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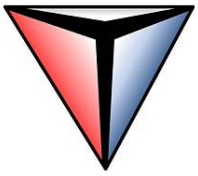

MALT-targeting transport peptides have the potential to make ALL vaccines better

EXECUTIVE SUMMARY

By using a new type of screening test to screen a billion candidates from a phage library, a biotech startup company has isolated and sequenced a set of highly aggressive "pathogen pattern" peptides. That is a highly useful result, since these peptide sequences can be added (in *low* copy numbers) to the surfaces of vaccine particles, to provide rapid, precision-guided, "targeted transport" of the resulting "MALT-targeting" vaccine particles, to make them appear to be very dangerous pathogens. That appearance of danger will cause specialized immune cells, mounted on the outer surfaces of mucosal membranes, to rapidly pull in and process those dangerous-looking particles, in ways that will trigger and drive – all the way to completion – an immune response that will form antibodies to ANY antigen sequence, derived from ANY pathogen, that is carried (in *high* copy numbers) on those "MALT-targeting" mucosal vaccines.

The mucosal membranes in all vertebrate animals have "surface mounted lymph nodes" (called MALT patches, for "mucosal-associated lymphoid tissues"), as a first line of defense against pathogens which attack and infect mucosal cells. Surface cells called "M cells" are adapted for "sampling" any particles that contact them, to identify – and pull in – those which appear to be dangerous. Rather than processing those particles, an M cell will rapidly hustle and push a particle (enclosed in a membrane bubble) through the cell, and it will eject that particle, in naked form again, into a "docking site" on its "basal" surface. When "dendritic" immune cells are formed, they are attracted to those docking sites, and large numbers of dendritic cells settle into those docking sites, to await delivery of a pathogen. If and when a particle is handed to a dendritic cell by an M cell, the dendritic cell will use its surface receptors to analyze that particle, and if the particle has certain types of "pathogen patterns" on its surface – causing it to appear to be both dangerous, and important – that "immature" dendritic cell will undergo an "activation" (aka "maturation") event, which will transform it into an "antigen-presenting cell". When that happens, the activated (maturing) dendritic cell will leave that docking site, and go searching for the "germinal center" of a lymph node; and, while it travels, it will semi-digest surface proteins on the particle, and mount "chunks" of those proteins on mounting-plaque proteins (MHC proteins). When that "antigen presentation" occurs inside a lymph node, B and T cells in the lymph node will take over, and will begin working together to create antibodies that will bind to those antigen sequences, and the dendritic cell will leave the lymph node (possibly in a way that might allow it to revert back into "immature status" again).

By studying the changes that occur when an immature dendritic cell commits to activation/maturation, and by creating a new type of screening test which could isolate dendritic cells which had commenced that process, we were able to isolate phage particles which

happened to be carrying small foreign peptide inserts, having "pathogen pattern" sequences that appear to be so aggressively dangerous that they can activate and drive M cells, and then dendritic cells, to perform every step in the sequence described above, and take the dendritic cells all the way to a full and irrevocable commitment to activation, maturation, and doing what it takes to launch an antibody-forming response.

We then hired a phage lab to create genetically-engineered phage particles, with 15 copies/particle of the "MALT-targeting" sequences, and hundreds of copies/particle of a well-known, easy-to-test antigen (the "HA-tag epitope"). "Antibody production tests", in both mice and pigs, showed that even at the lowest dosages tested, a single nasal infusion of those particles, with no adjuvants added, triggered "robust" formation of not just internal antibodes, but of secreted mucosal antibodies as well, which work via an entirely different mechanism.

We then shifted over to a different and better type of phage vehicle; we selected an antigen sequence found in numerous strains of influenza viruses which are actively causing problems around the world (the "FI-6" influenza antigen, described in Corti 2011); and, as this is being written, we are only a few days away from commencing the first-ever "pathogen challenge tests" to see if those vaccines can actually protect lab animals against a deadly pathogen.

We will not be ready to formally announce and publish the results of that work, until the results from the pathogen challenge tests become available, hopefully by mid-March 2026. However, we are using this website to begin quietly spreading information (about what we have done so far, and what we are planning to do next) among small numbers of animal vaccine companies, research experts, and government agencies, in an effort to help give them some advance notice, and an opportunity to begin considering whether they might want to weave this opportunity into their research plans.

