



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Review

The role of extracellular DNA in COVID-19: Clues from inflamm-aging

Gianluca Storci^{a,1,*}, Francesca Bonifazi^b, Paolo Garagnani^{a,c}, Fabiola Olivieri^{d,e},
Massimiliano Bonafè^{a,1,*}

^a Department of Experimental and Diagnostic Medicine, University of Bologna, Italy

^b IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

^c Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Huddinge University Hospital, Stockholm, Sweden

^d Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica delle Marche, Ancona, Italy

^e Center of Clinical Pathology and Regenerative Therapy, IRCCS INRCA, Ancona, Italy

ARTICLE INFO

Keywords:

Inflamm-aging
Misplaced DNA
COVID-19
DNA sensing receptors
Telomere

ABSTRACT

Epidemiological data convey severe prognosis and high mortality rate for COVID-19 in elderly men affected by age-related diseases. These subjects develop local and systemic hyper-inflammation, which are associated with thrombotic complications and multi-organ failure. Therefore, understanding SARS-CoV-2 induced hyper-inflammation in elderly men is a pressing need. Here we focus on the role of extracellular DNA, mainly mitochondrial DNA (mtDNA) and telomeric DNA (telDNA) in the modulation of systemic inflammation in these subjects. In particular, extracellular mtDNA is regarded as a powerful trigger of the inflammatory response. On the contrary, extracellular telDNA pool is estimated to be capable of inhibiting a variety of inflammatory pathways. In turn, we underpin that telDNA reservoir is progressively depleted during aging, and that it is scarcer in men than in women. We propose that an increase in extracellular mtDNA, concomitant with the reduction of the anti-inflammatory telDNA reservoir may explain hyper-inflammation in elderly male affected by COVID-19. This scenario is reminiscent of inflamm-aging, the portmanteau word that depicts how aging and aging related diseases are intimately linked to inflammation.

1. Introduction

The ongoing COVID-19 pandemic due to the SARS-CoV-2 coronavirus is causing a global health emergency (Callaway et al., 2020). The rapid SARS-CoV-2 infectivity and the appearance of serious and fatal respiratory complications in a significant fraction of patients represent unprecedented threats for all health systems worldwide. The extreme virulence of the infection in a substantial proportion of patients (about 6–12 % of lethality, <https://coronavirus.jhu.edu/map.html>), coupled with its impressive infectivity (an estimated reproduction number R_0 up to 2/3 in comparison to 1.4 of the seasonal flu), and the unprecedented worldwide spread (about 60.000.000 affected people on November the 30th 2020; <https://coronavirus.jhu.edu/map.html>), highlight the urgent need to understand the molecular basis of the most severe manifestations of COVID-19 disease. Data on COVID-19 pandemic show that the clinical outcome of SARS-CoV-2 infection is linked to the age and gender of the patients, irrespective of ethnicity (Ruan et al., 2020; Remuzzi and Remuzzi, 2020). In Italy, about 1.600.000 people infected

on November the 30th, with a median age of death of 80 years, which is 31 years older than that of the infected (49 years of age). Only 1.1 % of COVID-19 victims are less than 50 years of age. Most of them, (around 60 %), were affected by at least 3.3 age-related diseases (e.g. cardiac dysfunction, hypertension, diabetes, <https://www.epicentro.iss.it/>). The data available in Italy displays that the lethality is substantially higher in men than in women, in the age-range of 50–70 years of age, with a male to female death toll of 75% vs 25% (<https://www.epicentro.iss.it/coronavirus/sars-cov-2-decessi-italia>). Clinical manifestations in the most severe COVID-19 patients are characterized by an aberrant hyper-inflammatory response in which Interleukin-6 (IL-6), the so called cytokine for gerontologists (Ershler, 1993), stands out as major player (Chen et al., 2020; Zhou et al., 2020). Thanks to this evidence, some clinical trials suggested that patients with SARS-CoV-2 benefit of the administration of the monoclonal antibody against IL-6/IL-6 receptor that tapers the cytokine release syndrome (Xu et al., 2020; Luo et al., 2020; Zhang et al., 2020). Nevertheless, the debate on clinical significance of IL-6 pathway inhibition is still open (Salvarani et al., 2020).

* Corresponding authors.

E-mail addresses: gianluca.storci@unibo.it (G. Storci), massimiliano.bonafe@unibo.it (M. Bonafè).

¹ Equally contribution.

Importantly, the local (pulmonary) and systemic hyper-inflammatory status are involved in the extensive thrombotic phenomena that lead to ischemic damage and multi-organ failure in COVID-19 patients (Wu et al., 2020; Connors et al., 2020; Zuo et al., 2020). Therefore, the understanding of the biological events acting in the SARS-CoV-2-induced inflammation, particularly in male aged people is an urgent need.

2. COVID-19 and inflamm-aging

Nowadays, it is acknowledged that old people suffer of a state of progressive chronic inflammation called inflamm-aging (Franceschi et al., 2000). Inflamm-aging is closely related to age-related diseases, whether it is considered a cause, i.e. involved in the pathogenesis of diseases, or the consequence of them (Fulop et al., 2018). A plethora of biologic stimuli such as self and non-self molecules, nutrients and microbiota, nurture inflamm-aging by triggering the age-dependent activation of the innate immune system (Franceschi et al., 2018). Inflamm-aging is intimately linked to an age-associated immune metabolic remodeling that occurs in aging and age-related diseases (metainflammation) (Franceschi et al., 2018; Storci et al., 2018, 2020). In other words, if we consider inflamm-aging as an age-related inflammatory drift, specific diseases, such as COVID-19, may be considered spikes or bouts of inflammation that may accelerate the immune-metabolic derangement of aged people. We recently proposed that at least four mechanisms linked to aging, (i.e. the rate of inflamm-aging; the rate of immune-senescence; the loss of the anti-inflammatory activity of ACE2 receptor; the accrual of senescent cells and the shortening of telomeres), may affect COVID-19 severity (Bonafè et al., 2020a). Notably, most of these mechanisms are gender-biased, as they occur more overtly in men than in women (Bonafè et al., 2019; Márquez et al., 2020). Here, we focus on the role gambled by circulating cell-free DNA as a modulator of systemic inflammation in aged people (i.e. during inflamm-aging) and in COVID-19 patients. In this regard, two recent investigations identified extracellular mtDNA released by senescent cells as a key factor of inflamm-aging (Iske et al., 2020), as well as the level of circulating mtDNA as a predictor of COVID-19 severity (Scozzi et al., 2020). We also focus on extracellular telomeric DNA (telDNA) as crucial source of extracellular anti-inflammatory DNA exhaustible with aging (Storci et al., 2018; Bonafè et al., 2020b). A wealth of literature shows that telDNA shortening is a hallmark of cellular and systemic aging, and that it is associated with cellular and systemic inflammation (Aguado et al., 2019). Indeed, many phenomena related to aging, including a plethora of age-related diseases, are associated with telomere shortening (Aguado et al., 2019; Armanios et al., 2013; Herrmann et al., 2018). Consistently, a recent paper suggests that short telomeres are markers of an unfavorable outcome in COVID-19 patients (Aviv, 2020). However, a plenty of data show that extracellular telDNA may act as a powerful quencher of inflammation and we recently reported its possible role in inflamm-aging (Storci et al., 2018; Bonafè et al., 2020b; Kaminski et al., 2013). Moreover, recent literature shows that extracellular telDNA is actively shed from the chromosomal telomeric ends and that it is found in extracellular vesicles (Bonafè et al., 2020b; Mazzucco et al., 2020; Bruno et al., 2020). Under this perspective, the age-dependent shortening of telomeres represents a mechanism of depletion of extracellular anti-inflammatory DNA reservoir in aged people. Following this reasoning, it is not surprising that telomere shortening and high levels of extracellular mtDNA have been linked to a large number of age-related disease with inflammatory pathogenesis (Storci et al., 2018; Bonafè et al., 2020a; Iske et al., 2020; Aguado et al., 2019; Bruno et al., 2020). Hence, extracellular DNA in aged people is more likely to stimulate inflammation, due to a concomitant increase of extracellular mtDNA and a decrease in extracellular telDNA. Consequently, the release of pro-inflammatory mtDNA in aged people, exacerbated during COVID-19, paralleled by the age-dependent loss of anti-inflammatory extracellular telDNA reservoir may lead to an imbalance towards systemic hyper-inflammation, which becomes particularly life threatening

in aged males affected by age-related diseases. Therefore, we hypothesize that the pro-inflammatory baseline of inflamm-aging facilitates the severe and harmful evolution of SARS-CoV-2 disease in male aged people.

