When ISG15 was subjected to quantum biology (GB), the outcomes outlined in the document affixed to this article supports the QB assertion that the Coxsackie virus is not a viral infection.

https://phys.org/news/2020-03-protein-modification-isg15-blocks-coxsackievirus.html

MARCH 24, 2020 FEATURE

Protein modification with ISG15 blocks coxsackievirus pathology via antiviral and metabolic reprogramming

by Thamarasee Jeewandara , Phys.org

During early encounters between a pathogen and a cell, receptors located on the cell surface, in the cytosol or within endosomal (storage) compartments engage with the pathogen's nucleic acid (DNA/RNA) or pathogen-associated molecular patterns (PAMPs), as a host response to combat infection. The host response can initiate specific gene expression patterns and posttranscriptional (prior to translation of a gene into a protein product) control mechanisms at multiple levels and stages during disease development. The outcomes cause cells to produce type I interferons (IFNs) as a first line of defense to orchestrate a complex defense network in both infected and noninfected cells. As a classic example, the ubiquitin family protein IFN-stimulated gene of 15 kDa (known as ISG15) and its conjugation machinery including the enzyme Ube1L represents an IFN-induced broad spectrum antimicrobial system.

Protein modification with ISG15 known as ISGlyation is a major antimicrobial system; commonly perceived as a key to lock the cell gates and prevent the spread of threats—but scientists have yet to identify its common mechanism of action and the viral species-specific aspects of the process. In a new report in *Science Advances*, Meike Kespohl and an international research team in microbiology, biomolecular medicine, medical biotechnology and healthcare in Germany, U.S. and Belgium used a multiphase coxsackievirus B3 (CV) infection model to identify the host response. During CV infection, the first wave resulted in hepatic injury of the liver, followed by a second wave culminating in cardiac damage.

The scientists showed that ISGlyation activated ISG15 proteins to act on antiviral proteins, causing nonhematopoietic cells (which include airway epithelial cells or AECs; critical players in the inflammatory process initiated during airway infection) vital for CV control—into a resistant antiviral state. Due to altered energy demands, ISG15 also adapted liver metabolism during infection, which the scientists demonstrated using shotgun proteomics

combined with metabolic network engineering to reveal how ISG15 promoted gluconeogenesis (generation of glucose) in liver cells. In the absence of a protease or a protein enzyme known as the ubiquitin specific protein (USP18) that breaks down ISG15, the cells showed increased resistance to clinically relevant CV strains. The results therefore suggest inhibiting USP18 to stabilize ISGlyation and investigate treatments during CV-associated human disease.

CV disease is highly prevalent among newborn infants and young children, causing substantial medical and socioeconomic impact, with an etiology of hepatitis, myocarditis, encephalomyelitis and coagulopathy for multisystem sepsis. The disease can be mimicked in mice with similarities to humans, where mice show a robust systemic response on early CV infection. The biosynthesis of molecules required for an efficient antiviral response against CV consumes large portions of cellular energy packets or energy transfer molecules known as adenosine triphosphate (ATP). As a result, IFNs also activate the uptake and turnover of glucose within infected cells. IFN signaling can concurrently trigger cardioprotective effects but its molecular operation during CV infection remains elusive. To study the cellular response, Kespohl et al. used animal models in the lab to understand ISG15-mediated protection from viral toxicity.

Inhibiting CV burden via ISGlyation

The team investigated the viral burden during CV infection in gene knockout mice that lacked protein expression for ISG15 ($ISG15^{-/-}$) and Ube1L ($Ube1L^{-/-}$) proteins. Deleting Ube1L prevented ISGlyation but did not affect the function of freely available ISG15—allowing the scientists to distinguish between ISGlyation-dependent functions and those mediated by the free form of the protein. Approximately 36 hours after CV infection, they noted the formation of ISG15 conjugates in the liver, pancreas, spleen and heart tissue, and increased protein ISGlyation during disease progression. ISGlyation accelerated CV clearance from the liver and spleen due to higher CV titers in the knockout animal models compared to the wild-type (regular) controls. When they inactivated the protease (protein enzyme) USP18 specific to ISG15 breakdown, they saw increased cellular resistance toward CV infection.

