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Commentary

Nutraceuticals have potential for boosting the type 1 interferon response to RNA viruses including influenza and coronavirus

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NOX2-dependent oxidant production inhibits TLR7 signaling

In light of worldwide concern regarding the recent outbreak of a deadly novel strain of coronavirus in China, it is fortuitous that two recent discoveries point the way to effective nutraceutical measures for potentiating the type 1 interferon response to RNA viruses.

Activation of toll-like receptor 7 (TLR7) by single-stranded viral RNA trapped within endosomes provides a key stimulus to type 1 interferon induction by RNA viruses.[1] Selemidis and colleagues have recently demonstrated that, within the endosomes of human alveolar macrophages, such viruses evoke superoxide production by NOX2-dependent NADPH oxidase complexes; the presence of TLR7 is required for this effect.[2] This phenomenon was demonstrated with a wide range of RNA viruses, including rhinovirus, respiratory syncytial virus, human parainfluenza virus, human metapneumonia virus, Sendai virus, Dengue virus, and HIV. Furthermore, the subsequent generation of hydrogen peroxide within these endosomes leads to an oxidation of Cys98 on TLR7 that blocks the ability of this receptor to transmit a signal boosting type 1 interferon production. In macrophages deficient in NOX2 activity, either genetically or owing to administration of a targeted NOX2 inhibitor (gp91ds-TAT), the production of type 1 interferon was markedly higher in response to RNA virus infection. When genetically normal or NOX2 knockout mice were exposed to an inactive strain of influenza virus, the interferon-beta response and the antibody response evoked by this virus were markedly higher in the NOX2 knockout mice.

These findings point to the possibility that nutraceuticals capable of inhibiting NOX2, promoting clearance of hydrogen peroxide, or aiding restoration of the native structure of Cys98 in TLR7, might be expected to boost the TLR7-mediated induction of type 1 interferon and antiviral antibodies. The low nanomolar intracellular concentrations of unconjugated bilirubin generated by activation of heme oxygenase-1 (HO-1) are known to inhibit NOX2-depending NADPH oxidase activity; this likely is a key homeostatic mission of HO-1.[3,4] Moreover, biliverdin – the HO-1 product which is converted rapidly to bilirubin within cells – has been reported to boost the type 1 interferon response to hepatitis C RNA virus in hepatocyte cell lines.[5] Furthermore, HO-1 induction is reported to potentiate the type 1 interferon response to influenza virus.[6] Phase 2-inductive nutraceuticals – such as ferulic acid, lipoic acid, or sulforaphane – are known to promote induction of HO-1, and hence may have some utility for boosting type 1 interferon response.[7–9] The ability of sodium ferulate to activate TLR7, stimulate type 1 interferon production, and enhance survival in influenza A-infected mice, might be secondary to HO-1 induction, and possibly reflects an additional effect of ferulate per se (as TLR9 was also found to be activated).[10]

Moreover, the phycocyanobilin (PCB) chromophore of cyanobacteria (such as spirulina) and many types of blue-green algae, a biliverdin metabolite, has been shown to mimic the NAPDH oxidase inhibiting activity of unconjugated bilirubin, likely because it is rapidly converted within cells to phycocyanorubin, a compound very similar in structure to bilirubin.[11,12] This phenomenon likely explains many of the profound antioxidant and anti-inflammatory effects observed when spirulina, phycocyanin (the prominent spirulina protein incorporating PCB as a chromophore), or PCB itself are administered in rodent models of human pathology.[11,13] Hence, ingestion of spirulina or of spirulina extracts enriched in PCB may have potential for boosting type 1 interferon response in the context of RNA virus infection. Oral administration of a cold-water spirulina extract rich in phycocyanin has been found to decrease mortality in influenza-infected mice.[14]

The downstream consequences of hydrogen peroxide production might also be addressed by phase 2-inductive nutraceuticals, as these induce various peroxidase enzymes and promote the synthesis of glutathione, a cofactor for certain peroxidases and a catalyst in reactions that reconvert oxidized cysteine groups to their native form.[15] Glutathione production can also be promoted by administration of *N*-acetylcysteine (NAC), which has been shown to be protective in rodents infected with influenza.[16–18] In a little-noticed 6-month controlled clinical study enrolling 262 primarily elderly subjects, those receiving 600 mg NAC twice daily, as opposed to those receiving placebo, experienced significantly fewer influenza-like episodes and days of bed confinement.[19] Although the rate of seroconversion to influenza A was comparable in the two groups – indicating that they were exposed at the same frequency – only 25% of the virus-infected subjects in the NAC group developed symptoms, as contrasted to 79% of those of

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placebo. (Given the carnage that influenza wreaks among the elderly, it is most regrettable that no effort has been made to replicate this study, conducted over 20 years ago.) The particular utility of NAC in the elderly might reflect the fact that plasma cysteine levels and cellular glutathione levels tend to decline with advancing age.[20]

Since selenium is an essential cofactor for certain peroxidases, and selenium deficiency has been endemic in certain regions of China and other parts of the world, insuring adequacy of selenium nutrition might also be appropriate in this context.[21] Not surprisingly, influenza is more pathogenic in selenium-deficient mice, and selenium deficiency also increases the rate at which viruses can mutate, promoting the evolution of strains that are more pathogenic and capable of evading immune surveillance.[22]

Antioxidants can also protect by quelling excessive lung inflammation

Importantly, the anti-inflammatory impact of such antioxidant nutraceuticals might also be expected to quell the excessive inflammatory reaction within lung parenchyma evoked by viral infections whose lethality is mediated by an acute respiratory distress syndrome.[23,24] These nutraceuticals could decrease such as response both by suppressing viral spread, and by dampening pro-inflammatory signaling in endothelial cells that promotes influx of inflammatory cells.