3. Extracellular mtDNA release: a beneficial response to viral infections that turns into a detrimental booster of systemic inflammation

The release of mtDNA is an ancestral response mechanism to cell damage and it is nowadays regarded as major local and systemic trigger of inflammation (Bruno et al., 2020). The dominant role of extracellular mtDNA as pro-inflammatory trigger has been reported in different clinic-pathological settings (Zhang et al., 2010). This phenomenon recalls the concept that mtDNA must be kept into the organelle until this latter is in a "good shape" and its functioning is preserved (Bruno et al., 2020). Once damage has occurred, mtDNA leaks out into the cytoplasm and in the extracellular space, where it triggers inflammation, as well as the type-1 interferon (IFN-1) antiviral response (Barnes et al., 2020). Its activity is mainly due to the lack of methylation at CpG sites, which triggers the activation of Toll-Like-Receptor-9 (TLR-9) receptors, mimicking bacterial DNA (Riley et al., 2020). MtDNA also engages cytoplasmic DNA detectors, such as Absent in Melanoma-2 (AIM2) and cGAS (Bruno et al., 2020; Barnes et al., 2020; Dalpke et al., 2006; Bae et al., 2019; Itagaki et al., 2015; Puyo et al., 2019). Mitochondrial damage in the anti-viral response have been reported in a number of viral infections: such as Herpes simplex, hepatitis B, HIV, severe fever with thrombocytopenia syndrome, Dengue virus (a single-strand positive RNA virus) (Lai et al., 2018; Saffran et al., 2007; Cao et al., 2020; Pinti et al., 2012; Li et al., 2020). Many of these viruses set off a potent inflammatory response mediated by the release of mtDNA (Lai et al., 2018; Singh et al., 2020; Burtcher et al., 2020). Noteworthy, the pro-inflammatory capability of mtDNA is expected to be increased in aged people, owing to the fact that most of the released mtDNA is oxidized and therefore more resistant to cytoplasmic DNaseIII/TREX enzyme, thus being endowed with an increased stability (Gehrke et al., 2013). Interestingly, a large number of studies show that mtDNA is detectable in body fluids including plasma, in which it is contained in extracellular vesicles (EVs) (Guescini et al., 2010; Meddeb et al., 2019; Thurairajah et al., 2018). These latter are likely to preserve mtDNA stability and facilitate its systemic pro-inflammatory activity. Current data indicate that mtDNA levels increase and correlate with the extent of tissue damage, clinical evolution and the onset of multi-organ failure in patients affected by multiple systemic damage, sepsis and Acute Respiratory Distress Syndrome (Itagaki et al., 2015; Puyo et al., 2019; Simmons et al., 2013; Nakahira et al., 2013). Notably, increased levels of circulating mtDNA have been found in elderly people even in absence of any overt systemic inflammation (Dalpke, A., et al., 2006; Pinti et al., 2014). On the basis of its ability to activate TLR-9, AIM2 and cGAS, circulating mtDNA may be regarded as major pro-inflammatory stimulus in COVID-19 patients, particularly in aged ones (Zhang et al., 2010; Riley and Tait, 2020; Dalpke et al., 2006; Bae et al., 2019; Itagaki et al., 2015; Nakahira et al., 2013). Following this perspective, it is worth noting that the active release of (mt)DNA outward the cell may occur during the formation of NET (Neutrophil Extracellular Trap). This acronym refers to the capability of cells, especially myeloid ones, to extrude the genomic material contained in mitochondria and nuclei, both in vital conditions and during death (this latter being called NET-osis) (Zuo et al., 2020; Singh et al., 2020; Schönrich and Raftery, 2016). The NET mechanism has been studied in neutrophils and constitutes a major pathogenic step in the acute lung tissue injury, a life-threatening phenomenon that occurs in critically ill patients, including COVID-19 ones (Zuo et al., 2020; Barnes et al., 2020; Schönrich and Raftery, 2016). The capability of NETs to facilitate blood clotting has been regarded as a pathogenetic phenomenon in COVID-19-related thrombosis (Barnes et al., 2020). Currently, NET

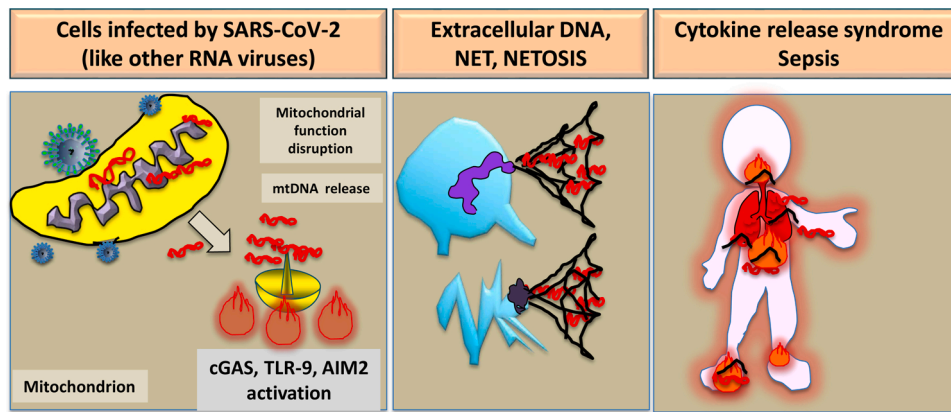


Fig. 1. mtDNA dependent hyper-inflammation induced by SARS-CoV-2 infection. SARS-CoV-2 (RNA)-viral infection thwarts mitochondrial function and sets off strong local and systemic pro-inflammatory response via the release of mtDNA, which engages DNA receptors AIM2, cGAS and TLR-9.

represents a therapeutic target for anti-thrombotic strategies to treat COVID-19 complications (Zuo et al., 2020; Barnes et al., 2020) (Fig. 1).

4. Telomere shortening at the basis of the progressive decay of anti-inflammatory extracellular telDNA reservoir

Genomic (g)DNA leakage outside the nucleus following cell death or consequent to NETs is a major trigger of inflammatory response (Storci et al., 2018). However, gDNA is not pro-inflammatory in all the experimental settings, since it has been demonstrated that gDNA has a limited ability to induce inflammation through TLR-9, but this activity is greatly increased after the cleavage of telomeric ends (Storci et al., 2018; Goldfarb et al., 2018). This finding pinpoints that the pro-inflammatory capability of extracellular gDNA depends upon its telDNA content, which in turn may vary upon the cell of origin and the age of the individual (Bonafè et al., 2020b; Goldfarb et al., 2018). Indeed, a wealth of literature shows that telDNA fragments are endowed with anti-inflammatory activity, being capable to inhibit TLR-9, cGAS and AIM2 (Kaminski et al., 2013; Goldfarb et al., 2018; Storci et al., 2019; Gursel et al., 2003). Telomeres are well recognized as double-stranded DNA sequences that protect the open ends of chromosomes (Palm and De Lange, 2008). Moreover, the shortening of the telomeres has long been recognized as a hallmark of cellular aging (Aguado et al., 2019; Hayflick and Moorhead, 1961). Notably, the length of the telomere decreases not only during cellular aging but also in cells from aged