Every result we have been seen to date indicate that this new approach to creating "MALT-targeting" vaccines can provide three new and extremely useful benefits:

1. When applied topically, as "mucosal vaccines" (such as by nasal spray, or via lollipops, or lozenges), they will trigger the formation of both the normal, well-known types of antibodies that function inside the body, as well as an entirely different class of "secreted antibody dimers" (which work by an entirely different mechanism), into saliva, nasal mucus, lung and genital fluids, and digestive juices, to provide a "first line of defense" against mucosal pathogens (which includes all upper respiratory tract infections, including COVID, and influenza).
2. By using a "targeted transport" system, these vaccines completely eliminate any need for the types of harsh, unpleasant, muscle-irritating "adjuvants" that are required to make *injected* vaccines effective; and,
3. These vaccines can completely eliminate needles, injections, and medical waste, and they do not even require refrigeration. Instead of requiring people to make an appointment, get a shot, and feel soreness at the injection site for 2-3 days afterward, any nurse or group administrator can pass around a bowl of tasty lollipops, to a group of people.

The startup company which created this new approach to vaccine design has no desire or intent to become a manufacturing company, and we do not have the "biosafety labs" or expertise to perform "pathogen challenge tests". Instead, we intend to become a licensing company, and will offer (at low cost) customized MALT-targeting phage constructs – carrying any antigen sequence designated by the requester – to any animal vaccine company, vet school research group, government agency, or other qualified research group that will commit to testing those phage constructs in "pathogen challenge tests" in one or more types of animals. To provide

incentives and motivation for that type of testing, we hereby offer a worldwide exclusive license – covering MALT-targeting vaccines against one or more specific diseases, in one or more designated types of animals – to the first company or research group which generates enough positive data to support an "animal vaccine registration" (i.e., an approval for sale) by the US Department of Agriculture. More information on that is available via the "Goals and Plans" button in the footer.

[end of Executive Summary box]

The two "PDF" buttons below will enable anyone to download, at no charge: (i) a complete copy of this website, in pdf format; and, (ii) the entire Background section, and list of cited references, from one of several pending (but not-yet-published) patent applications that describe and claim MALT-targeting vaccines, and the methods used to create them.

The next page contains a summary list of relevant topics (e.g., What are MALT patches? What are secreted IgA dimers?). If you click on any heading (in red), it will take you to a different page with more information on that topic.

The final page in this website contains a way to get in touch with us, if you want more information, and/or if your company or group might be interested in getting some low-cost MALT-targeting phages, with any antigen sequence you specify (however, that offer is available only to companies or groups that are able and willing to do pathogen challenge tests, with those particles, and that antigen).

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NEXT PAGE: www.tetraheed.com/overview-list-pages-topics

This page contains a list, with brief overviews, of topics that are addressed in more detail on the pages that follow. **If you click on any red header, below, it will take you to a page with more info on that topic.**

1. BRIEF INTRODUCTION TO MUCOSAL MEMBRANES, AND MUCOSAL PATHOGENS

This page describes how mucous membranes are very different from "dry skin", why they are major targets for pathogenic microbes, and why "mucosal pathogens" are – by far – the most important pathogens on this planet.

2. MALT PATCHES, AND HOW "M CELLS" SAMPLE AND PULL IN APPARENT PATHOGENS

MALT ("mucosal associated lymphoid tissue") patches are surface-mounted lymph nodes, and they are a crucial part of the "first line of defense" against pathogens that try to infect mucous membrane cells.

M cells are "sampling cells" on the surfaces of MALT patches. If they detect a particle carrying a "pathogen pattern", they will grab it and pull it in; however, rather than processing it,

they will push it through the cell, as rapidly as possible, and eject it into a "docking site" where a different type of immune cell – a dendritic cell – is waiting for that type of "pathogen delivery".

Therefore, "MALT-targeting" peptides are "pathogen pattern" peptides that can be attached to the surfaces of vaccine particles, so that they will "trick" the M cells into pulling them in, and passing them on to dendritic cells.

3. DENDRITIC CELLS ARE THE "GENIUS" CELLS WHICH CONTROL ANTIBODY-FORMING RESPONSES. THEREFORE, THEY ARE THE PERFECT TARGETS FOR "TARGETED TRANSPORT" VACCINES CARRYING PATHOGEN SIGNALS WHICH MAKE THOSE VACCINE PARTICLES LOOK LIKE DANGEROUS PATHOGENS.

