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Previews

CD4⁺ T Cells at the Center of InflammagingGuido Kroemer^{1,2,3,4,5,*} and Laurence Zitvogel^{4,6,7,8,9}¹Equipe Labellisée par la Ligue Contre le Cancer, Université de Paris, Sorbonne Université, Inserm U1138, Centre de Recherche des Cordeliers, Paris, France²Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France³Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France⁴Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China⁵Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden⁶Faculty of Medicine, Université Paris Saclay, Le Kremlin-Bicêtre, France⁷Gustave Roussy Comprehensive Cancer Institute, Villejuif, France⁸INSERM U1015, Villejuif, France⁹Center of Clinical Investigations in Biotherapies of Cancer (CICBT) 1428, Villejuif, France*Correspondence: kroemer@orange.fr<https://doi.org/10.1016/j.cmet.2020.04.016>

Bharath et al., 2020 report that CD4⁺ T lymphocytes from aged individuals exhibit defective mitochondrial autophagy, resulting in altered redox metabolism and upregulation of TH17 cytokines, which in turn may contribute to aging-associated chronic inflammation or “inflammaging.” Of note, the antiaging drug metformin reverses this autophagy defect and rejuvenates CD4⁺ T cell function.

The aging organism develops a chronic state of initially smoldering and then progressively overt inflammation that contributes to the aging process and thus has been nicknamed “inflammaging” (López-Otín et al., 2013). A recent paper by Bharath et al., 2020 reveals that, upon α CD3/ α CD28 stimulation, purified CD4⁺ T lymphocytes from healthy, lean, older (57–68 years) donors produce more TH17-associated/supportive cytokines (IL-6, IL-17A, IL-17F, IL-21, and IL-23) than cells from younger (28–38 years) subjects. This effect could be inhibited by the addition of a clinically achievable concentration (100 μ M) of metformin, an oral antidiabetic that has multiple effects on aging-related processes and is currently undergoing clinical evaluation in the TAME (Targeting Aging by Metformin) trial for its capacity to prevent the manifestation of age-associated diseases (Kulkarni et al., 2020).

In a series of elegant experiments performed on primary CD4⁺ T cells stimulated with α CD3/ α CD28, Bharath et al., 2020 elucidated a plausible mechanism for the old age-associated, metformin-treatable TH17-linked cytokine hyperproducer phenotype (TH17-CHP). Indeed, the overarching cause of TH17-CHP appears to be reduced autophagy of mitochondria, which compromises mitochondrial turnover and quality control, as indicated by an increase in mitochondrial mass, an increase in the proton leak, and a reduction in the mitochondrial inner transmembrane

potential. In addition, mitochondria contained in CD4⁺ T cells from older donors exhibited an enhanced basic and maximal oxygen consumption, correlating with reduced glycolytic lactate production, enhanced production of reactive oxygen species (ROS), and activating phosphorylation of the transcription factor STAT3, with enhanced STAT3 binding to the promoters of the *IL17A* and *IL17F* genes. Metformin reactivated autophagy through an AMPK-independent pathway and reversed most of the aforementioned alterations (Figure 1A). Conversely, knockdown of the essential autophagy gene *ATG3* (but not that of *PINK1*, a gene specifically involved in mitophagy) inhibited autophagy in CD4⁺ T cells from younger subjects, inducing TH17-CHP similar to the one spontaneously found in CD4⁺ T cells from older donors (Figure 1B). Tempol, a membrane-permeable radical scavenger, was able to reduce some aspects of the TH17-CHP, yet did not show a clear epistasis with respect to metformin, suggesting that tempol-quenchable ROS somehow contribute to, but are not entirely responsible for, the TH17-CHP (Bharath et al., 2020).

Of note, in a cohort of obese, pre-diabetic patients, a 3-month-long treatment with metformin (1 g/day) changed the phenotype of purified CD4⁺ T cells stimulated with α CD3/ α CD28, causing a reduction in the lipidated (autophagy-associated) form of LC3 and a reduction of organellar markers (m-aconitase for mito-

chondria and GFP78 for the endoplasmic reticulum, ER), suggesting that metformin can enhance mitochondria and ER clearance in CD4⁺ T cells *in vivo*. In contrast, the *in vitro* result obtained with metformin on CD4⁺ T cells from young subjects did not reveal any sign of autophagy enhancement (Bharath et al., 2020).

Altogether, these results have important conceptual and clinical implications at several levels.

First, they suggest yet another causal link between “normal” aging and deficient autophagy involving a vicious cycle in which aging causes an autophagy defect that then aggravates the aging phenotype (Rubinsztein et al., 2011). Here, it appears that aging compromises autophagy in CD4⁺ T lymphocytes to stimulate the secretion of several pro-inflammatory interleukins, thus contributing to inflammaging (Bharath et al., 2020). However, it remains to be determined in preclinical experiments, in mouse models, whether a selective autophagy (or mitophagy) defect solely affecting CD4⁺ cells would be sufficient to cause TH17-CHP *in vivo* and accelerate the aging process. As it stands, it appears that autophagy has rather broad anti-inflammatory effects, notably by avoiding the spill of mitochondrial or nuclear DNA into the cytoplasm (to avoid activation of the cGAS/STING pathway) or by inhibiting excessive activation of the NLRP3 inflammasome (Galluzzi et al., 2012; Mathur et al., 2018).