subjects particularly those affected by age-related diseases (Aguado et al., 2019; Armanios et al., 2013; Herrmann et al., 2018; Campisi, 2001; Hayflick and Moorhead, 1961). Upon telomere shortening, a state of replicative arrest with consequent cellular senescence ensues that is followed by the release of a set of inflammatory mediators and cytokines, called Senescence Associated Secretory Phenotype, SASP (Armanios et al., 2013; Herrmann et al., 2018). Nowadays, cell senescence and SASP are considered contributors of inflamm-aging (Franceschi and Campisi, 2014). Importantly, telomere shortening also occurs when cells are exposed to a variety of stressors, including viral infections (Dowd et al., 2017). Noteworthy, the phenomenon of telomere shortening occurs even in absence of any cell proliferation (Wang et al., 2017). The shortening of telomeres has been proposed as the consequence of the chromosome ends trimming off by a specific enzymatic machinery (Bonafè et al., 2020b; Mazzucco et al., 2020; Bruno et al., 2020). Excised telDNA may be kicked-out of the nucleus and found in the cytoplasm, in extracellular fluids including plasma EVs (Byrd et al., 2016; Wang et al., 2015). The generation of telomeric DNA fragments detached from chromosome ends and their biologic significance are a cutting edge of telomere biology. Intriguingly, it has been recently reported that antigen-presenting cells (i.e. dendritic cells) release telDNA containing EVs which sustain the life-span of interacting T lymphocytes (Bruno et al., 2020). Free telDNA molecules may exert their intrinsic anti-inflammatory function by engaging DNA receptors, such as cGAS, AIM2, TLR-9 (Gursel et al., 2008). Notably, this is the same set of

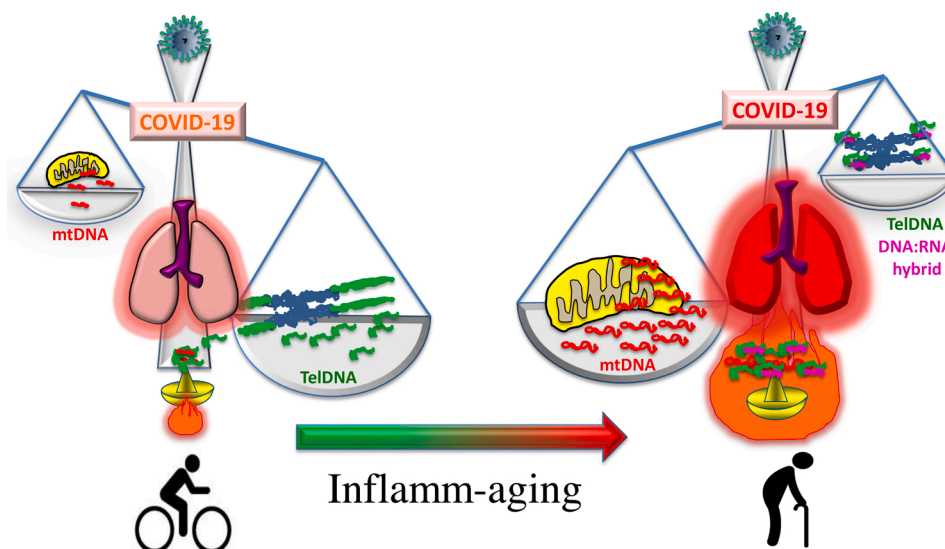


Fig. 2. Telomere shortening at the basis of the progressive decay of anti-inflammatory extracellular telDNA reservoir.

In young people the telomeric anti-inflammatory telDNA reservoir quenches the pro-inflammatory activity of mtDNA; in aged people, owing to telomere attrition, the amount of extracellular telDNA is reduced and it is partially replenished by pro-inflammatory DNA:RNA hybrids. This age-dependent unbalancing favors the onset of hyper-inflammation in aged people.

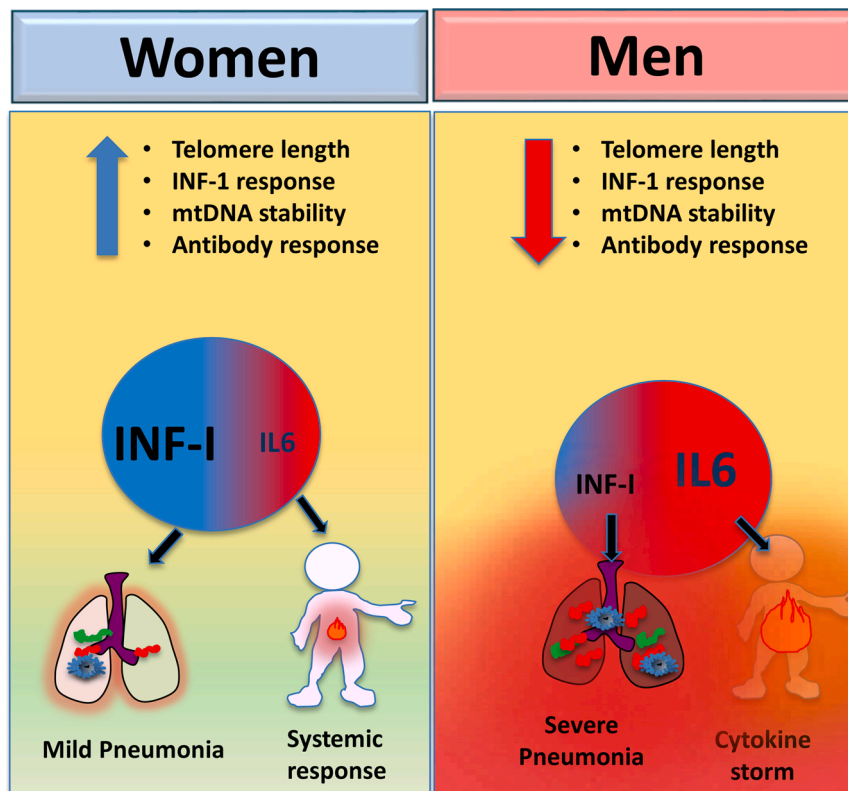


Fig. 3. Gender bias in extracellular mtDNA /telDNA content in COVID-19 pathogenesis.

Women are endowed with enhanced IFN-1 response, higher antibody response, less mtDNA instability and increased telomere length compared to men.

molecules that are engaged by the mtDNA recognition (Guescini et al., 2010; Simmons et al., 2018; Nakahira et al., 2013; Pinti et al., 2014). Therefore, it is not surprising that the free telDNA fragment can favorably switch off the mtDNA/TLR-9/NF-kappaB axis during ssRNA virus infection (Kindler et al., 2014). In addition, free telDNA molecules dampen the activation of the mtDNA/TLR-9/NF-kappaB axis in the respiratory system, where the massive release of mtDNA following endotracheal intubation has been described (Itagaki et al., 2015). Following the reasoning above, the progressive loss of the anti-inflammatory telDNA reservoir with aging may be a mechanism that depletes the circulating telDNA pool and facilitates the onset of systemic hyper-inflammation in COVID-19 patients, particularly in aged people (Froidure et al., 2020). Therefore, we expect that young subjects are equipped with an abundant anti-inflammatory telDNA reservoir that would, on average, worn-off in the elderly, especially in those suffering of comorbidities associated with shortened telomeres (Armanios et al., 2013; Herrmann et al., 2018; Zhao et al., 2013). On one hand, this scenario envisages also that young people, endowed with short telomeres due to environmental (e.g. sedentary/stressful life-style) and/or genetic reasons will be more likely to undergo severe outcome of SARS-CoV-2 infection. On the other hand, people endowed with long telomeres may experience an advantage when facing the SARS-CoV-2 infection (Lapham et al., 2015). We refer to extremely aged people such as centenarians that have been reported to carry longer than expected telomeres (Storci et al., 2019; Marcon et al., 2020) and show a more plastic and adaptive response against SARS-CoV-2 infection (Balk et al., 2013). The above picture may even more complex, by taking into account that the shortening of telomeres triggers the transcription of an mRNA named as Telomere repeat-containing RNA (TERRA) (Balk et al., 2013). When TERRA mRNA binds to telomeric ends it forms an DNA:RNA hybrid region (Balk et al., 2013). Notably, DNA:RNA hybrids, irrespective of their sequence, are powerful activators of inflammation via TLR-9 and cytoplasmic DNA sensors (Rigby et al., 2014; Mankan

et al., 2014). These findings suggest that an extracellular gDNA enriched in shortened telomeres, not only is devoid of its intrinsic anti-inflammatory reservoir, but also contains increased levels of pro-inflammatory telDNA:RNA hybrids. This scenario is more likely to occur in aged people and in individuals affected by genetic/environmental causes of telomere shortening (Fig. 2). Notably, EVs that contain TERRA and its cognate telDNA sequence are likely to carry substantial amounts of DNA:RNA hybrids and have been demonstrated to exert a potent pro-inflammatory activity.