Glucosamine administration may up-regulate MAVS activation

Another key mediator of type 1 interferon response is the mitochondrial antiviral-signaling protein (MAVS), which oligomerizes in response to activation of cytosolic RNA virus detectors RIG-1 and MDA5, and subsequently participates in the activation of the transcription factor interferon regulatory factor 3 (IRF3).[25] (TLR7 signaling likewise contributes to activation of this factor; both pathways promote the K63-linked polyubiquitination and activation of the tank-binding kinase-1 – TBK1 – which in turn activates IRF3 via phosphorylation.[26–28]) Duan and colleagues have recently shown that RNA virus infection promotes O-GlcNacylation of MAVS on multiple sites, and that this renders MAVS susceptible to the K63-linked ubiquitination that enables it to activate IRF3.[29] Moreover, they show that, the more extensive this O-GlcNacylation is, the more effectively MAVS is activated. Hence, they are able to demonstrate that measures which suppress or amplify the cellular pool of UDP-*N*-acetylglucosamine – the substrate for O-GlcNacylation – correspondingly suppress or amplify the activation of MAVS. They then proceed to demonstrate that feeding mice a glucosamine-enriched diet (2.5% by weight) markedly enhances the survival of wild-type mice infected with influenza virus, whereas this provided no protection in mice in which MAVS, type 1 interferons, or O-GlcNac transferase (the mediator of O-GlcNacylation) were genetically absent.

This striking new finding points to the possibility that high-dose glucosamine supplementation might aid prevention and control of RNA virus infections. Whereas the hexosamine biosynthesis pathway is capable of generating UDP-*N*-acetylglucosamine in the absence of exogenous glucosamine, glucosamine administration can further enhance the intracellular pool of this compound, thereby boosting the extent of O-GlcNacylation evoked by viral infection.[30] The dietary dose employed in this study is quite high in the context of previous clinical experience – 2.5% of a human diet providing 400 g dry weight daily would correspond to 10 g glucosamine – but an intake of 3 g daily would be practical and is within the range of previous clinical experience.[31] Rather high intakes may be required for significant clinical benefit, inasmuch as this compound is rather inefficiently absorbed after oral administration.[32]

Table 1
Provisional daily dosage suggestions for nutraceuticals that might aid control of RNA viruses including influenza and coronavirus

Ferulic acid	500-1,000 mg
Lipoic acid	1,200-1,800 mg (in place of ferulic acid)
Spirulina	15 g (or 100 mg PCB)
N-Acetylcysteine	1,200–1,800 mg
Selenium	50-100 mcg
Glucosamine	3,000 mg or more
Zinc	30-50 mg
Yeast Beta-Glucan	250-500 mg
Elderberry	600–1,500 mg

Toward a practical nutraceutical strategy for coping with RNA virus infections

In light of the foregoing, administration of spirulina (or a spirulina extract enriched in PCB), a phase 2 inducer (such as ferulic acid, lipoic acid, or sulforaphane), *N*-acetylcysteine, selenium, and high-dose glucosamine, in adequate doses, might be expected to help prevent and control RNA virus infections by amplifying the signaling functions of TLR7 and MAVS in evoking type 1 interferon production.

With respect to practical efforts to prevent and control RNA virus infections, nutraceutical preparations intended to provide protection in this respect might reasonably also include brewer's yeast beta-glucan — which can amplify dendritic cell activation via dectin-1 and CR3 receptors; this agent has clinically documented immunostimulant effects, and has been shown to protect mice challenged with influenza virus.[33,34] (Polysaccharide cell wall preparations from certain mushrooms and sea weeds have comparable activity). Insurance of good zinc status, particularly in the very young and the elderly, would likewise seem to be prudent, as zinc supports the effective function and proliferation of various immune cells.[23,35] This effect might be pertinent to the significant 27% reduction in total mortality observed in elderly subjects who received high-dose zinc in the AREDS1 multicenter trial.[36] And it should be acknowledged that certain herbal preparations have shown potential for controlling or mitigating the symptoms of infections with influenza and other RNA viruses, for reasons that remain obscure[37]; extracts of elderberry, in particular, have received considerable clinical evaluation, and have been found to be symptomatically beneficial in influenza and the common cold.[38] Given that elderberry is a very rich source of anthocyanins, there is reason to suspect that its impact on viruses might be mediated, at least in part, by ferulic acid, a prominent metabolite that appears in plasma following anthocyanin ingestion.[39] Careful research evaluation of the most promising of these preparations may reveal specific phytochemicals which can influence the pathogenicity of viruses by addressing novel targets.

Table 1 offers some preliminary suggestions for the dosage levels of the agents discussed above that might be expected to be worthwhile for controlling RNA viruses.

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