ISGlyation within nonhematopoietic cells can protect from CV pathology.

MCFIP - The following document was extracted from our initial modeling for quantum biology.

Using bioinformatics, Coxsackie virus is attributable to BCR-Abl (the constituent of vitamin B3 ---- Abl1 - Abl2 - BCR-Abl.)

In other words, Coxsackie virus is attributable to vitamin B3 mutation and it is not viral.

Protein proves to be vital in immune response to bacteria

August 13th, 2012 in Medical research

(Medical Xpress) -- A team of researchers led by scientists at Rockefeller University have discovered that a protein once thought to be mainly involved in antiviral immunity is in fact more important in fighting bacterial infections and could provide new mechanisms for treating diseases like tuberculosis, which is increasingly becoming resistant to antibiotic medication.

A mutation in the gene that codes for the protein ISG15 was found to increase susceptibility to infection by mycobacteria, a group of bacteria that cause a range of disorders, the most common of which are tuberculosis and leprosy. MCFIP – Our modeling of the interactions between bacteria indicates mycobacteria are actually fungal organisms. We can provide substantial scientific support for that assertion.
Furthermore, our modeling of IFN-gamma has identified it has three families of byproducts that are primary immune defense regulators; the three lactoferrins, hololactoferrins and aoplactoferrins. Based on our preliminary review, it appears ISG15 is one of the members of the hololactoferrin (the tyrosine member). It is also noteworthy that our modeling of vitamins as signaling molecules indicates ISG15 is also likely to be one of the three forms of the body's natural B3. Furthermore, a quick check of the literature supports the likelihood of these findings; i.e. B3 is known for its antimicrobial properties.

"We were very surprised by this," says Dusan Bogunovic, a postdoctoral associate in the St. Giles Laboratory of Human Genetics of Infectious Diseases at Rockefeller and lead author on the study, which appeared in *Science* in August. "There were about 300 articles published on ISG15 before this, and I'd say 295 of them looked at viral disease. This connection to bacterial infection wasn't known."

The studies Bogunovic refers to tested only viruses and used mouse models — scientists can't knock out the ISG15 gene in humans to see what happens. But the lab, headed by Senior Attending Physician Jean-Laurent Casanova, tapped into their database of 300 people with what's known as Mendelian susceptibility to mycobacterial disease, a rare disorder that predisposes individuals to become severely ill from exposure to mycobacteria, even weak strains that would not affect healthy people. Whole exome sequencing revealed that ISG15 was mutated in three children whose diseases had no known cause. ISG15 proved to be a new genetic link to the disease, which has several other genes attributed to it.

"What's interesting here, in addition to finding a new genetic mutation that causes susceptibility to mycobacterial disease, is that while these children became very, very sick from a bacterium

that would not harm you or me, they had normal reactions to viral infection," says Bogunovic. "They've seen flu, chicken pox, and they're fine. So it turns out that this gene is essential for immunity against bacteria. It could be important in fighting viruses, but it's not essential."

ISG15 is a protein involved in a cascade of protein-cell interactions that help drive the immune system to eliminate a pathogen from the body. In studies spanning two and a half years, Bogunovic and colleagues worked out the protein's function. They found it was secreted by granulocytes, a type of white blood cell, and that it prompted another white blood cell, called a natural killer cell, to release interferon-gamma, a protein crucial to fighting mycobacterial infections. MCFIP – If our findings are correct, theranostic testing for ISG15 and its cell surface counterparts may establish deficiencies in immune defenses that could be remediated to prevent "superbug" infections.

To test whether this genetic mutation was, in fact, causing mycobacterial disease among the three children, Bogunovic exposed samples of their blood to a tuberculosis vaccine routinely given to children in Europe and elsewhere. Using a mild form of the tuberculosis bacterium, the vaccine exposes children to the disease and prompts the immune system to fight it off and remember it, reducing the chances of getting sick from full-blown tuberculosis in the future. The patients in this study had low levels of interferon-gamma in response to the vaccine, indicating their immune systems weren't fighting it. When Bogunovic added the ISG15 protein to the blood samples, interferon-gamma levels shot up. This and other tests proved that the genetic mutation was causing the children's immune deficiency.