5. Gender bias in extracellular DNA mitochondrial/Telomeric content in COVID-19 pathogenesis

The interpretative model hereby proposed, provides the opportunity to interpret also the above reported gender bias in COVID-19 lethality. Indeed, elderly men, especially those affected by age-related diseases, are endowed with shortened telomeres compared to aged matched women (Axson et al., 2018). Moreover, despite the still unknown mechanism, the rate of telomere shortening in men is steeper than in women, especially above the age of fifty, i.e. the age at which SARS-CoV-2 infection starts to impinge upon population mortality (Marcon et al., 2020) (<https://www.epicentro.iss.it/coronavirus/sars-cov-2-decessi-italia>). Furthermore, extremely old women are endowed with longer than expected telomeres, in respect to male age-matched subjects (Lapham et al., 2015). Molecular mechanisms at the basis of such a reproducible gender bias are still unclear (Ly et al., 2019; Di Florio et al., 2020). As far as mtDNA is concerned, there are hints suggesting that males release more mtDNA than females upon RNA viral infection (Klein and Flanagan, 2016). Hence, the higher chance for the mtDNA to engage DNA sensors in males may explain the gender bias in some inflammatory phenomena (Anker and Arima, 2011). The gender difference in response to viral infection has long been reported and holds across all ages and evolution (Gebhard et al., 2020; Griesbeck et al.,

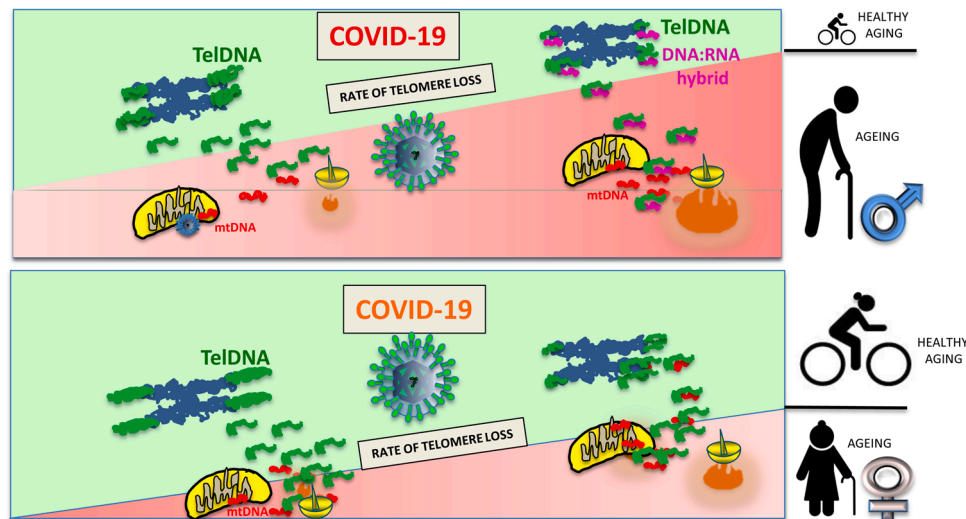


Fig. 4. The anti-inflammatory telDNA reservoir decay with aging. The age-dependent decline of the anti-inflammatory extracellular telDNA is steeper in aged men compared to aged women. This imbalance creates a condition that, according to our hypothesis, favors aged women over aged men in dealing with COVID-19 related complications.

2015). Notably, females have long been reported to make a higher antibody response against viruses and vaccinations (Anker and Arima, 2011). Furthermore, the onset of specific immunity (antibody production) and the capability to induce specific anti-viral response is more pronounced in women than in men (Anker and Arima, 2011) (Fig. 3). This gender bias is due to the higher capability of plasmacytoid dendritic cells to produce IFN-1 in women, a gender skewing that has been regarded as a major pathogenetic mechanism for the onset of autoimmune diseases (Smits et al., 2010). Notably, this gender difference is wider in aged people (Smits et al., 2010). Conversely, innate pro-inflammatory response is promoted over the set-off of specific immunity in men more than in women. In this regard, it has been recently reported that inflamm-aging, due to the activation of the myeloid pro-inflammatory compartments, is characterized by a strong preponderance in male subjects (Márquez et al., 2020). At least, in the early stages of viral infection, inflammation and IFN-1 anti-viral response are synergistic: the IFN-1 antiviral response aims at promoting local mechanisms that prevent the spread of the viral agent, whilst the inflammatory response promotes the local recruitment of inflammatory cells and facilitates systemic response. Thus, the prevailing of IFN-1 response over inflammation is more likely to occur in women where the clearance of the virus is more likely to occur without the involvement of inflammation. Therefore, inflammatory phenomena such as neutrophil infiltration, NETs production and systemic inflammatory response are less likely to occur in women than in men. In other words, the outcome of SARS-CoV-2 infection is expected to be more favorable in women as they are more capable to restrict inflammation via IFN-1 pathway activation (Anker and Arima, 2011). In fact, a reciprocal inhibitory effect between IFN-1 anti-viral response and inflammation has been extensively reported (Banerjee et al., 2017; Menachery et al., 2014; Chan et al., 2013; Lau et al., 2013; Zielecki et al., 2013; Aman et al., 1996; Ganster et al., 2005; Pauli et al., 2008; Wei et al., 2006; Nagata et al., 2008; Hadjadj et al., 2020). Notably, the unbalancing between IFN-1 mediated anti-viral innate immunity and inflammation was reported in SARS-CoV-1 infected macaques: the IFN-1 antiviral response was hampered in aged animals that develop a massive pro-inflammatory response and was dampened by the administration of IFN-1, which reversed the unfavorable course of the infection (Banerjee et al., 2017). Moreover, it has been found that genetically determined or immune-mediated curtailment of IFN-1 response is a risk for COVID-19 poor outcome (Bastard et al., 2020; Thoms et al., 2020). Under this perspective, the capability of SARS-CoV-2 proteins (i.e. Nsp1) to inhibit the IFN-1 mediated anti-viral

response, suggests that the impairment of IFN-1 is a condition that favors severe COVID-19 outcome (Burtscher et al., 2020; Bayik et al., 2016). Indeed, several data show that SARS-CoV-2 encoded proteins are capable to affect the mitochondrial function and the innate immune response mediated by mitochondria (Burtscher et al., 2020). Furthermore, the mitochondria integrity is a pivotal factor affecting the COVID-19 outcome (Burtscher et al., 2020). Overall, the gender difference above described allows to depict a scenario in which women are more capable to clear out the viral infection by means of IFN-1 mediated pathway, that can also keep inflammation under control. Conceivably, in presence of inflamm-aging, SARS-CoV-2 response is more likely to drift towards an uncontrollable local and systemic inflammation that may turn to be detrimental and potentially lethal (Fig. 4).

