COVID Lux - **March 2021**

The most commanding aspect of the ongoing COVID-19 pandemic now is probably the interplay of viral *mutation* and human *vaccination.* Almost anything I write today about this interaction will be superseded by the time this newsletter is available. But we'll be better able to understand the evolving story if we try and clarify some of the concepts and nomenclature. Therefore I want to review the vocabulary of "mutations" in context . And though it is (mostly) SARS CoV 2 mutations that are in the News, I will start more generally because it's easy to forget what an integral part of Life's variety is attributable to mutation - they are not just the sinister ploys of a menacing virus!

I'll assume a basic working knowledge of the virus which, if you have the time can be reviewed on this video by clicking on the graphic above.

Anyone interested in a deeper dive into vaccination and the immune system itself will find a lucid article in The Atlantic from last summer:
https://www.theatlantic.com/health/archive/2020/08/covid-19-immunity-is-the-pandemics-central-mystery/614956/

Mutant Spawn

My brothers like to refer to my sons as Mutant Spawn. And I bet any fifth grader would define a *Mutant* more or less as someone with an inborn error of development that results in the ability to emit magnetic rays or fire or some kind of freezing substance - always from the extended hands, never from the elbow or ankle as near as I can tell. But what is it REALLY?

(Spoiler alert: *mutations* are alterations in genetic code that lead to altered proteins with or without significant alteration of their structure of function…. 'Genetics' is the science of heredity and the source of terms we will encounter such as 'gene', 'genome' and 'genotype'. "Proteins" are the basic structural building blocks of living things, though they may be embroidered with carbohydrates and fats. And "Enzymes" are a group of workhorse proteins that each facilitate a specific chemical reaction - the broker, you might say, that enables and speeds a reaction that synthesizes a sugar or regulates a hormone, or transfers a chemical messenger from one nerve ending to another resulting in motion, or occasionally, thought etc…)

Cellular Operating System - Alle Menschen werden Bruder?

Our starting point is the simple central premise of Life's molecular biology: A plan, coded in *nucleic acids* (DNA in the human *Genome*; RNA in SARS CoV 2) is translated into proteins by which cells do their business. The segment of our DNA that encodes a particular protein is called its '*gene*'; all the human genes together represent our human '*genome*'. These gene-governed proteins (made of linked amino acids) including the enzymes that promote our complex chemistry, then pretty much do all the rest for us. And for animals and plants. And for viruses - though they don't schlepp around ALL the enzymes or raw materials they need, pillaging living cells to make up for what they lack. But we are ALL creatures of protein arranged per our DNA (or RNA) blueprints.

All humans share the same Human Genome - the same set of genes encoding the same set of proteins that act both as structure - the collagens of the skin for instance - and function - those enzymes. But….. if we are identical in our individual *genotypes*, ((the specific gene collection of each individual human) why do we not all look alike? Hold that thought….

- Morse than meets the eye…

How do humans get from DNA and genes to proteins (made of amino acids)? It starts with that famous double Helix of DNA (the molecular structure really more of a spiral staircase), the blueprint, containing the code for the construction of <u>all</u> our human proteins. The way you 'read' this DNA is in groups of three 'rungs' on this staircase. A marker identifies the start point for a given gene and after that, every three -rung -triad represents the code for one unique Amino Acid (of which there are about 20). So DNA represents a continuous inventory

of all our proteins in a long sequence separated by some rungs of punctuation. Not really a book of protein plans - more like a scroll.
Think of this like Morse code - the DNA triad-rungs are the dots & dashes; the "letter" each triplicate defines is an Amino Acid. In Morse code "Dot Dash Dot" = the letter R. In DNA, rungs CGT = the amino acid Arginine. So if you - or one of your cells - started to decode a sequence of DNA , each triplet would call for an Amino Acid and as each new triplet was read and that AA added to the elongating chain, eventually - when you get to the rung sequence that says "The End" - you'd have a long strand of Amino Acids, and voila! Strong work! You made a protein! Just as a sequence of Morse Code generates a series of alphabetical letters that carry abstract meaning, the DNA has coded for a string of Amino Acids making a very concrete protein.
Imagine you could make a video game that rewarded for protein construction instead of Zombie destruction! You could have kids reading an un-scrolling helix of DNA as easily as they read a long line of text, putting each Amino Acid in its place like so many proteinaceous letters! They be whizzes as protein chemists!