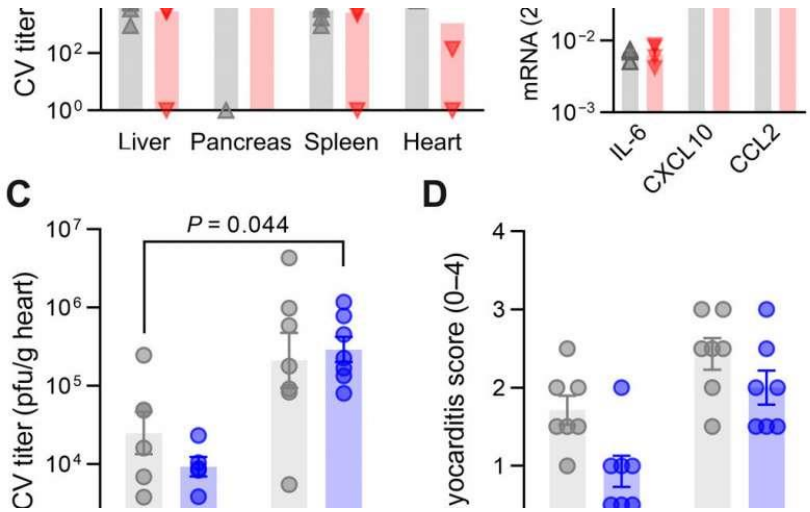
"We know interferon-gamma is vital to fighting tuberculosis, and now we know that ISG15 is one of the proteins that brings it into action," says Bogunovic.

That means the proteins could be used in a treatment for tuberculosis and other mycobacterial diseases, rather than antibiotics. It's an approach in line with the growing field of personalized medicine, whereby a patient's genetic makeup is considered alongside clinical and environmental information to produce more effective treatment.

"Of course we are not there yet," says Bogunovic. "But this is an indication that we might not have to rely on antibiotics one day."

In the meantime, Bogunovic and his fellow lab members will continue to investigate ISG15's cell-signaling functions — they're currently looking for its receptor — and to search for more genetic errors that lead to mycobacterial disease.

More information: Mycobacterial Disease and Impaired IFN-γ Immunity in Humans with Inherited ISG15 Deficiency, by Dusan Bogunovic et al., *Science* online: August 2, 2012.
www.sciencemag.org… 224026.short
Provided by Rockefeller University
"Protein proves to be vital in immune response to bacteria." August 13th, 2012.
http://medicalxpress.com/news/2012-08-protein-vital-immune-response-bacteria.html