6. Conclusions

The imbalance towards a hyper-inflammatory state caused by the accumulation of extracellular pro-inflammatory mtDNA and the reduction of anti-inflammatory extracellular telDNA in elderly subjects is hypothesized to play a pivotal role in COVID-19 poor outcome. Following this rationale, it would be worthwhile to test *in vitro* and in animal models exogenous telDNA repeats which may taper or/halt SARS-CoV-2 induced inflammation. Since telDNA is composed of -TTAGGG- repeats, it belongs to a large family of poly-guanosine rich (G-rich) DNA oligonucleotide that exert anti-inflammatory activities (Ohto et al., 2015; Römmeler et al., 2015; Lenert, 2010). Hence, this “potential” DNA drugs may be worth to be taken into consideration for the treatment of COVID-19 complications. In conclusion, the above-described scenario depicts how studies on extracellular DNA (e.g. mtDNA and telDNA) may help to understand the link between inflamm-aging and COVID-19 pathogenesis. Hopefully, this approach will help the ongoing struggling against the unprecedented pandemic that is spreading worldwide in 2020.

References

- Aguado, J., Sola-Carvajal, A., Cancila, V., Revéchon, G., Ong, P.F., Jones-Weinert, C.W., Wallén Arzt, E., Lattanzi, G., Dreesen, O., Tripodo, C., Rossiello, F., Eriksson, M., d'Adda di Fagagna, F., 2019. Inhibition of DNA damage response at telomeres improves the detrimental phenotypes of Hutchinsonin-Gilford Progeria Syndrome. *Nat. Commun.* 10 (1), 4990.
- Aman, M.J., Tretter, T., Eisenbeis, I., Bug, G., Decker, T., Aulitzky, W.E., Tilg, H., Huber, C., Peschel, C., 1996. Interferon-alpha stimulates production of interleukin-10 in activated CD4+ T cells and monocytes. *Blood* 87 (11), 4731–4736.

