heritable factor influenced by environmental cues such as the availability of food and pathogens²⁰.

Anatomical consideration

The 'anatomical tango' between lymphoid organs and adipose tissue, which has been known for decades, should be revisited in a new light. The lymph nodes, thymus and bone marrow are in tight contiguity with adipose tissue⁶. This suggests an anatomical link among nutritional status, metabolism and immune responses that could be mediated by adipocytokines produced by both adipocytes and activated immune cells⁶. Also, the thymus is surrounded by adipose tissue that might influence thymocytes and differentiating T cells in response to nutritional and/or metabolic status. In this sense, it is noteworthy that the thymus, whose atrophy is associated with lower leptin concentrations, has been considered a 'barometer of malnutrition', as it is small in conditions of hyponutrition^{21,22}. Adipocytes are also abundant in the bone marrow, where the differentiation of lymphoid and myeloid precursor cells occurs, together with hematopoiesis, lymphopoiesis, and memory B cell and T cell responses²³.

The autoimmune link

The abnormal accumulation of adipose tissue in overweight and/or obese people is associated with metabolic changes that include altered glucose tolerance, insulin resistance, hyperlipidemia and metabolic syndrome. That is paralleled by an increased incidence of autoimmune diseases²⁴, although other factors can influence these changes^{25–27}.

Type 1 diabetes and multiple sclerosis show a general trend toward developing in overweight and/or obese people, with a high BMI at

birth being particularly relevant for type 1 diabetes²⁵. An elevated BMI might also represent a causal risk factor for multiple sclerosis, in which an environmentally determined change in BMI from normal to overweight and/or obese is associated with a substantially increased risk of disease²⁸. Moreover, there is a strong association between psoriasis and an elevated BMI and metabolic syndrome²⁹. Together these epidemiological data indicate that environmental factors, and in particular metabolic pressure, are associated with obesity and autoimmunity, although the mechanistic determinants that link those aspects remain mostly elusive³⁰.

Low-grade inflammation induced by a high adipocyte mass, together with an elevated abundance of pro-inflammatory cytokines and adipocytokines produced by fat tissue, promote immune responses by the T_H1 and T_H17 subsets of helper T cells, decrease the frequency of T_{reg} cells and enhance M1-macrophage-type pro-inflammatory responses^{30–33}. That favors bystander activation of pro-inflammatory immune cells, including those with an autoreactive potential. Notably, obesity itself displays features of an autoimmune disease in which adipose-tissue antigens are targets of autoreactive T cells³⁰. Overweight and/or obese people in particular exhibit the following autoimmune characteristics^{10,34}: the presence of inflammatory-cell infiltrates, including T_H1 cells, T_H17 cells, CD8⁺ T cells and M1 inflammatory macrophages, in the target organ (adipose tissue); a reduction in the abundance of anti-inflammatory and/or tolerogenic T_{reg} cells in the adipose tissue; and circulating autoantibodies to adipose tissue and apoptosis of adipocytes^{35–38}. Also of note is the observation that CD4⁺ T cells infiltrating adipose tissue display T cell antigen receptor (TCR) oligoclonality, suggestive of a response driven by autoantigens³⁵. While the identification of putative self antigens in the fat represents a challenge, it is conceivable that high levels of oxidized lipids might modify self proteins and thus generate autoimmune responses³⁹. The progression of obesity after ingestion of a high-fat diet increases the concentration of natural immunoglobulin M via a stimulation of the receptor TLR4 on B cells³⁸. Immunoglobulin M and the macrophage-apoptosis inhibitor AIM have a key role in obesity-associated insulin resistance. Obesity often coexists with autoimmune disorders in the same person, possibly as result of concurrent analogous mechanisms of altered immunotolerance that operate in that person but attack different organs^{10,34}.