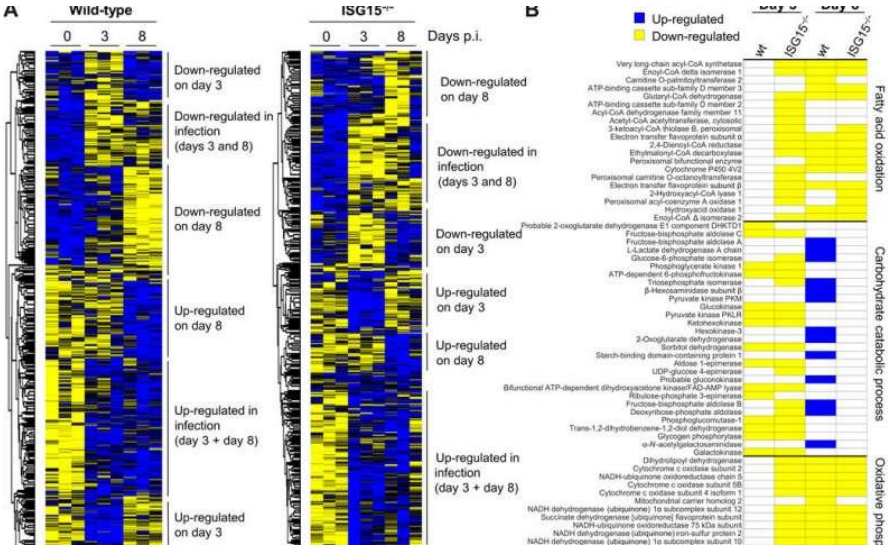


Protection from CV pathology requires ISGylation in nonhematopoietic cells. (A and B) ISG15−/− (CD45.2) mice were reconstituted with bone marrow cells from either ISG15−/− (CD45.2) or wild-type (wt, CD45.1) donors before CV infection, and mice were sacrificed 3 days after infection. (A) Infectious viral particles were quantified by plaque assay (wild-type ➔ ISG15−/−, n = 6; ISG15−/− ➔ ISG15−/−, n = 4). Data are summarized as median. (B) Splenic mRNA expression of the indicated cytokines and chemokines was determined by TaqMan qPCR. (C to F) Chimeric wild-type and Ube1L−/− mice were generated upon transfer of wild-type or Ube1L−/− bone marrow cells into lethally irradiated wild-type or Ube1L−/− recipients, respectively. Mice were infected with CV and sacrificed after 8 days (n = 7 in all four groups). (C) Infectious viral particles were quantified in heart tissue by plaque assay. Data are summarized as means ± SEM. (D) Myocarditis was scored microscopically by a blinded pathologist based on cardiac hematoxylin and eosin staining. (E) Representative histopathologic stains of heart tissue of each group are shown. (F) mRNA levels of the indicated genes in heart tissue were determined by TaqMan qPCR. Unequal variance versions of two-way ANOVA were performed, followed by a Sidak-Holm's multiple comparison test. Data were summarized as means ± SEM if applicable. Credit: Science Advances, doi: 10.1126/sciadv.aay1109

The team hypothesized that ISG15 protein offered protection from CV through nonhematopoietic cell types and tissues. Kespohl et al. tested the hypothesis using a genetically modified mouse model that did not express the Ube1L protein (Ube1L$^{-/-}$) so as to prevent ISGlyation and compared the results with wild-type bone marrow chimeras. They observed an increased CV load in the gene knockout mice, causing high-grade inflammation and tissue destruction as well as increased chemokine expression. The results demonstrated the protective role of ISGlyation to control the CV-triggered disease. They then reconstituted the compromised mice with wild-type bone marrow cells with functional ISGlyation machinery, but their condition did not improve. The work highlighted the role of non-bone-marrow-derived somatic cells to prevent viral cytotoxicity and inflammatory tissue damage during CV compared to bone marrow-derived immune cells.

ISGlyation increased the expression levels of antiviral proteins and ISG15 reprogrammed central liver metabolism during CV infection.

The scientists then studied and identified molecular mechanisms of protein ISGlyation that suppressed the virus in cells targeted by CV infection. They profiled the proteins inside infected liver tissue using mass-spectrometry (MS)-based proteomics (the study of proteins). The analysis showed the upregulation of antiviral vectors; IFN-induced proteins with tetratricopeptide repeats (IFIT) 1 and 3, alongside the ISG15 protein. Extensive findings proved that the ISG15 system also regulated protein expression levels of antiviral proteins (IFIT 1/3) post-transcriptionally, i.e., between transcription and translation—at the RNA level.



