JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS Vol. 34, no. 3, 1125-1129 (2020)

LETTER TO THE EDITOR

A THEORY ON SARS-COV-2 SUSCEPTIBILITY: REDUCED TLR7-ACTIVITY AS A MECHANISTIC LINK BETWEEN MEN, OBESE AND ELDERLY

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Received April 16, 2020 – Accepted May 14, 2020

To the Editor,

Conti and Younes have pointed to a connection between lower TLR7 expression in men and their increased vulnerability towards a severe form of COVID-19 infection (1). I had started to investigate the same idea and think that I have discovered a wealth of additional supporting information that renders the hypothesis plausible and, if correct, could establish lower TLR7 activity/sensitivity as a common molecular characteristic of several high-risk patient groups.

As Conti and Younes have already pointed out, in the current COVID-19 epidemic about twice as many men seem to die as women. This has been attributed to lifestyle by our mass media, but this would not explain a ratio that is quite uniform across societies. In addition, the same quantitative imbalance between male and female deaths was already observed for the 2003 SARS outbreak (2), which was caused by a closely related Coronavirus.

For SARS, the receptor ACE2, encoded on the X-chromosome, was investigated as a possible reason for the gender difference, but there is no established link between different ace2-alleles and disease outcome, making this explanation less likely. However, TLR7 (toll-like receptor 7) is also encoded on the X-chomosome, and it is a key regulator of pathogen detection and the resulting innate immune response. TLR7 detects viral RNA in the endosome and then stimulates the production of inflammatory cytokines and interferons. It is a key component of our immune response to infection by RNA-viruses. SARS-CoV-2 is such an RNA virus. Importantly, TLR7 is one of few genes that evade X-chromosome silencing in immune cells (3). It is known that this biallelic expression of TLR7 in immune cells of women can have physiological consequences, which can be positive, but also negative, as manifest in female predisposition to systemic lupus erythematosus (SLE). The higher gene dosage of TLR7 has been shown to be the reason for increased female susceptibility to this autoimmune disease. TLR7 has been described to bind and respond strongly to SARS-RNA (4). It is altogether plausible that higher TLR7 expression is the reason why women are better equipped to deal with SARS-CoV-2. If this is correct, a prediction is that patient groups with lower TLR7 expression or sensitivity should be more prone to develop a severe form of COVID-19.

There are patient groups who are emerging as carrying increased risk: obese patients and old people with a senescent immune system. One characteristic that obese and old people have in common is their physiological state of low-level, chronic inflammation. I speculated that this could lead to a common molecular characteristic with men, low TLR7 expression or sensitivity, and analyzed the literature for supporting or contradictory evidence. There is crosstalk between obesity and the innate immune system. Obesity is characterised by a chronic state of low inflammation (for a review see 5). A role of inflammation in the development of obesity-induced metabolic syndrome and insulin resistance via the pro-inflammatory

Key words: Covid-19; tlr-7; SARS-CoV-2; tlr tolerance; obesity; inflammaging; X-chromosome inactivation

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0393-974X (2020)

cytokine TNF-a was already suggested in the 1990s and resident immune cells in the adipose tissue are responsible for that response.

While TLR4 has been particularly examined for its role in the development of obesity-induced insulin resistance, it is interesting to appreciate that there is a high prevalence of insulin resistance and metabolic syndrome in SLE patients (6), indicating that also chronic inflammation triggered by overactive TLR7 increases the risk to develop metabolic syndrome. The increased prevalence of insulin resistance and metabolic syndrome in SLE patients is not linked to obesity.

Revelo et al. (7) have shown that in high-fat-diet (HFD)-fed mice metabolic parameters worsen through mechanisms including activation of macrophages in visceral adipose tissue and expansion of plasmacytoid dendritic cells in the liver. HFD-fed mice lacking TLR7 and TLR9, however, showed reduced metabolic inflammation and improved glucose homeostasis. Treatment of HFD-fed mice with inhibitors against TLR7 and 9 improved metabolic disease. The authors conclude that obesity leads to metabolic inflammation via the activation of TLR7 and 9. Hanna Kazazian et al. (8) have come to a very similar conclusion. TLR8-deficient mice, which develop spontaneous lupus-like disease due to increased TLR7-signalling by dendritic cells, show an aggravated SLE pathogenesis when fed with a high-fat-diet. Taken together, these two studies show that obesity leads to a chronic, low-level activation of the TLR7 receptor.