Metabolic pathways affect immunotolerance: the role of mTOR

The discovery of intracellular nutrients and energy sensors that perceive changes in the extracellular and intracellular milieu has defined additional molecular links between metabolism and immunotolerance^{40,41}. All organisms and cells have the ability to sense the presence of nutrients necessary for the generation of energy and maintenance of cellular processes. These pathways, which are conserved from yeast to humans, are represented mainly by mTOR ('mechanistic (formerly 'mammalian') target of rapamycin'), AMPK ('AMP-activated protein kinase') and GCN2 ('general-controlled non-repressed kinase'), a key sensor of amino-acid deprivation^{40,41}. These sense not only essential nutrients, such as glucose, amino acids and lipids, but also growth factors (linked to nutrition) and have a considerable effect on metabolism, inflammation and immune responses both at the intracellular level and at the systemic level^{40,41}.

In the context of immunotolerance to self, studies showing that mTOR regulates its protein complexes (mTORC1 and mTORC2) to control cell growth and metabolism are particularly interesting. mTOR is involved in the control of anabolic pathways, including protein synthesis, ribosome production, lipogenesis, glycolysis and nucleotide synthesis. It also suppresses the key catabolic process of

autophagy and maintains homeostasis in response to changing availability of nutrients, growth factors (i.e., insulin) and adipocytokines (i.e., leptin)⁴⁰. This macromolecular complex is involved in the differentiation of pro-inflammatory T_H1 and T_H17 cells and anti-inflammatory T_{reg} cells^{42–48}.

Notably, depending on the strength and duration of signaling via the TCR, naive conventional T cells (T_{conv} cells) in humans can differentiate either into inflammatory T_H1 and T_H17 cells or into tolerogenic T_{reg} cells^{49–53}. Strong stimulation of the TCR results in the generation of inducible T_{reg} cells (iT_{reg} cells) only in the presence of exogenous cytokine TGF- β *in vitro*. In contrast, weak and suboptimal engagement of the TCR leads to the generation of iT_{reg} cells even in the absence of exogenous TGF- β ⁴⁹. From a molecular point of view, this phenomenon is the result of a combined effect of activation of both the TCR and the nutrient-energy-sensing pathway. Specifically, during suboptimal stimulation of the TCR, activation of the mTOR pathway occurs at a relatively low level but is necessary for T_{conv} cells to undergo both glycolysis and fatty-acid oxidation, proliferate and differentiate. Weak engagement of the TCR represents a permissive temporal window that supports the epigenetic program required for *de novo* expression of the gene encoding the transcription factor FOXP3 and consequent induction of iT_{reg} cells. Notably, inhibition of glycolysis with the glucose analog 2DG (2-deoxy-D-glucose) blocks the generation of iT_{reg} cells from

T_{conv} cells⁴⁹. Conversely, strong engagement of the TCR results in rapid overactivation of mTOR and glycolysis, which are both deleterious to the transcription of FOXP3 and its maintenance over time^{42,50}. The addition of either TGF- β or rapamycin (a selective inhibitor of mTOR) to cultures results in the generation of iT_{reg} cells only after strong activation of the TCR, because these molecules act as inhibitory signals for the TCR or mTOR and thus mimic weak activation of the TCR, which is permissive for the induction of FOXP3 and its stable transcriptional program. Many studies have confirmed that a crucial factor for the outcome of T cell differentiation is strength of the TCR signals and associated costimulatory signals^{51,54}. In short, TCR signal strength and strong activation of the kinase PI3K–kinase Akt–mTOR pathway can restrain the induction of FOXP3 and consequently the generation of iT_{reg} cells, while weak or suboptimal activation of the TCR would be needed to engage both the activation of mTOR and glycolysis and thus promote the epigenetic program that maintains FOXP3 expression over time⁵³. As antigen-specific stimulation of the TCR *in vivo* is weak (relative to that in *in vitro* studies), mTOR activity must be oscillatory, requiring alternating periods of high activity and low activity that reflect fluctuations in nutrition and diet (but also leptin)^{43,55,56} (Fig. 2a). However, constitutive activation of mTOR would be a dominant factor that perturbs the TCR's signal strength. In this case, overactivation of mTOR (as happens during nutritional overload, when fluctuations in diet and leptin are lost)