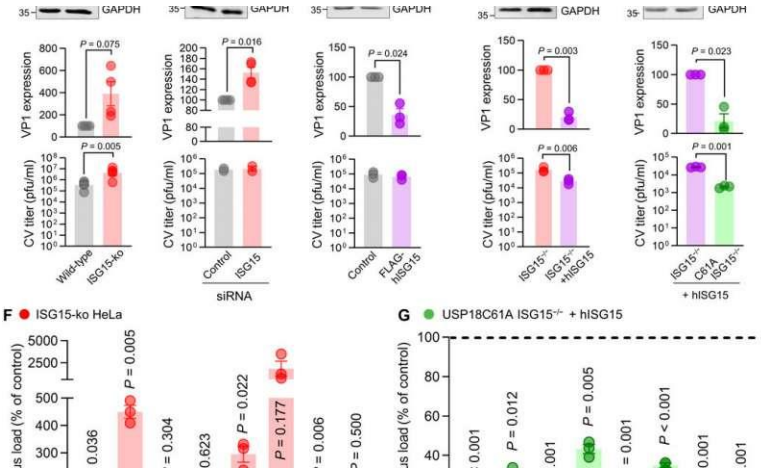
ISG15 reprograms the central liver metabolism during CV infection. (A) Hepatic tissue obtained from wild-type (wt) and ISG15−/− mice (n = 3) during early (day 3) and late (day 8) state of CV infection was subjected to LC-MS/MS analysis. Heatmaps summarizing all differentially regulated hepatic proteins during infection for both strains are depicted. The relative abundance of each protein is color-coded based on the z score normalized log2-transformed LFQ intensities. Blue color indicates proteins of high abundance, and yellow color indicates proteins of low abundance as compared to row means. A hierarchical clustering resolved six distinct clusters, with annotation shown on the right. (B) Heatmap-based clusters were subjected to Gene Ontology (GO) analysis, and proteins involved in selected enriched metabolic GO terms with catabolic ATP-generating function (FA oxidation, carbohydrate catabolic process, and OXPHOS) are depicted at an early and late state of CV infection, applying the same color code as used in (A) (blue, up-regulation; yellow, down-regulation). If the GO term of interest was not found within a dataset, individual proteins were not plotted. (C) At the indicated time points of infection, liver biopsies were obtained from wild-type and ISG15−/− mice. The basal oxygen consumption (top) and extracellular acidification (bottom) rates were monitored using a Seahorse Biosciences extracellular flux analyzer. Values were normalized to protein content in the biopsies. Data of at least six mice per group were summarized as means ± SEM. A one-way ANOVA was performed followed by a Tukey's multiple comparison test. (D and E) Liver

proteome data together with HEPATOKIN1, a model of central liver metabolism , were used to assess the metabolic alterations in liver tissue of wild-type and ISG15−/− mice during viral infection. Metabolic models for the different conditions were constructed by scaling the maximal activity for each enzyme using the LFQ intensities for each protein obtained from MaxQuant analysis at the respective point in time. (D) For a standard 24-hour profile metabolite plasma profile, diurnal glucose exchange fluxes were simulated in wild-type and ISG15−/− mice at each time point of viral infection. Negative exchange fluxes indicate net release from the liver to the plasma (gluconeogenesis), while positive values indicate hepatic glucose uptake (glycolysis). (E) For each condition, experimentally determined blood glucose levels were used as model input to calculate realistic exchange fluxes and glycogen levels. Credit: Science Advances, doi: 10.1126/sciadv.aay1109

When viral infections activate the host defense pathways, cellular demands for ATP (adenosine triphosphate) will alter and remodel central metabolic processes. In this study, IFN treatment (precursor pathway to ISG15) reduced CV replication in cell cultures and decreased cellular glucose consumption back to control levels. CV infection in whole organisms lead to elevated glucose uptake by infected cells, while impairing the function of the exocrine pancreas—demanding metabolic reprogramming for recovery. The scientists observed infection-triggered hypoglycemia, increased energy demand, malnutrition and lower glucose storage in the liver of CV infected mouse models. ISG15 influenced the central liver metabolism at multiple stages of infection by increasing the capacity of liver tissue to produce endogenous glucose and conduct efficient glycolysis during early and late stages of disease. Using metabolic models, Kespohl et al. illustrated how ISG15 reprogrammed the central liver metabolism during infection for efficient glucose production and storage.