(There IS some protein folding to finalize form and in humans there is an intermediary between the DNA and the protein construction called RNA - its as if the DNA is a Prima Donna and doesn't want to leave the cell nucleus for the vulgar cell cytoplasm where all the protein construction takes place so it sends its RNA servant - but we don't have to get into all that.)

Polly want a Morphism?

So back to the question: We share the Human Genome so why don't we all look alike? The answer is (at least in part) because while every protein shared amongst humans has to perform its assigned task common to us all, the proteins might differ just a little in Amino Acid sequence person to person… and yet still function. Maybe for a given protein - say myosin, one of the proteins in active muscle - two people differ in the code at triad # 77 and therefore have different Amino Acids at place # 77 in that protein. Yet those amino acids were 'close enough' in configuration that the protein works well enough in both - or maybe it works <u>better</u> in one form than the other and contributes to, say, better endurance. (Note to self - review Natural Selection….) These small differences mean we **don't** all have exactly the same *genotype*, we don't have the identical final protein construction - and we don't all look act and behave in the same way. Without these minor differences in Genomic blueprint and protein construct (called polymorphisms , meaning 'many forms') people-watching would be a lot less interesting. While we all share the same Human Genome, we each have our own individual version of it, our own unique variant - identical twins being the exception.

Of course most of this individuality is passed on from conception - the familial polymorphisms that we're talking about when we say a baby 'has his grandmother's eyes". We are so used to this that if that baby's sister had her Grandfather's eyes, we wouldn't identify her as a mutant! These manifestations of individuality arise from small differences in DNA coding - some

mixing, some mutations - in the DNA of conception. (Mutations in humans can occur after birth and represent potential causes of cancer and of aging itself, not relevant here. But in any case most of them arise as errors occurring during the process of copying the DNA for cell replication.) Humans tend to keep conception apart from all the other cellular processes of day to day life(!) But for viruses, their day to day life is ALL about replication. each viral genome is unpacked and replicated thousands or tens of thousands of times per day. So for them, mutations that alter proteins that (potentially) alter function are far more frequent than in humans.

By the way, humans have "proof reading' functions to minimize the likelihood of a mutation in our DNA. But just as medieval monks copying manuscripts made errors - a word dropped or repeated, a mis-spelling, perhaps even an entire line misplaced - errors DO creep into the system as DNA (or RNA in Corona virus) is copied over and over. MOST monkish errors left the text still intelligible to the educated reader, but some have been the source of considerable confusion - the same is true of the protein constructed from a mutation-afflicted blueprint.

So errors in the genetic code accumulating randomly represent *mutations* - but since most never come to light its not surprising that the term is commonly associated with the more obvious cases with FUNCTIONAL significance. (Like emitting fire from your palm.)
So on the one hand there is a kind of normal genetic wobble that meets tolerance specs and allows function more or less as expected. But on the other hand there are the alterations in genetic material that result in something outside of expectations. Or to be more precise, any of us might have had a cell division that included a random mistake in copying one rung of the ladder - after all there are about 3 BILLION rungs in the human genome, and the best of Monkish transcriptionists would find it daunting to copy that many characters without a single error. For human DNA the mistake rate is about 1 in 100,000 rungs. Mistakes DO happen - but most will have no consequence and so we won't 'notice'.