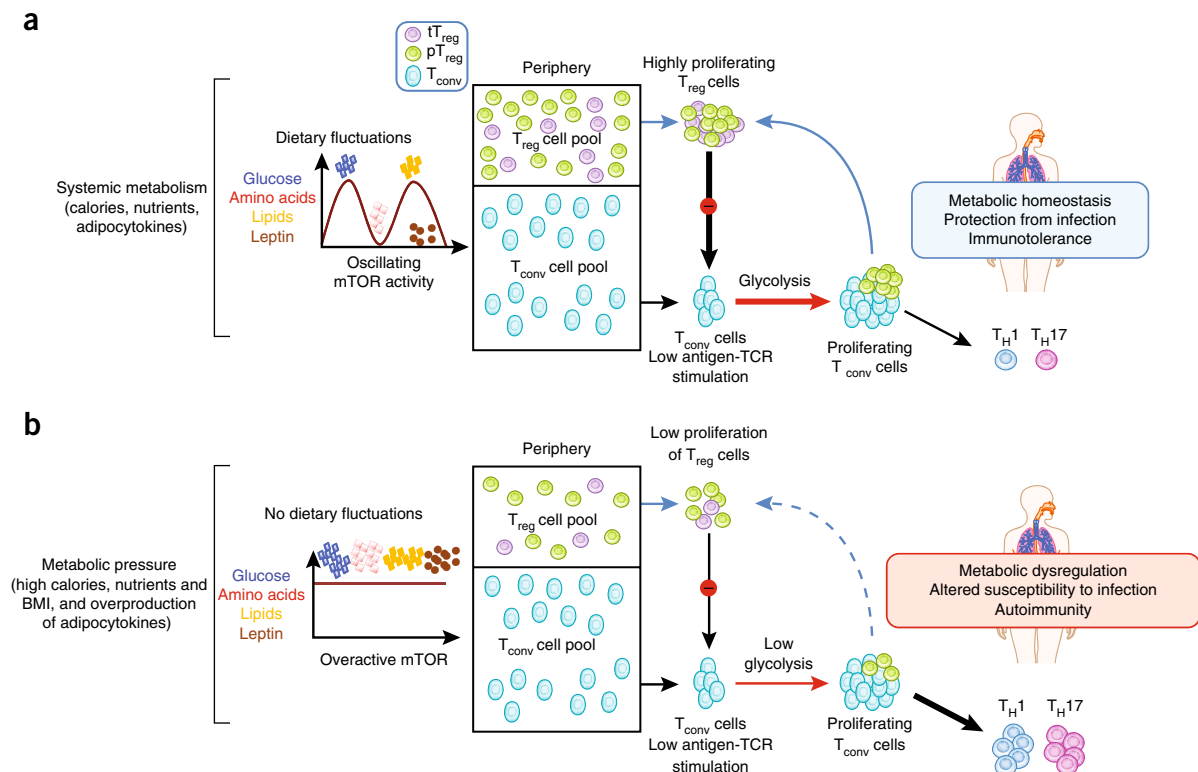


Figure 2 Metabolic pressure and immunotolerance to self. Immunotolerance is maintained because of interactions among genetics, environment and metabolism. The peripheral pool of T_{reg} cells is composed of thymus-derived T_{reg} cells (tT_{reg} cells) generated at very early stages of life, together with peripheral T_{reg} cells (pT_{reg} cells) generated in the periphery throughout life after weak antigenic stimulation of T_{conv} cells. Glycolysis has a key role in the activation and proliferation of T_{conv} cells. When glycolysis is induced by weak stimulation of the TCR, most metabolically active and proliferating $CD25^+$ T cells will differentiate into T_{reg} cells and replenish the peripheral pool of T_{reg} cells. (a) In physiological conditions, due to dietary fluctuations, several metabolic stimuli (for example, nutrients and leptin) activate the mTOR pathway in T_{reg} cells in an oscillatory manner, which supports the proliferation of T_{reg} cells *in vivo* and the maintenance of immunotolerance and metabolic homeostasis. (b) Chronic overactivation of the mTOR pathway due to nutritional overload, calories and obesity is associated with impaired proliferative potential of T_{reg} cells and diminished glycolytic induction by T_{conv} cells. This results in less induction of T_{reg} cells in the periphery and the differentiation of pro-inflammatory T_H1 and T_H17 cells, which favors metabolic dysregulation, altered susceptibility to infection and autoimmunity.