Identifying the antiviral capacity of human ISG15

The team then examined the concept of stabilizing ISGlyation by inhibiting the protease USP18 for therapeutic applications during CV infection. Kespohl et al. investigated if the antiviral capacity in the mouse also applied to humans to show there was no barrier between the two (in human cell culture or mouse models) during ISGlyation to effectively counteract CV infection. While the results were based on a laboratory CV strain, little was known of the impact of ISGlyation on a clinical viral counterpart. The researchers tested clinical viral variants from patients to determine viral sensitivity to ISG15 in cell culture and noted that improving ISGlyation could inhibit viral replication for all clinical CV isolates tested in the work.



Human ISG15 suppresses CV replication. (A) ISG15 expression was deleted in HeLa cells using CRISPR-Cas9 gene editing. ISG15-ko cells and wild-type cells were infected with CV (MOI 0.1) for 16 hours. Expression of CV VP1 was determined by Western blot analysis in four independent experiments, and the obtained signal normalized to GAPDH was compared to wild-type samples. Infectious virus particles were quantified in five independent experiments by plaque assay. Data are summarized as means ± SEM. (B) HeLa cells were transfected with siRNA targeting human ISG15 or a nontargeting control siRNA. Cells were subsequently infected with CV (MOI 0.01) for 16 hours. VP1 protein expression was determined and normalized to the control sample in four independent experiments. Plaque assays were performed in two independent experiments. Data are summarized as described in (A). (C) HeLa cells stably expressing FLAG-tagged human ISG15 and respective control cells were infected with CV (MOI 0.1) for 16 hours. VP1 expression and virus titer were determined as described in (A) in three independent experiments. (D) Primary embryonic cardiomyocytes obtained from ISG15−/− mice were transduced with Ad5 vectors expressing human ISG15 (hISG15) or control for 48 hours at MOI 25 before CV infection (MOI 0.1) for 24 hours. VP1 levels were determined by Western blot analysis, and infectious viral particles were quantified by plaque assay in three independent experiments. (E) Cardiomyocytes derived from USP18C61A ISG15−/− and ISG15−/− embryos were transduced with Ad5 vectors encoding hISG15 and infected with CV in three independent experiments for detection of the viral load by Western blot analysis of VP1 as well as plaque assay. One-sample t tests were performed for all summarized VP1 data. Unpaired t tests were conducted for all plaque assay data. (F and G) CVB isolates were obtained from patients presenting with neurological symptoms that may have been of infectious origin. (F) ISG15-ko HeLa cells and control cells were infected with the indicated CV serotypes (MOI 0.1), and infectious virus particles were determined after 16 hours by plaque assay. The relative increase of the viral titer in ISG15-ko HeLa cells as compared to control cells is depicted for a representative experiment. Three independent experiments demonstrated similar results. (G) The ISG15 system was induced in USP18C61A ISG15−/− and ISG15−/− cardiomyocytes by transduction of Ad5 vectors encoding hISG15. Cells were infected with CV serotypes as follows: CVB1 425, MOI 1 (2 days); CVB1 506, MOI 1 (1 day); CVB3 1072, MOI 10 (2 days); CVB3 180, MOI 10 (1 day); CVB4 686, MOI 10 (2 days); CVB4 120, MOI1 (2 days); CVB5 679, MOI 1 (1 days); CVB5 800, MOI 10 (1 day). Infectious viral particles were quantified by plaque assay. Relative reduction of the viral load in USP18C61A ISG15−/− cells as compared to ISG15−/− cardiomyocytes with restored

ISG15 expression is depicted for a representative of at least three independent experiments. Data are means ± SEM; one-sample t tests were performed, and P values are depicted. Credit: Science Advances, doi: 10.1126/sciadv.aay1109

In this way, Meike Kespohl and colleagues precisely understood the functions of the ISG15 system during antiviral and metabolic rewiring to combat CV infection. Despite the impressive antiviral activity observed for CV, ISG15 is not effective against all viruses since viral resistances evolved due to a constant battle of immune evasion mechanisms of the pathogen and the corresponding host immune response. However, inactivating the ISG15-degradation-specific protease USP18 can enhance antiviral capacity of the ISG15 system against clinical CV serotypes with relatively minimal side-effects. The data support inhibiting USP18 protease as a host-directed antiviral approach to combat CV pathology in man.