There is also a link between immune senescence and TLR7. It is well established that there are age-related changes in the immune system function which shows a functional decline in its ability to respond to new pathogens during aging. This functional decline includes the innate immune system, and Metcalfe et al. (9) found that peripheral blood mononuclear cells isolated from old individuals showed a delayed response to stimulation with TLR4, TLR7/8 and RIG-1 agonists compared to corresponding cells from young adults. This alone might explain the lower initial response and thus the higher vulnerability of old people towards SARS-CoV-2 infection.

On the other hand, serum levels of inflammatory cytokines increase with age. This age-related, systemic, chronic inflammation is called inflammaging. Increased levels of circulating inflammatory mediators such as interleukin-6 (IL-6) and C-reactive protein (CRP), which increase in an age-dependent manner, are indicators of inflammaging. While diverse underlying reasons for inflammaging have been discussed, it is interesting that TLR7-activation has been implied in it. Levels of miR-146a are significantly increased in senescent cells that have a senescence associated secretory phenotype. miR-146a is a well-known negative regulator of tlr-signalling acting downstream of MyD88 by decreasing signalling through IRAK1 and TRAF-6. However, ectopic localization of miR-146a in the extracellular space triggers an innate immune response, via - interestingly! - TLR7 (10). It has been suggested that it is the chronic tlr-activation by endogenous tlr ligands in the absence of pathogen infection - such as miR-146a activation of TLR7? - which contributes to the phenotype of "inflammaging" in old people.

Thus, a chronic, low level activation of TLR7 could link obesity and "inflammaging", and thus SARS-CoV-2 sensitive groups. Over time, however, chronic low-level TLR7 activation should result in acquired tolerance to TLR7 signalling, similar to the homologous immune tolerance which Michaelis et al. (11) have observed upon repeated TLR7 stimulation with the TLR7 agonist R848 (resiquimod).

I therefore hypothesize that high-risk patients - obesity, inflammaging - have in common that there is a chronic, low-level activation of TLR7 by intrinsic substrates. This leads to desensitization of TLR7-signalling and, more generally, to a state of tlr-tolerance. Upon SARS-CoV-2 infection the induced TLR7 activation in high-risk patient groups can be expected to be weaker and delayed and, consequently, these patients will show a weaker or delayed initial innate antiviral response immediately after infection. In men, this is then exacerbated further by the naturally lower expression level of TLR7.

It is tempting to speculate that another high-risk group, people who are exposed to continuous air pollution, might also show decreased sensitivity towards SARS-CoV-2 induced TLR7 activation by adaptive responses to chronic stimulation of the innate immune system (Fig. 1), possibly by heterologous

PAMP tolerance reciprocal to the R848-induced cross-tolerance towards TLR4 signalling observed by Michaelis et al. If so, people exposed to continuous air pollution would also be characterized by a state of acquired tlr-tolerance, and thus by a physiological state, where the signal of a new viral infection is initially not detected (see the figure for the proposed mechanistic model). The proposed mechanistic model allows several predictions to be made. First, the gender difference in developing a severe SARS-CoV-2 infection should be independent of age, i.e. prepubertarian boys should be more susceptible than prepubertarian girls, as the different sensitivity would be a primary consequence of different TLR7 expression levels and not a secondary consequence due to sex hormone regulation.

Also, untreated SLE patients, where TLR7 activity is high, should show a stronger initial innate immune response against infection by SARS-CoV-2, which should protect them. However, such patients will be hard to find, as the treatment against SLE typically focuses on suppressing the exaggerated, pathogenic TLR7-signalling. For example hydroxychloroquine, which is frequently used in SLE-patients, reduces TLR7-signalling in SLE-patients by inhibiting autophagy and thus preventing the delivery of extracellular TLR7-substrates to endosomal TLR7. While protection from viral infection is probably not present in human SLE-patients, where their respective immunosuppressing treatments would more than counteract their inherently overactive innate immune system, experiments in untreated lupus-prone mice are in line with the proposed model. Lupus-prone MRL/MpJ-Fas(lpr) mice did show accelerated viral clearance upon influenza infection (12), and thus demonstrated a beneficial role of high TLR7-activity in the initial phase of virus infection. The same mouse model also showed a severe disadvantage of high TLR7-activity after viral clearance since the lupus-prone mice had severe complications during the contraction and resolution phase of the infection with widespread pulmonary inflammation. This points to a positive effect of high TLR7-activity during the early stages of infection, but to a negative effect during the late stages. What is the