reduces the proliferation of T_{reg} cells, inhibits the differentiation of T_{conv} cells into T_{reg} cells, and promotes the differentiation of T_{conv} cells into the T_H1 cell– T_H17 cell lineage and thus leads to imbalanced immunotolerance^{42,57–59} (Fig. 2b). It follows that alternating periods of high mTOR activity and low mTOR activity (or oscillations in this activity), which happen with normal feeding and fasting, are essential for the maintenance of optimal homeostatic mechanisms. This would also apply to T_{reg} cells, for which optimal growth requires oscillations and alternating periods of high mTOR activity and low mTOR activity, whereas overactivation or chronic inhibition of mTOR would lead to a reduction in the *in vivo* proliferation and homeostasis of T_{reg} cells^{43,55,56} (Fig. 2). Indeed, when elevated and tonic activity of mTOR and the S6 ribosomal kinase is maintained in naive $CD4^+$ T cells by genetic manipulation, there is a reduction in the number of T_{reg} cells with an accumulation of autoreactive T cells that leads to systemic autoantibody production and autoimmunity⁶⁰.

T_{reg} cells as metabolic sensors

How is immunotolerance to self maintained in the context of metabolic regulation? The environment, via nutrient availability, certainly has a pivotal role in this homeostatic process. In this context, T_{reg} cells seem to be involved not only in the maintenance of immunotolerance to self but also in the control of metabolic homeostasis. T_{reg} cells are generally categorized according to their origin into two main subgroups, both of which express FOXP3 as a pivotal element for their suppressive function⁶¹. One subgroup arises from the thymus as a distinct lineage, and the other is derived from the peripheral conversion of naive $CD4^+CD25^-$ T cells⁶². These processes rely on multiple signaling pathways, TCR signal strength, costimulatory molecules and the cytokine milieu^{61,62}. For FOXP3, its expression and function are regulated at transcriptional, post-transcriptional and post-translational levels, with at least twelve different FOXP3 transcripts described in humans, as well as four to eight different splicing variants ranging from 18 kDa to 49 kDa (refs. 63–65). Advances in T_{reg} cell biology have shown that specific splicing variants of FOXP3 encode products with a dominant role in the control of transcriptional programs linked with immunotolerance and T_{reg} cell function^{63–65}.

T_{reg} cells are anergic and/or hyporesponsive to TCR stimulation *in vitro*, and these cell populations are difficult to expand unless they are cultured with high doses of exogenous cytokine IL-2 and strong ligation of the TCR^{61,62} (Fig. 3). Despite being anergic *in vitro*, T_{reg} cells have an intact proliferative potential *in vivo*. Indeed, studies using deuterium labeling have shown that human T_{reg} cells divide rapidly, with a doubling time of 8 days, much faster than the 24-day doubling time of memory $CD4^+$ T cells or the 199-day doubling time of naive $CD4^+$ T cells^{66,67}. Thus, T_{reg} cells have the dichotomous ability to be both anergic *in vitro* and proliferative *in vivo*, displaying an activated surface phenotype typical of activated and proliferative cells^{61,62} (Fig. 3). T_{reg} cells also require continuous TCR stimulation to maintain their activated phenotype and suppressive activity *in vivo*⁶⁸. In summary, T_{reg} cells are highly proliferative and very active metabolically^{48,69} and express a proteomic signature *ex vivo* that is biased toward glycolysis and lipid synthesis, reflective of their state of active proliferation and synthesis of membranes and organelles⁶⁹. For these reasons, they respond quickly to metabolic environmental signals that trigger proliferation and suppress and modulate FOXP3, which makes them metabolic sensors that perceive local and systemic nutritional and/or metabolic changes (Fig. 3). That concept has been reinforced by studies showing the following: nutrients and leptin render T_{reg} cells anergic *in vitro* via overactivation of mTOR signaling; the *in vitro* anergy of T_{reg} cells is associated with high expression of the leptin