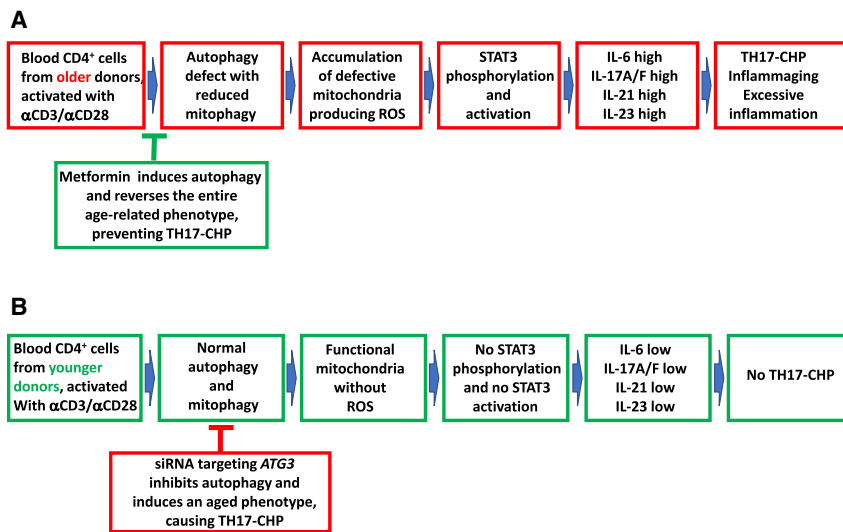


Figure 1. Differences between CD4⁺ T Cells from Older Donors and Younger Donors Revealed by Polyclonal *In Vitro* Activation

The phenotype of T cells from older donors (A) can be rejuvenated by metformin treatment, while that of T cells from younger donors (B) can be jeopardized by inhibition of autophagy using a small interfering RNA (siRNA) that depletes ATG3 mRNA. TH17-CHP, TH17-linked cytokine hyperproducer phenotype.

Second, [Bharath et al., 2020](#) reveal potential biomarkers of biological aging. Chronological and biological aging can be dissociated from each other to some extent, meaning that simple metabolic parameters, such as obesity (with the associated metabolic syndrome) and caloric restriction can accelerate and decelerate, respectively, the aging process ([Kroemer et al., 2018](#); [López-Otín et al., 2016](#)). For this reason, it is important to measure biological age on cell types that are (relatively) accessible such as circulating CD4⁺ T lymphocytes. At this stage, the methodology developed by [Bharath et al., 2020](#) requires *in vitro* stimulation of these cells with α CD3/ α CD28 to reveal differences between CD4⁺ T cells from older and younger donors. Ever-advancing single-cell “omics” approaches might allow researchers to retrieve and characterize blood CD4⁺ T cells that have been naturally activated *in vivo*, hoping to confirm the observations by [Bharath et al., 2020](#) on freshly obtained blood samples and to refine the analyses to unprecedented levels by obtaining the sequences (and hence the specificities) of the T cell receptors that contribute to inflammaging. Indeed, it might be highly informative whether TH17-CHP affects all TCR clonotypes in a similar fashion or whether it preferentially occurs in autoreactive T cells.

Third, [Bharath et al., 2020](#) confirm prior evidence that metformin might mediate (part of) its healthspan-extending effects through the induction of autophagy, in line with prior speculations that any kind of antiaging manipulation must involve a pro-autophagic component to be successful ([Rubinsztein et al., 2011](#)). Metformin is used at high doses (in the gram per day range), mediates cellular effects at rather elevated concentrations (typically in the \sim 100 μ M range), and influences all hallmarks of aging at multiple levels, including at the level of the intestinal microbiota ([Kulkarni et al., 2020](#)). Hence, the pharmacological targets of metformin have not been defined in molecular terms. The finding that metformin can impact the aging-related phenotype of CD4⁺ T cells in a direct fashion (without an action on the microbiota or on other cell types) might facilitate the search for molecular metformin target(s), hence paving avenues toward the development of other (perhaps more efficient) antiaging molecules.

Moreover, the observation that metformin can blunt TH17-CHP might open the possibility to repurpose this drug for the treatment of excessive acute inflammatory reactions as they occur in COVID-19, especially in aged individuals. Indeed, the ongoing COVID-19 crisis dramatically reveals major age-related differences in the antiviral response. In

most cases, the immune system of the young eliminates the virus, while older subjects (>60 years) exhibit a reduced antiviral defense, as well as a tendency toward exaggerated inflammatory responses resulting in lethal lung damage ([Zhou et al., 2020](#)). Of note, Tocilizumab, an anti-IL-6 receptor antibody, has successfully been used to treat some cases of COVID-19-associated pneumonitis ([Mehta et al., 2020](#)), establishing a cause-effect relationship between inflammation and organ damage. It remains to be explored whether metformin might have similar beneficial effects in the context of acute life-threatening inflammatory reactions.

DECLARATION OF INTERESTS

G.K. is a scientific co-founder of Samsara Therapeutics and Therafast Bio.

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