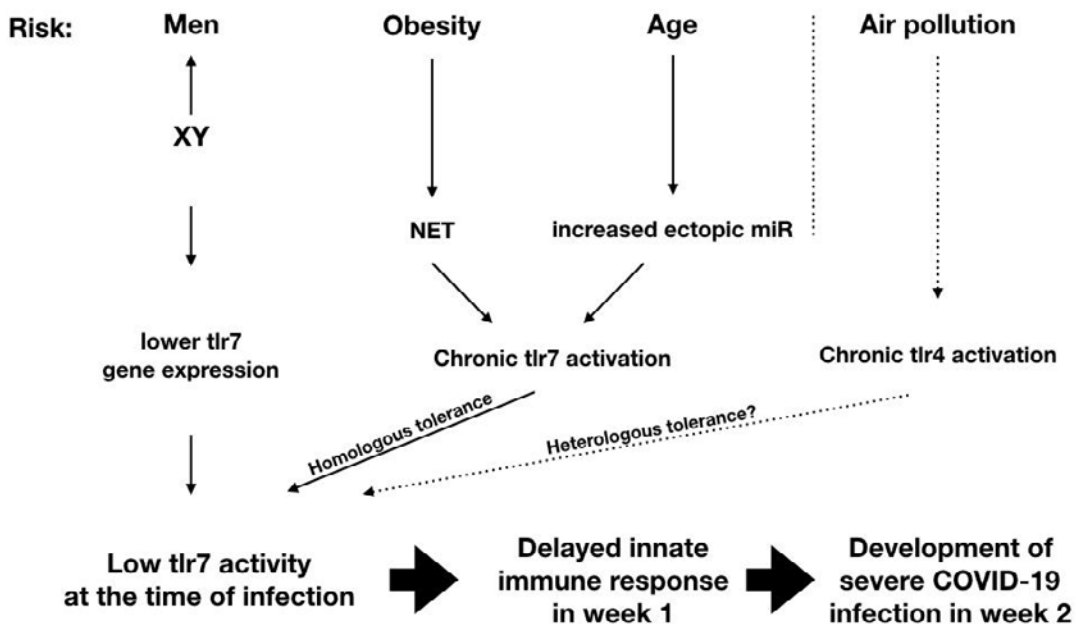

Fig. 1. *Reduced TLR7 activity/sensitivity at the time of SARS-CoV-2 infection as a mechanistic link between high-risk patient groups NET: Neutrophil extracellular traps*

underlying reason for the negative effect of high TLR7-activity during the late stage of infection, even after the virus has been cleared? It may be that the continued TLR7-activity in the lupus-prone mice after virus clearance prevents their immune system from realizing that the virus is indeed gone and that it is now time to contract and resolve.

If the innate immune system of obese people and elderly people reacted to the establishment of a severe SARS-CoV-2 infection (due to an initially laggard TLR7-response, as suggested by this model) by restoring normal TLR7-sensitivity after the initially delayed recognition of the viral infection, then they would find themselves in a situation which is similar to the lupus-prone mice (12): TLR7-signalling from the chronic intrinsic TLR7-substrates to a TLR7, which has regained normal sensitivity (to better fight the virus), would continue even after virus clearance and the innate immune system would effectively continue fighting a virus that is no longer there.

At an intermediate stage of the infection TLR7-signalling would even be overactive in obese and old SARS-CoV-2 patients, as not only the viral RNA, but also the chronic intrinsic TLR7-substrates would signal to TLR7, which has regained normal sensitivity (to better fight the virus).

It is interesting to note that high TLR7 activity has been implied in triggering blood-clotting during sepsis. Thus, there might also be a link between TLR7 and the pathological changes – thrombosis, embolism - which can be observed in the seriously ill. Overall the symptoms which have been described for COVID-19 patients show a striking similarity to the symptoms of SLE patients: overactive TLR7-signalling at the intermediate and late stage of COVID-19 could be a reason for this similarity. It is also interesting that vitamin D3, which has recently been described to protect severely ill COVID-19 patients from thrombosis and embolism, has been also described as downregulating TLR7 gene expression in SLE-patients.

For the use of hydroxychloroquine, the model would predict that it should not be used as an antiviral agent in the early stages of an infection - where any positive effects on viral replication would by counteracted by its interference with a proper innate antiviral immune response - but rather be used to suppress the negative effects of TLR7-signalling during the contraction and resolution phase of the infection and thus with a mechanistic logic that is very similar to its use in SLE patients.

The proposed mechanistic model also confirms the significance of the early phase of SARS-CoV-2 infection for disease progression. If obese people and people of old age have a significantly higher risk than others to develop severe COVID-19 because of an initially weak and/or delayed antiviral response, then therapeutic interventions that aim at improving that initial response should have the most visible effects and antiviral therapy should thus start very early for at risk patients. This is in line with what others have suggested previously, however based on a different logic.