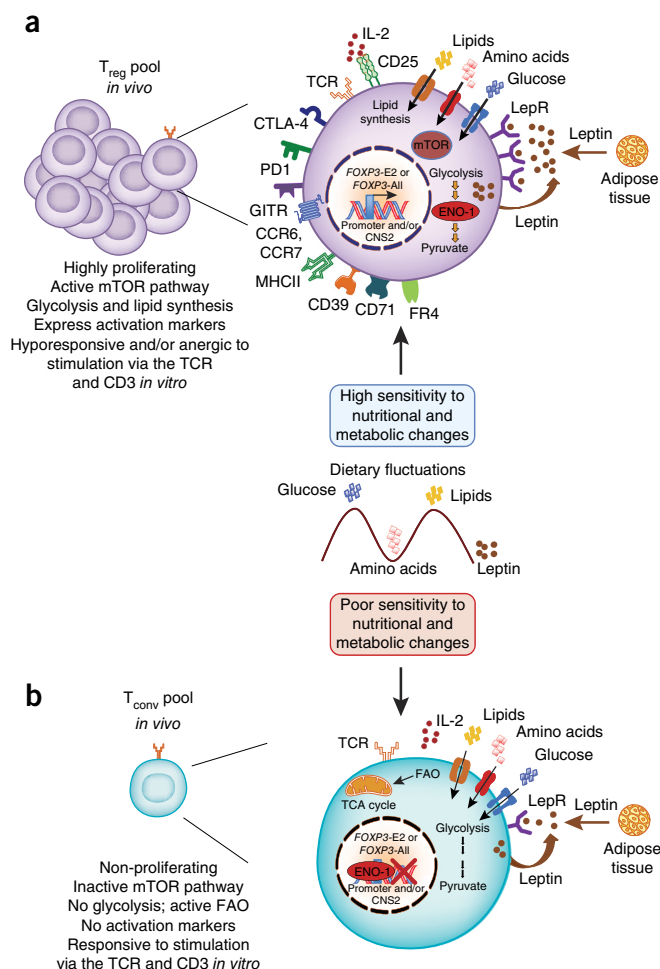


Figure 3 Metabolic differences between T_{reg} cells and T_{conv} cells, and T_{reg} cells as metabolic sensors. (a) T_{reg} cells are highly sensitive to metabolic changes. Since they are very active metabolically (they engage both glycolysis and lipid synthesis) and are highly proliferative, they can quickly sense and adapt to metabolic changes induced by fluctuations in nutrients and adipocytokines such as leptin. On their surface, they express activation markers. Intracellularly, their active mTOR makes them highly sensitive to metabolic and/or nutritional perturbations. Through transcriptional control of FOXP3 by the glycolytic enzyme ENO-1, glycolysis supports the induction and maintenance of human FOXP3 splice variants containing exon 2 (FOXP3-E2) or all exons (FOXP3-All). CD3, TCR invariant chain; CD25, IL-2 receptor- α ; CTLA-4, cell-surface marker; PD1, costimulatory molecule; GITR, immunomodulatory receptor; CCR6 and CCR7, chemokine receptors; MHCII, major histocompatibility complex class II; CD39, ATP ectonucleotidase; CD71, transferrin receptor; FR4, folate receptor; LepR, leptin receptor. (b) Peripheral T_{conv} cells are in a non-proliferative and/or resting state, do not express surface activation markers or mTOR and rely on fatty-acid oxidation (FAO) and mitochondrial metabolism, which makes them less sensitive to metabolic changes. In resting conditions, nuclear ENO-1 is bound to the FOXP3 promoter and its CNS2 regulatory regions and represses FOXP3 expression. After weak stimulation of the TCR, ENO-1 is engaged in cytosolic glycolysis and allows FOXP3 transcription and, consequently, the induction of T_{reg} cells. TCA, tricarboxylic acid.

receptor and mTOR; transient acute inhibition of the leptin–mTOR axis via leptin-neutralizing monoclonal antibodies or pre-treatment with rapamycin (both signals of pseudo-starvation) reverses the anergy and proliferation of T_{reg} cells; mTOR signaling needs to be rapid, oscillatory and of low or intermediate strength to support the

proliferation and homeostasis of T_{reg} cells *in vivo* and *in vitro*; T_{reg} cells are very sensitive to oscillatory fluctuations in leptin and circulating nutrients *in vivo*; and T_{reg} cells rapidly adapt to microenvironmental changes such as low glucose and high lactate (typical of inflamed or ischemic tissues)^{35,43,70,71} (Figs. 2 and 3).