Going Viral

The story in SARS CoV 2 is (mostly) the same. Of course this virus uses RNA instead of DNA so its genetic code is translated into proteins with fewer steps than in humans in ways we don't really need to discuss. But its proof-reading function is not very good and its numbers are great. There can be 500 BILLION SARS CoV 2 in a teaspoon of pharyngeal secretions! If all of them are replicating several times per day, a thousand or more new progeny at a time, no wonder even last fall there were already 12,000 *mutations* catalogued for this virus - though none were clearly of functional significance (https://www.nature.com/articles/d41586-020-02544-6) And no doubt there were many MORE mutations that actually resulted in DECREASED replication competence or even proved 'fatal'. We're talking only about the mutations to which the replication process is indifferent - that occur randomly but provide no obvious benefit or harm to that version of the Virus.

How would we know if there was a mutation - or more likely a cluster of mutations - that actually DID have functional significance? Because that virus variant would out-compete other viral strains to become a dominant form of the infection. It could be because mutations made for more 'sticky' interactions between virus and human cell so the pharynx simply had more virus to cough sneeze or sing out. A change in just one or two Amino Acids making up the Spike protein might do this - make its fit to the ACE 2 receptor more stable and make successful cell penetration more likely (as the British B. 1.1.7 strain seems to do and perhaps the South African B.1.351). Or perhaps mutations changing a few amino acids in the protein of the viral shell makes it a little more durable outside the human body, or changes its profile like 'stealth' technology delaying immune recognition. An easily overlooked consequence of an unchecked pandemic is the sheer number of mutations SARS CoV 2 can try out, those most advantageous being naturally selected.

Great Britain has updated its recommendations on mitigation as a result of its experience with its now dominant variant strain B.1.1.7 that seems to be more infectious than the 'original' strain(s).
(https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/948607/s0995-mitigations-to-reduce-transmission-of-the-new-variant.pdf) There is a cluster of 13 specific mutations, including mutations of the spike protein, that characterize this variant . Early reports DO suggest the *possibility* of higher viral loads and a greater chance of serious illness and death but this variant also seems to be prominent in nursing homes with more vulnerable patients so there is no certainty about lethality per case even if we don't know know all the ways this set of mutations confers replication advantage. It seems likely that OUR CDC recs on mitigation will need to be tightened up as this variant among others begins to be seen in the US - 15 minutes of 6-feet or less single-masked exposure over 24 hours may not be strict enough.

On the other hand, all things being equal, a mutation that did NOT improve replication but made the virus more lethal would NOT be a good choice for SARS CoV2. The death of its host means the death of the virus - its a goner unless it can evade the masks and the distance and the air movement and get into another host. Increased deaths associated with a newly recognized viral variant is far more likely to be due to more efficient replication and more total infections than to pure "lethality" per infection. There ARE reports now of viral strains first identified in Britain, South Africa and Brazil claiming the resultant illness to be more lethal than the 'original' SARS CoV 2 first identified in China. As I write data are scanty but of course this could be true. But it will be difficult to be sure without tracking a LOT of infections with the full spectrum of outcomes while sequencing each virus (i.e. mapping each virus genotype) in every infection. Early on its just difficult to distinguish an increase in disease severity from an increase in disease incidence, both of which could result in an increase of deaths. But because a virus with mutations that result in it being more lethal to humans is not advantageous, it seems more likely that a higher death rate from one or more recently described mutant strains reflect some advantage in infectivity or replication efficiency resulting in more viri per infected human or higher efficiency of spread between humans.

Mutations and Vaccines

But "successful" enduring mutations aren't really just random. Some parts of the virus just can't be fiddled with and still preserve function. If cars were living things, and the position of the back bumper was mistakenly (mutantly) altered to be a quarter of an inch longer than specified in the plans, its not likely to be noticed. But if the inner diameter of the engine cylinders was a quarter inch longer than plans, the car probably wouldn't work at all. For the SARS CoV 2 one of the areas least likely to tolerate mutations is the Spike Protein. This is the viral site that docks with the cellular ACE 2 receptor by which the virus sneaks aboard to hijack the cell. There is a precise fit between spike and receptor and though there are portions of the protein that tolerate a little variance in Amino Acid sequence, the actual Binding Domain, perfected over trillions of mutations, is unlikely to be greatly improved by any more. Unlikely but not impossible….