Zhao et al. (13) observed a protective prophylactic effect of the TLR7/8 agonist R848 (resiquimod) (and tlr-agonists in general) in aged mice from SARS-CoV-1 infection. A clinical trial with the tlr3 agonist PrEP-001 administered as a nasal powder prior to exposure to influenza-A (14) reduced the number of individuals with clinical illness and attenuated severity and duration of infection without compromising seroconversion and was well tolerated.

To date, tlr-agonists have not led to approved prophylactics against viral infection, but this is probably due to trying to shoehorn them into the context of continuous dosage regimens and hoping that their administration would give prolonged protection (which will not happen because repeated tlr-agonist administration induces tlr-tolerance). Based on the results of Zhao (13) and Malcolm (14) one would predict that if tlr-agonists are used in the proper context, such as in a one-time rescue prophylactic treatment - for example, by providing PrEP-001 to yet unsymptomatic inhabitants of an old people care facility where a very first case with Covid-19 symptoms has just been detected - they should be beneficial by attenuating severity and duration of infection in those whose innate immune system was boosted just in time.

If tlr-agonists proved protective against SARS-CoV-2 when administered immediately prior to virus exposure, and if attempts to develop a vaccine against SARS-CoV-2 should fail, then tlr-agonists

might provide an option - albeit suboptimal - to immunization of selected parts of the population, for example by protecting a group with already high resistance against the virus, such as people under the age of 40, even further by tlr-agonist administration prior to controlled virus exposure. While this approach would still raise serious ethical and political questions, it might be less undesirable than allowing SARS-CoV-2 to spread through an unprotected population or shutting down societies for months or years.

ACKNOWLEDGEMENTS

I would like to particularly thank Iain Mattaj for comments on the manuscript, discussion and support. I would also like to thank Matthias Hentze and Günter Weiss for helpful initial discussions.

REFERENCES

1. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. J Biol Regul Homeost Agents 2020; 34(2).
2. Karlberg J, Chong DS, Lai WY. Do men have a higher case fatality rate of severe acute respiratory syndrome than women do? Am J Epidemiol 2004; 159(3):229-31.
3. Heintze JM. Autoimmunity: TLR7 evades silencing in human immune cells. Nat Rev Nephrol 2018; 14(4):214.
4. Li Y, Chen M, Cao H, Zhu Y, Zheng J, Zhou H. Extraordinary GU-rich single-strand RNA identified from SARS coronavirus contributes an excessive innate immune response. Microbes Infect 2013;15(2):88-95.
5. Lee BC, Lee J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim Biophys Acta. 2014; 842(3):446-62.
6. Chung CP, Avalos I, Oeser A, et al. High prevalence of the metabolic syndrome in patients with systemic lupus erythematosus: association with disease characteristics and cardiovascular risk factors. Ann Rheum Dis 2007; 66(2):208-14.
7. Revelo XS, Ghazarian M, Chng MH, et al. nucleic acid-targeting pathways promote inflammation in obesity-related insulin resistance. Cell Rep 2016; 16(3):717-30.
8. Hanna Kazazian N, Wang Y, Roussel-Queval A, et al. Lupus autoimmunity and metabolic parameters are exacerbated upon high fat diet-induced obesity due to TLR7 signaling. Front Immunol 2019; 10:2015.
9. Metcalf TU, Cubas RA, Ghneim K, et al. Global analyses revealed age-related alterations in innate immune responses after stimulation of pathogen recognition receptors. Aging Cell 2015;14(3):421-32.
10. Feng Y, Zou L, Yan D et al. Extracellular MicroRNAs induce potent innate immune responses via TLR7/MyD88-dependent mechanisms. J Immunol 2017; 199(6):2106-17.
11. Michaelis KA, Norgard MA, Levasseur PR, et al. Persistent Toll-like receptor 7 stimulation induces behavioral and molecular innate immune tolerance. Brain Behav Immun 2019; 82:338-53.
12. Slight-Webb SR, Bagavant H, Crowe SR, James JA. Influenza A (H1N1) virus infection triggers severe pulmonary inflammation in lupus-prone mice following viral clearance. J Autoimmun 2015; 57:66-76.
13. Zhao J, Wohlford-Lenane C, Zhao J, et al. Intranasal treatment with poly(I•C) protects aged mice from lethal respiratory virus infections. J Virol 2012; 86(21):11416-24.
14. Malcolm BA, Aerts CA, Dubois KJ, et al. PrEP-001 prophylactic effect against rhinovirus and influenza virus - results of 2 randomized trials. Antiviral Res 2018; 153:70-77.