The concept of T_{reg} cells as metabolic sensors is strengthened by the fact that they also reside in the adipose tissue, where leptin and adipocytokines reach high concentrations. Thus, both their functional characteristics and their anatomical location allow T_{reg} cells to link local metabolism with systemic metabolism^{35,69,72}. Since leptin can promote the entry of glucose into the tissues and reduce hyperglycemia, its secretion by T_{reg} cells locally and systemically might contribute to metabolic homeostasis and glucose tolerance^{73,74}. Also, adipose-tissue-resident T_{reg} cells might control systemic glucose homeostasis and tolerance via secretion of anti-inflammatory cytokines and expression of the transcription factor PPAR- γ ('peroxisome proliferator-activated receptor- γ ')^{35,72}. In conclusion, T_{reg} cells can be considered metabolic sensors that perceive nutritional and metabolic changes and in turn modulate leptin production and affect metabolic homeostasis and immunotolerance (Fig. 3).

Metabolic pressure and susceptibility to autoimmunity

We propose that the hygiene hypothesis should be complemented by recognition of the influence of environmental triggers that lead to loss of immunological self-tolerance⁷⁵. Specifically, prolonged metabolic overload of peripheral tissues, influenced by genetic and epigenetic components, might affect immunological self-tolerance through changes in the energy metabolism of immune cells, particularly T_{reg} cells.

The hygiene hypothesis postulates that the immune system, soon after birth and the microbial colonization of organs such as the gut, skin and lungs, undergoes constant antigenic challenge, which creates a broad TCR repertoire and constant replenishment of peripheral immune cells, including those with regulatory function^{4,5,75}. If a highly hygienic environment is not stimulatory enough for the innate and adaptive immune systems early in life, immunological fitness and tolerance to self will be compromised. The adaptive immune system has best evolved to fight infection. Once an organism grows through infancy and puberty (when thymic involution begins), the environment becomes a major participant in the control of immunotolerance to self, mainly via induced immune responses driven by colonizing microorganisms^{76–79}. The colonization of the gut and other tissues exposed to the outside world via the microbiota might indicate that T_{reg} cells can also be induced by exposure to antigens and metabolites such as butyrate and short-chain fatty acids produced in the gut⁷⁶. In support of that proposal, the non-obese diabetic mouse model develops spontaneous type 1 diabetes under specified pathogen-free conditions in which exposure to commensal microorganisms is extremely low^{80,81}. When such mice are moved to a conventional animal facility, the frequency of type 1 diabetes drops substantially, due to the induction of T_{reg} cells as a counter-regulatory mechanism for controlling production of the cytokines IFN- γ and IL-17. The same result is obtained when IFN- γ is injected into mice of the non-obese diabetic strain early in life or if these mice are immunized with complete Freund's adjuvant or with bacillus Calmette-Guérin, both strong inducers of IFN- γ that mimic activation of the innate immune system^{82–87}.

Preventive immunization with complete Freund's adjuvant or bacillus Calmette-Guérin also provides protection against experimental autoimmune encephalomyelitis^{88–90}, which suggests that T_{reg} cell-mediated suppression operates best after activation of the immune