- Anker, M., Arima, Y., 2011. Male-female differences in the number of reported incident dengue fever cases in six Asian countries. *Western Pac. Surveill. Response J.* 2 (2), 17–23.
- Armanios, M., 2013. Telomeres and age-related disease: how telomere biology informs clinical paradigms. *J. Clin. Invest.* 123 (3), 996–1002.
- Aviv, A., 2020. Telomeres and COVID-19. *FASEB J.* 34 (6), 7247–7252.
- Axon, E.L., Petersen, K.E., Tellez-Rojo, G.M., Goodrich, J.M., Meeker, J., Mercado-Garcia, A., Solano, M., Needham, B., 2018. Sex differences in telomere length are not mediated by sex steroid hormones or body size in early adolescence. *Genom. Genome* 2, 68–75.
- Bae, J.H., Jo, S.L., Kim, S.J., Lee, J.M., Jeong, J.H., Kang, J.S., Cho, N.J., Kim, S.S., Lee, E. Y., Moon, J.S., 2019. Circulating cell-free mtDNA contributes to AIM2 inflammasome-mediated chronic inflammation in patients with type 2 diabetes. *Cells* 8 (4).
- Balk, B., Maicher, A., Dees, M., Klermund, J., Luke-Glaser, S., Bender, K., Luke, B., 2013. Telomeric RNA-DNA hybrids affect telomere-length dynamics and senescence. *Nat. Struct. Mol. Biol.* 20 (10), 1199–1205.
- Banerjee, I., Vanaja, S.K., Fitzgerald, K.A., Rathinam, V., 2017. Control of type I interferon responses by the AIM2 inflammasome. *J. Immunol.* 198 (1 Supplement), 3, 64.
- Barnes, B.J., Adrover, J.M., Baxter-Stoltzfus, A., Borczuk, A., Cools-Lartigue, J., Crawford, J.M., Daßler-Plenker, J., Guerci, P., Huynh, C., Knight, J.S., Loda, M., Looney, M.R., McAllister, F., Rayes, R., Renaud, S., Rousseau, S., Salvatore, S., Schwartz, R.E., Spicer, J.D., Yost, C.C., Weber, A., Zuo, Y., Egeblad, M., 2020. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J. Exp. Med.* 217 (6) e20200652.
- Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.H., Zhang, Y., Dorgham, K., Philippot, Q., Rosain, J., Béziat, V., Manry, J., Shaw, E., Haljasmägi, L., Peterson, P., Lorenzo, L., Bizien, L., Trouillet-Assant, S., Dobbs, K., de Jesus, A.A., Belot, A., Kallaste, A., Catherinot, E., Traidjoui-Lambiotte, Y., Le Pen, J., Kerner, G., Bigio, B., Seeleuthner, Y., Yang, R., Bolze, A., Spaan, A.N., Delmonte, O.M., Abers, M.S., Aiuti, A., Casari, G., Lampasona, V., Piemonti, L., Ciceri, F., Bilguvar, K., Lifton, R.P., Vasse, M., Smadja, D.M., Migaud, M., Hadjadj, J., Terrier, B., Duffy, D., Quintana-Murci, L., van de Beek, D., Roussel, L., Vinh, D.C., Tangye, S.G., Haerynck, F., Dalmau, D., Martinez-Picado, J., Brodin, P., Nussenzweig, M.C., Boisson-Dupuis, S., Rodríguez-Gallego, C., Vogt, G., Mogensen, T.H., Oler, A.J., Gu, J., Burbelo, P.D., Cohen, J.L., Biondi, A., Bettini, L.R., D'Angio, M., Bonfanti, P., Rossignol, P., Mayaux, J., Rieux-Laucat, F., Husebye, E.S., Fusco, F., Ursini, M.V., Imberti, L., Sottini, A., Paghera, S., Quiros-Roldan, E., Rossi, C., Castagnoli, R., Montagna, D., Licari, A., Marsaglia, G.L., Duval, X., Ghosn, J., HGID Lab, NIAID-USUHS Immune Response to COVID Group, COVID Clinicians, COVID-STORM Clinicians, Imagine COVID Group, French COVID Cohort Study Group, Milieu Intérieur Consortium, CoV-Contact Cohort, Amsterdam UMC Covid-19 Biobank, COVID Human Genetic Effort, Tsang, J.S., Goldbach-Mansky, R., Kisand, K., Lionakis, M.S., Puel, A., Zhang, S.Y., Holland, S.M., Gorochov, G., Jouangué, E., Rice, C.M., Cobat, A., Notarangelo, L.D., Abel, L., Su, H.C., Casanova, J.L., 2020. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 370 (6515) eabd4585.
- Bayik, D., Gursel, I., Klinman, D.M., 2016. Structure, mechanism and therapeutic utility of immunosuppressive oligonucleotides. *Pharmacol. Res.* 105, 216–225.
- Bonafé, M., Praticchi, F., Giuliani, A., Storci, G., Sabbatinelli, J., Olivieri, F., 2020a. Inflamm-aging: why older men are the most susceptible to SARS-CoV-2 complicated outcomes. *Cytokine Growth Factor Rev.* 53, 33–37.
- Bonafé, M., Sabbatinelli, J., Olivieri, F., 2020b. Exploiting the telomere machinery to put the brakes on inflamm-aging. *Ageing Res. Rev.* 59, 101027.
- Bruno, V., Vuotto, C., Valvo, S., D'Ambrà, C., Esposito, F.M., Chiurchiù, V., Devine, O., Sanchez, M., Borsellino, G., Gilroy, D., Akbar, A.N., Dustin, M.L., Karin, M., Lann, A., 2020. Intercellular telomere transfer extends T cell lifespan. *BioRxiv*. <https://doi.org/10.1101/2020.10.09.331918>.
- Burtscher, J., Cappellano, G., Omori, A., Koshiba, T., Millet, G.P., 2020. Mitochondria: in the cross fire of SARS-CoV-2 and immunity. *iScience* 23 (10), 101631.
- Byrd, A.K., Zybailov, B.L., Maddukuri, L., Gao, J., Marecki, J.C., Jaiswal, M., Bell, M.R., Griffin, W.C., Reed, M.R., Chib, S., Mackintosh, S.G., MacNicol, A.M., Baldini, G., Eoff, R.L., Raney, K.D., 2016. Evidence that G-quadruplex DNA accumulates in the cytoplasm and participates in stress granule assembly in response to oxidative stress. *J. Biol. Chem.* 291 (34), 18041–18057.
- Callaway, E., Cyranoski, D., Mallapaty, S., Stoye, E., Tollefson, J., 2020. The coronavirus pandemic in five powerful charts. *Nature* 579 (7800), 482–483.
- Campisi, J., 2001. Cellular senescence as a tumor-suppressor mechanism. *Trends Cell Biol.* 11 (11), S27–31.
- Cao, H., Krueger, E.W., Chen, J., Drizyte-Miller, K., Schulz, M.E., McNiven, M.A., 2020. The anti-viral dynamism family member MxB participates in mitochondrial integrity. *Nat. Commun.* 11 (1), 1048.
- Chan, R.W., Chan, M.C., Agnihothram, S., Chan, L.L., Kuok, D.I., Fong, J.H., Guan, Y., Poon, L.L., Baric, R.S., Nicholls, J.M., Peiris, J.S., 2013. Tropism of and innate immune responses to the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. *J. Virol.* 87 (12), 6604–6614.
- Chen, X., Zhao, B., Qu, Y., Chen, Y., Xiong, J., Feng, Y., Men, D., Huang, Q., Liu, Y., Yang, B., Ding, J., Li, F., 2020. Detectable serum SARS-CoV-2 viral load (RNAemia) is closely associated with drastically elevated interleukin 6 (IL-6) level in critically ill patients with Coronavirus Disease. *Clin. Infect. Dis.* 71 (8), 1937–1942.