If you DID improve that fit by the pure luck of a few random mutations that improved the alignment even just a bit (without screwing anything else up in the process), making binding a little more efficient, you might become a dominant viral variant. And that is of great concern because it is that binding we disrupt with vaccine-induced antibodies. The concern is that mutational monkeying with that part of the Spike protein might change it such that our antibodies can no longer recognize it, no longer glom onto the spike protein, no longer interfere with cellular docking to prevent replication.
The good news, conceptually, is that the very reason vaccine-induced antibodies 'work' is because they recognize the binding domain on the spike protein and occupy that spot, ruining the chance for the Spike protein to dock with its usual target ACE 2 receptor. If the spike protein is the 'key', the Antibodies encrust the business end so it no longer fits into that receptor 'lock'. If you imagine mutations effecting the binding domain so severely that is no longer recognizable by antibodies, it is very likely that the alteration means that binding site is also too distorted to recognize and dock with ACE 2 either. So it is unlikely that mutations in SARS CoV 2 will leave a virus that utterly evades antibodies and yet remains competent to infect a cell.

Unlikely is good, but there have been reports that vaccines deployed to date show lower rates of disease prevention in British and South African viral variants. Unfortunately these trials so far are small, have looked at mild and moderate disease only, and have been reported for young patients. But it is important to note that while the elimination of INFECTION by vaccine is the ultimate goal, the elimination of severe ILLNESS is a not- so- distant second. And to date in clinical trials NO properly vaccinated patient with ANY of the vaccines available in the US has died of any strain of COVID 19. Furthermore, the post-vaccination data available to date for all vaccines in millions of vaccinated patients show subsequent serious infection or death to be a small fraction of a percent . There may be more post-vaccination patients found to be positive for variant SARS CoV 2 , but so far these patients do not seem to be getting

seriously ill. (https://www.nytimes.com/2021/02/01/briefing/vaccination-myanmar-coup-rochester-police.html) So while we may still find that variants of SARS CoV 2 with characteristic mutations are more infectious or even more dangerous, it is not possible to conclude at this time that any particular vaccine will prove inadequate for any particular viral strain.

TAKE HOME POINTS AND WHAT TO WATCH FOR:

Mutations occur when mistakes creep into DNA (or RNA) blueprints as they ARE replicateD. Most are tolerable, many are lethal, but a few confer advantage in replication and over time are 'selected' as they out-compete other variants.

Some sites in an organism's genome are more tolerant of mutations than others, but in general it's unusual to "improve" a critical function.

Nevertheless mutations that facilitate viral replication or transmissibility are more likely to be sustained than any that might increases lethality.

Mutations of the viral spike protein that made vaccine-induced antibodies less able to recognize and attach to it probably make the spike protein less able to recognize and attach to the ACE 2 receptor and invade the cell. Therefore it is unlikely - though not impossible - that viral variants will become impervious to vaccination-induced immunity. Nevertheless, mutations of a minor nature might reduce vaccine efficacy in a minor way and obligate us to revising the vaccine.

(Bear in mind that once this virus is being pressured by Vaccine-induced antibodies around the world, variants that have ANY degree of greater resistance to current antibodies will become dominant.

In the next few months I will be looking for the impact of each vaccine on 1) ICU admissions and 2) death for patients with each significant variant strain - currently from Britain, South Africa and Brazil but there will be more. I'd rather see elimination of mild disease and asymptomatic disease for every strain since that would mean lower rates of transmission would follow. But we MUST see severe disease reduction for all strains. I am also watching for robust data on re-infections and on persistence of immunity - these are the factors that will ultimately determine if boosters will be needed and/or boosters adjusted to optimize antibodies to variant strains.

The arising of viral variants by mutation is no surprise - its an annual event with the influenza virus - but the dramatic reduction in cases we now see as vaccination climbs dramatically is extremely promising.
Keep distanced, keep masked, and get vaccinated!