system and concomitantly with ongoing immune responses. In the absence of activation of the immune system and antigenic challenge, suppression and counter-regulation are limited. This dynamic scenario considers autoimmunity a result of the impaired generation of thymus-derived T_{reg} cells early in life or a result of a reduction in the induction and/or replenishment of T_{reg} cells by environmental factors (Fig. 2a). Metabolic pressure would affect both the thymic generation of T_{reg} cells and the peripheral generation of T_{reg} cells by overstimulating nutrient-energy-sensing pathways, which would lead to loss of T_{reg} cell homeostasis and thus trigger autoimmunity (Fig. 2b). Consistent with that, peripheral T_{reg} cells from patients with multiple sclerosis have an overactive mTOR pathway associated with altered expression of defined FOXP3 splice variants and impaired proliferative potential of T_{reg} cells⁹¹. Such alterations are associated with reduced responsiveness to IL-2, which would suggest that impaired T_{reg} cell homeostasis in multiple sclerosis might be secondary to metabolic overload. On the other hand, in human multiple sclerosis, glycolysis in T_{conv} cells is diminished after suboptimal engagement of the TCR, despite their preserved proliferative capacity⁷⁰. That deficit in glycolysis, together with a reduced ability to induce defined FOXP3 splice variants containing exon 2, as well as a stable T_{reg} cell phenotype in people with autoimmunity, are associated with reduced proliferative potential and suppression linked to overactivation of mTOR (Figs. 2 and 3).

The impaired ability to generate T_{reg} cells and the subsequent increase in the activity of pro-inflammatory T_{conv} cells, whose populations are generally expanded in autoimmune and obese people, have been analyzed at the molecular level. The glycolytic enzyme ENO-1 ('enolase-1') links T_{conv} cell activation, glycolysis and FOXP3 induction. ENO-1 is a multifunctional complex that also displays non-glycolytic activity ('moonlighting functions') and can act as a transcriptional repressor and as a cell-surface receptor for plasminogen⁹². Of note, ENO-1 can also act as functional repressor of FOXP3, being bound to its promoter and CNS2 regulatory regions in resting T_{conv} cells⁷⁰ (Fig. 3). After weak stimulation of the TCR and the induction of glycolysis in T_{conv} cells, ENO-1 cannot bind the FOXP3 promoter and/or CNS2 regions and resides in the cytosol, where it supports the oxidation of glucose. Thus, glycolysis controls the generation of T_{reg} cells from T_{conv} cells, and this process involves engagement of ENO-1 (Figs. 2 and 3). Notably, defective induction of glycolysis in CD4⁺ T_{conv} cells has been described not only in multiple sclerosis but also in other autoimmune diseases, such as rheumatoid arthritis, in which T cells exhibit diminished glycolysis, low ATP levels and enhanced shunting of glucose into the pentose-phosphate pathway⁹³. Also, in the glycogen-storage disease GSD-1b, which is characterized by impaired use of glycogen and glucose, a higher incidence of autoimmune disease has been observed. T_{conv} cells from patients with GSD-1b show reduced induction of glycolysis following TCR stimulation, reduced expression of FOXP3 in T_{reg} cells derived from the peripheral conversion of naive CD4⁺CD25[–] T cells, and an impaired ability of T_{conv} cells to induce FOXP3 after suboptimal stimulation of the TCR⁹⁴. In conclusion, the induction of T_{reg} cells from the activation of T_{conv} cells occurs via the induction of glycolysis, and when this process is impaired (either due to metabolic pressure or genetically), the efficiency with which T_{reg} cells are generated in the periphery is altered and so is immunotolerance.

Concluding remarks

The link between chronic metabolic pressure and susceptibility to autoimmune disease is fascinating and involves a global workload on tissues and immune cells that fail to maintain their highly tuned

homeostatic mechanisms because of the persistence of the process (Fig. 2b). We note that the central nervous system also has a potential role in mediating signals of metabolic pressure in T_{reg} cells and T_{conv} cells under conditions of chronic overnutrition. Indeed, specific hypothalamic feeding circuits can provide signals of metabolic overload via the sympathetic nervous system and affect T_{reg} cell function and susceptibility to autoimmune disease^{95,96}.

In conclusion, genetic predisposition, epigenetics, regulation of the immune system and the environment have long been considered key pathogenic participants in autoimmunity. Among the environmental factors, metabolic overload should be included as a newly identified contributor. Notably, this raises the possibility of intervention via an early reduction in the metabolic pressure associated with autoimmunity through metabolic signals of starvation either non-pharmacologically^{97–99} (i.e., based on behavioral changes and diet or caloric restriction) or pharmacologically¹⁰⁰ (i.e., ‘pseudo-starvation’, in which drugs convey signals of starvation and metabolic adjustments that mimic fasting).

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COMPETING FINANCIAL INTERESTS

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