- Connors, J.M., Levy, J.H., 2020. COVID-19 and its implications for thrombosis and anticoagulation. *Blood* 135 (23), 2033–2040.
- Dalpke, A., Frank, J., Peter, M., Heeg, K., 2006. Activation of toll-like receptor 9 by DNA from different bacterial species. *Infect. Immun.* 74 (2), 940–946.
- Di Florio, D.N., Sin, J., Coronado, M.J., Atwal, P.S., Fairweather, D., 2020. Sex differences in inflammation, redox biology, mitochondria and autoimmunity. *Redox Biol.* 31, 101482.
- Dowd, J.B., Bosch, J.A., Steptoe, A., Jayabalasingham, B., Lin, J., Yolken, R., Aiello, A.E., 2017. Persistent herpesvirus infections and telomere attrition over 3 years in the whitehall II cohort. *J. Infect. Dis.* 216 (5), 565–572.
- Ersler, W.B., 1993. Interleukin-6: a cytokine for gerontologists. *J. Am. Geriatr. Soc.* 41 (2), 176–181, 1993.
- Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci. Suppl.* 1, S4–9.
- Franceschi, C., Bonafé, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908, 244–254.
- Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14 (10), 576–590.
- Froidure, A., Mahieu, M., Hoton, D., Laterre, P.F., Yombi, J.C., Koenig, S., Ghayé, B., Defour, J.P., Decottignies, A., 2020. Short telomeres increase the risk of severe COVID-19. *Ageing (Albany N. Y.)* 12 (20), 19911–19922.
- Fulop, T., Witkowski, J.M., Olivieri, F., Larbi, A., 2018. The integration of inflammaging in age-related diseases. *Semin. Immunol.* 40, 17–35.
- Ganster, R.W., Guo, Z., Shao, L., Geller, D.A., 2005. Differential effects of TNF-alpha and IFN-gamma on gene transcription mediated by NF-kappaB-Stat1 interactions. *J. Interferon Cytokine Res.* 25 (11), 707–719.
- Gebhard, C., Regitz-Zagrosek, V., Neuhauser, H.K., Morgan, R., Klein, S.L., 2020. Impact of sex and gender on COVID-19 outcomes in Europe. *Biol. Sex Differ.* 11 (1), 29.
- Gehrke, N., Mertens, C., Zillinger, T., Wenzel, J., Bald, T., Zahn, S., Tuting, T., Hartmann, G., Barchet, W., 2013. Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. *Immunity* 39 (3), 482–495.
- Goldfarb, I.T., Adeli, S., Berk, T., Philippe, M., 2018. Fetal and placental DNA stimulation of TLR9: a mechanism possibly contributing to the pro-inflammatory events during parturition. *Reprod. Sci.* 25 (5), 788–796.
- Griesbeck, M., Ziegler, S., Laffont, S., Smith, N., Chauveau, L., Tomezsko, P., Sharei, A., Kourjian, G., Porichis, F., Hart, M., Palmer, C.D., Sirignano, M., Beisel, C., Hildebrandt, H., Cénac, C., Villani, A.C., Diefenbach, T.J., Le Gall, S., Schwartz, O., Herbeval, J.P., Autran, B., Guéry, J.C., Chang, J.J., Altfeld, M., 2015. Sex differences in plasmacytoid dendritic cell levels of IRF5 drive higher IFN- α production in women. *J. Immunol.* 195 (11), 5327–5336.
- Guescini, M., Guidolin, D., Vallorani, L., Casadei, L., Gioacchini, A.M., Tibollo, P., Battistelli, M., Falcieri, E., Battistin, L., Agnati, L.F., Stocchi, V., 2010. C2C12 myoblasts release micro-vesicles containing mtDNA and proteins involved in signal transduction. *Exp. Cell Res.* 316 (12), 1977–1984.
- Gursel, I., Gursel, M., Yamada, H., Ishii, K.J., Takeshita, F., Klinman, M.D., 2003. Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. *J. Immunol.* 171 (3), 1393–1400.
- Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H., Charbit, B., Bondet, V., Chenevier-Gobeaux, C., Breillat, P., Carlier, N., Gauzit, R., Morbieu, C., Pène, F., Marin, N., Roche, N., Szwebel, T.A., Merklind, S.H., Treliuyer, J.M., Veyer, D., Mouthon, L., Blanc, C., Tharaux, P.L., Rozenberg, F., Fischer, A., Duffy, D., Rieux-Laucat, F., Kernéis, S., Terrier, B., 2020. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* 369, 718–724.
- Hayflick, L., Moorhead, P.S., 1961. The serial cultivation of human diploid cell strains. *Exp. Cell Res.* 25, 585–621.
- Herrmann, M., Pusceddu, I., Marz, W., Herrmann, W., 2018. Telomere biology and age-related diseases. *Clin. Chem. Lab. Med.* 56 (8), 1210–1222.
- Iske, J., Seyda, M., Heinbokel, T., Maenosono, R., Minami, K., Nian, Y., Quante, M., Falk, C.S., Azuma, H., Martin, F., Passos, J.F., Niemann, C.U., Tchkonja, T., Kirkland, J.L., Elkhail, A., Tullius, S.G., 2020. Senolytics prevent mt-DNA-induced inflammation and promote the survival of aged organs following transplantation. *Nat. Commun.* 27 (1), 4289, 11.
- Itagaki, K., Kaczmarek, E., Lee, Y.T., Tang, I.T., Isal, B., Adibnia, Y., Sandler, N., Grimm, M.J., Segal, B.H., Otterbein, L.E., Hauser, C.J., 2015. Mitochondrial DNA released by trauma induces neutrophil extracellular traps. *PLoS One* 10 (3) e0120549.
- Kaminski, J.J., Schattgen, S.A., Tzeng, T.C., Bode, C., Klinman, D.M., Fitzgerald, K.A., 2013. Synthetic oligodeoxynucleotides containing suppressive TTAGGG motifs inhibit AIM2 inflammasome activation. *J. Immunol.* 191 (7), 3876–3883.
- Kindler, E., Thiel, V., 2014. To sense or not to sense viral RNA—essentials of coronavirus innate immune evasion. *Curr. Opin. Microbiol.* 20, 69–75.
- Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. *Nat. Rev. Immunol.* 16 (10), 626–638.
- Lai, J.H., Wang, M.Y., Huang, C.Y., Wu, C.H., Hung, L.F., Yang, C.Y., Ke, P.Y., Luo, S.F., Liu, S.F., Liu, S.J., Ho, L.J., 2018. Infection with the dengue RNA virus activates TLR9 signaling in human dendritic cells. *EMBO Rep.* 19 (8).
- Lapham, K., Kvale, M.N., Lin, J., Connell, S., Croen, L.A., Dispensa, B.P., Fang, L., Hesselson, S., Hoffmann, T.J., Iribarren, C., Jorgenson, E., Kushi, L.H., Ludwig, D., Matsuguchi, T., McGuire, W.B., Miles, S., Quisenberry Jr., C.P., Rowell, S., Sadler, M., Sakoda, L.C., Smethurst, D., Somkin, C.P., Van Den Eeden, S.K., Walter, L., Whitmer, R.A., Kwok, P.Y., Risch, N., Schaefer, C., Blackburn, E.H., 2015. Automated assay of telomere length measurement and informatics for 100,000 subjects in the genetic epidemiology research on adult health and aging (GERA) cohort. *Genetics* 200 (4), 1061–1072.

- Lau, S.K.P., Lau, C.C.Y., Chan, K.H., Li, C.P.Y., Chen, H., Jin, D.Y., Chan, J.F.W., Woo, P. C.Y., Yuen, K.Y., 2013. Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. *J. Gen. Virol.* 94 (Pt 12), 2679–2690.
- Lenert, P.S., 2010. Classification, mechanisms of action, and therapeutic applications of inhibitory oligonucleotides for Toll-like receptors (TLR) 7 and 9. *Mediators Inflamm.* 2010, 986596.
- Li, S., Li, H., Zhang, Y.L., Xin, Q.L., Guan, Z.Q., Chen, X., Zhang, X.A., Li, X.K., Xiao, G.F., Lozach, P.Y., Cui, J., Liu, W., Zhang, L.K., Peng, K., 2020. SFTSV infection induces BAK/BAX-dependent mitochondrial DNA release to trigger NLRP3 inflammasome activation. *Cell Rep.* 30 (13), 4370–4385 e7.
- Luo, P., Liu, Y., Qiu, L., Liu, X., Liu, D., Li, J., 2020. Tocilizumab treatment in COVID-19: a single center experience. *J. Med. Virol.* 92 (7), 814–818.
- Ly, K., Walker, C., Berry, S., Snell, R., Marks, E., Thayer, Z., Atatoa-Carr, P., Morton, S., 2019. Telomere length in early childhood is associated with sex and ethnicity. *Sci. Rep.* 9 (1), 10359.
- Mankan, A.K., Schmidt, T., Chauhan, D., Goldeck, M., Höning, K., Gaidt, M., Kubarenko, A.V., Andreeva, L., Hopfner, K.P., Hornung, V., 2014. Cytosolic RNA: DNA hybrids activate the cGAS-STING axis. *EMBO J.* 33 (24), 2937–2946.
- Marcon, G., Tettamanti, M., Capacci, G., Fontanel, G., Spanò, M., Nobili, A., Forloni, G., Franceschi, C., 2020. COVID-19 mortality in Lombardy: the vulnerability of the oldest old and the resilience of male centenarians. *Ageing (Albany NY)*. 12 (15), 15186–15195.
- Márquez, E.J., Chung, C.H., Marches, R., Rossi, R.J., Nehar-Belaid, D., Eroglu, A., Mellert, D.J., Kuchel, G.A., Banchereau, J., Ucar, D., 2020. Sexual-dimorphism in human immune system aging. *Nat. Commun.* 11 (1), 751.
- Mazzucco, G., Huda, A., Galli, M., Piccini, D., Giannattasio, M., Pessina, F., Doksani, Y., 2020. Telomere damage induces internal loops that generate telomeric circles. *Nat. Commun.* 20 (1), 5297, 11.
- Meddeb, R., Dache, Z.A.A., Thezenas, S., Otandault, A., Tanos, R., Pastor, B., Sanchez, C., Azzi, J., Tusch, G., Azan, S., Mollevi, C., Adenis, A., El Messaoudi, S., Blache, P., Thierry, A.R., 2019. Quantifying circulating cell-free DNA in humans. *Sci. Rep.* 9 (1), 5220.
- Menachery, V.D., Eisfeld, A.J., Schäfer, A., Josset, L., Sims, A.C., Prohl, S., Fan, S., Li, C., Neumann, G., Tilton, S.C., Chang, J., Gralinski, L.E., Long, C., Green, R., Williams, C. M., Weiss, J., Matzke, M.M., Webb-Robertson, B.J., Schepmoes, A.A., Shukla, A.K., Metz, T.O., Smith, R.D., Waters, K.M., Katze, M.G., Kawaoka, Y., Baric, R.S., 2014. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. *mBio*. 5 (3), e01174–14.
- Nagata, N., Iwata, N., Hasegawa, H., Fukushi, S., Harashima, A., Sato, Y., Saijo, M., Taguchi, F., Morikawa, S., Sata, T., 2008. Mouse-passaged severe acute respiratory syndrome-associated coronavirus leads to lethal pulmonary edema and diffuse alveolar damage in adult but not young mice. *Am. J. Pathol.* 172 (6), 1625–1637.
- Nakahira, K., Kyung, S.Y., Rogers, A.J., Gazourian, L., Youn, S., Massaro, A.F., Quintana, C., Osorio, J.C., Wang, Z., Zhao, Y., Lawler, L.A., Christie, J.D., Meyer, N. J., Mc Causland, F.R., Waikar, S.S., Waxman, A.B., Chung, R.T., Bueno, R., Rosas, I. O., Fredenburgh, L.E., Baron, R.M., Christiani, D.C., Hunninghake, G.M., Choi, A.M., 2013. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. *PLoS Med.* 10 (12) e1001577.
- Ohto, U., Shibata, T., Tanji, H., Ishida, H., Krayukhina, E., Uchiyama, S., Miyake, K., Shimizu, T., 2015. Structural basis of CpG and inhibitory DNA recognition by Toll-like receptor 9. *Nature* 520 (7549), 702–705.
- Palm, W., de Lange, T., 2008. How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* 42, 301–334.
- Pauli, E.K., Schmolke, M., Wolff, T., Viemann, D., Roth, J., Bode, J.G., Ludwig, S., 2008. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. *PLoS Pathog.* 4 (11), e1000196.
- Pinti, M., Mussini, C., Cossarizza, A., 2012. Mitochondrial DNA: a proinflammatory' enemy from within' during HIV infection? *Cell Death Dis.* 3 (5), 307.
- Pinti, M., Cevenini, E., Nasi, M., De Biasi, S., Salvioli, S., Monti, D., Benatti, S., Gibellini, L., Cotichini, R., Stazi, M.A., Trenti, T., Franceschi, C., Cossarizza, A., 2014. Circulating mitochondrial DNA increases with age and is a familiar trait: implications for "inflamm-aging. *Eur. J. Immunol.* 44 (5), 1552–1562.
- Puyo, C.A., Earhart, A., Staten, N., Prince, O.A., Haug, C., Kollef, M., Awad, M., 2019. Endotracheal intubation results in acute tracheal damage induced by mtDNA/TLR9/NF-κB activity. *J. Leukoc. Biol.* 105 (3), 577–587.
- Remuzzi, A., Remuzzi, G., 2020. COVID-19 and Italy: what next? *Lancet* S0140-6736 (20), 30627–30629.
- Rigby, R.E., Webb, L.M., Mackenzie, K.J., Li, Y., Leitch, A., Reijns, M.A., Lundie, R.J., Revuelta, A., Davidson, D.J., Diebold, S., Modis, Y., MacDonald, A.S., Jackson, A.P., 2014. RNA:DNA hybrids are a novel molecular pattern sensed by TLR9. *EMBO J.* 33 (6), 542–558.
- Riley, J.S., Tait, S.W., 2020. Mitochondrial DNA in inflammation and immunity. *EMBO Rep.* 21 (4) e49799.
- Römmel, F., Hammel, M., Waldhuber, A., Müller, T., Jurk, M., Uhlmann, E., Wagner, H., Vollmer, J., Miethke, T., 2015. Guanine-modified inhibitory oligonucleotides efficiently impair TLR7- and TLR9-mediated immune responses of human immune cells. *PLoS One* 10 (2) e0116703.
- Ruan, Q., Yang, K., Wang, W., Jiang, L., Song, J., 2020. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* 1–3.
- Saffran, H.A., Pare, J.M., Corcoran, J.A., Weller, S.K., Smiley, J.R., 2007. Herpes simplex virus eliminates host mitochondrial DNA. *EMBO Rep.* 8, 188–193.
- Salvarani, C., Dolci, G., Massari, M., Merlo, D.F., Cavuto, S., Savoldi, L., Bruzzi, P., Boni, F., Braglia, L., Turrà, C., Ballerini, P.F., Sciascia, R., Zammarchi, L., Para, O., Scotton, P.G., Inojosa, W.O., Ravagnani, V., Salerno, N.D., Sainaghi, P.P., Brignone, A., Codeluppi, M., Teopompi, E., Milesi, M., Bertomoro, P., Claudio, N., Salio, M., Falcone, M., Cenderello, G., Donghi, L., Del Bono, V., Colombelli, P.L., Anghelone, A., Passaro, A., Secondo, G., Pascale, R., Piazza, I., Facciolo, N., Costantini, M., RCT-TCZ-COVID-19 Study Group, 2020. Effect of tocilizumab vs standard care on clinical worsening in patients hospitalized with COVID-19 pneumonia: a randomized clinical trial. *JAMA Intern. Med.* 20 (October) e206615. Epub ahead of print.
- Schönrich, G., Rafferty, M.J., 2016. Neutrophil extracellular traps go viral. *Front. Immunol.* 7, 366 eCollection. Review.
- Scuzzi, D., Cano, M., Ma, L., Zhou, D., Zhu, J.H., O'Halloran, J.A., Goss, C., Raueo, A.M., Liu, Z., Peritore, V., Rocco, M., Ricci, A., Amodeo, R., Aimati, L., Ibrahim, M., Hachem, R., Kreisel, D., Mudd, P.A., Kulkarni, H.S., Gelman, A.E., 2020. Circulating mitochondrial DNA is an early indicator of severe illness and mortality from COVID-19. *bioRxiv*. 30, 2020.07.30.227553.
- Simmons, J.D., Lee, Y.L., Mulekar, S., Kuck, J.L., Brevard, S.B., Gonzalez, R.P., Gillespie, M.N., Richards, W.O., 2013. Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. *Ann. Surg.* 258 (4), 591–596.
- Singh, K.K., Chaubey, G., Chen, J.Y., Suravajhala, P., 2020. Decoding SARS-CoV-2 hijacking of host mitochondria in pathogenesis of COVID-19. *Am. J. Physiol. Cell. Physiol.* Jun 8.
- Smits, S.L., de Lang, A., van den Brand, J.M., Leijten, L.M., van IJcken, W.F., Eijkemans, M.J., van Amerongen, G., Kuiken, T., Andeweg, A.C., Osterhaus, A.D., Haagmans, B.L., 2010. Exacerbated innate host response to SARS-CoV in aged non-human primates. *PLoS Pathog.* 6 (2), e1000756.
- Storci, G., De Carolis, S., Olivieri, F., Bonafè, M., 2018. Changes in the biochemical taste of cytoplasmic and cell-free DNA are major fuels for inflamm-aging. *Semin. Immunol.* 40, 6–1.
- Storci, G., De Carolis, S., Papi, A., Bacalini, M.G., Gensous, N., Marasco, E., Tesei, A., Fabbri, F., Arienti, C., Zanoni, M., Sarnelli, A., Santi, S., Olivieri, F., Mensà, E., Latini, S., Ferranti, M., Salvioli, S., Garagnani, P., Franceschi, C., Bonafè, M., 2019. Genomic stability, anti-inflammatory phenotype, and up-regulation of the RNaseH2 in cells from centenarians. *Cell Death Differ.* 26 (9), 1845–1858.
- Storci, G., Bacalini, M.G., Bonifazi, F., Garagnani, P., De Carolis, S., Salvioli, S., Olivieri, F., Bonafè, M., 2020. Ribosomal DNA instability: an evolutionary conserved fuel for inflammaging. *Ageing Res. Rev.* 58, 101018.
- Thoms, M., Buschauer, R., Ameismeier, M., Koepeke, L., Denk, T., Hirschenberger, M., Kratzat, H., Hayn, M., Mackens-Kiani, T., Cheng, J., Straub, J.H., Stürzel, C.M., Fröhlich, T., Berninghausen, O., Becker, T., Kirchhoff, F., Sparrer, K.M.J., Beckmann, R., 2020. Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2. *Science* 369 (6508), 1249–1255.
- Thurairajah, K., Briggs, G.D., Balogh, Z.J., 2018. The source of cell-free mitochondrial DNA in trauma and potential therapeutic strategies. *Eur. J. Trauma Emerg. Surg.* 44 (3), 325–334.
- Wang, Z., Deng, Z., Dahmane, N., Tsai, K., Wang, P., Williams, D.R., Kossenkov, A.V., Showe, L.C., Zhang, R., Huang, Q., Conejo-Garcia, J.R., Lieberman, P.M., 2015. Telomeric repeat-containing RNA (TERRA) constitutes a nucleoprotein component of extracellular inflammatory exosomes. *Proc Natl Acad Sci U S A.* 112 (46), E6293–300.
- Wang, Z., Deng, Z., Tutton, S., Lieberman, P.M., 2017. The telomeric response to viral infection. *Viruses* 9 (8), 218.
- Wei, L., Sandbulte, M.R., Thomas, P.G., Webby, R.J., Homayouni, R., Pfeffer, L.M., 2006. NF-kappaB negatively regulates interferon-induced gene expression and anti-influenza activity. *J. Biol. Chem.* 281 (17), 11678–11684.
- Wu, Y., Ho, W., Huang, Y., Jin, D.Y., Li, S., Liu, S.L., Liu, X., Qiu, J., Sang, Y., Wang, Q., Yuen, K.Y., Zheng, Z.M., 2020. SARS-CoV-2 is an appropriate name for the new coronavirus. *Lancet.* 395 (10228), 949–950.
- Xu, X., Han, M., Li, T., Sun, W., Wang, D., Fu, B., Zhou, Y., Zheng, X., Yang, Y., Li, X., Zhang, X., Pan, A., Wei, H., 2020. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc. Natl. Acad. Sci. U S A.* 117 (20), 10970–10975.
- Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K., Hauser, C.J., 2010. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464 (7285), 104–107.
- Zhang, C., Wu, Z., Li, J.W., Zhao, H., Wang, G.Q., 2020. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int. J. Antimicrob. Agents* 55 (5), 105954.
- Zhao, J., Miao, K., Wang, H., Ding, H., Wang, D.W., 2013. Association between telomere length and type 2 diabetes mellitus: a meta-analysis. *PLoS One* 8 (11), e79993.
- Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., Cao, B., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395 (10229), 1054–1062.
- Zielecki, F., Weber, M., Eickmann, M., Spiegelberg, L., Zaki, A.M., Matrosovich, M., Becker, S., Weber, F., 2013. Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. *J. Virol.* 87 (9), 5300–5304.
- Zuo, Y., Yalavarthi, S., Shi, H., Gockman, K., Zuo, M., Madison, J.A., Blair, C., Weber, A., Barnes, B.J., Egeblad, M., Woods, R.J., Kanthi, Y., Knight, J.S., 2020. Neutrophil extracellular traps in COVID-19. *JCI Insight* 24, 138999.