Dendritic cells play the absolutely crucial role, in determining: (i) which foreign particles are not really important and dangerous, and should be gobbled up and digested on the spot, without further ado; versus (ii) which foreign particles appear to be dangerous and important pathogens, which need to be taken to a lymph node (while being broken apart and semi-digested along the way), so that T and B cells in a lymph node can make antibodies that will bind to those alien/invading particles. With the help of "chemo-attractant" signals, newly-formed dendritic cells find and settle into the "docking sites" on the undersides of M cells, to await a pathogen delivery that will cause them to transform from an "immature" dendritic cell, into an "antigen-presenting" cell. Therefore, "MALT-targeting peptides" – when attached to the surfaces of mucosal vaccine particles – offer an ideal way to get those vaccine particles rapidly delivered to dendritic cells, and to get the dendritic cells which receive those particles to do exactly what is needed, to launch an antibody-forming response to any antigens on the surfaces of those vaccine particles.

4. ANIMALS USE "SECRETED MUCOSAL ANTIBODIES" TO FIGHT OFF MUCOSAL PATHOGENS, BUT MUCOSAL ANTIBODIES HAVE VERY DIFFERENT STRUCTURES AND FUNCTIONS THAN INTERNAL ANTIBODIES, AND THEY ARE NOT CREATED IN RESPONSE TO INJECTED VACCINES.

The Y-shaped internal IgG antibodies are not large or powerful enough to hinder a virus, let alone a bacterial cell, so they fight pathogens by using a shape-changing "tag and flag" process. When either "sticky arm" latches on to a particle, the antibody "stem" changes from a "Leave me alone, I'm an antibody" shape, into a different shape that signals, "I've latched onto something important, so somebody go find an immune cell, and tell it to come here and help me." Guided to that location by "complement proteins" which "amplify" that signal, the immune cells do the actual work of fighting and killing pathogens.

However, in secreted mucosal fluids that are outside of any cells or tissues, there are not enough immune cells (or complement proteins) to respond to any such signals. So, "secreted mucosal antibodies" needed a totally different structure, and function, to be able to function effectively, and the mucosal immune system developed a remarkably clever way to strap two antibodies to each other, via their stem components, in a way that creates an "antibody dimer", with FOUR different "sticky arms" (with two, at each end of an elongated molecular complex). These IgA antibody dimers perform a "grab-and-drag" function, which prevents pathogens from penetrating into any mucous membrane cells. If a pathogen is grabbed in the mouth or nasal cavity, it will be dragged down into the stomach acids, which will kill nearly all pathogens; if

grabbed in the intestines, it will be kept suspended in the food that is being digested, until it is "pooped out" and eliminated.

5. VACCINE "ADJUVANTS" ARE "IMMUNO-STIMULATORY" ADDITIVES. IN REALITY, THEY ARE INFLAMMATORY, IRRITATING, AND TOXIC.

"Adjuvants" are compounds added to injected vaccines, to make them more effective and potent. However, in practical terms, they are harsh, irritating, inflammatory, distress-causing agents, and they are added to injectable vaccines, to cause the muscle cells, at the injection site, to send out distress signals, to recruit any nearby immune cells to come to the injection site, before the vaccine particles can be diluted, diffused, or degraded. Because of how they work, MALT-targeting vaccines have the potential to completely eliminate any need for harsh and irritating adjuvants, and if that turns out to be the case, they will offer a major benefit, for all vaccines.

6. THERE ARE MORE THAN TWICE AS MANY SECRETED ANTIBODIES, IN A FEW POUNDS OF MUCOSAL FLUIDS IN A HEALTHY ADULT, THAN ALL THE INTERNAL ANTIBODIES IN THE ENTIRE BODY OF THAT PERSON. THAT IS A POWERFUL INDICATOR THAT MUCOSAL ANTIBODIES ARE VERY, VERY IMPORTANT IN FIGHTING MUCOSAL PATHOGENS. BUT, UNTIL NOW, THERE WERE NO RELIABLE, NON-TOXIC WAYS TO USE VACCINES TO CREATE MUCOSAL ANTIBODIES.

Most people have never even heard of "secreted antibody dimers", but there are more than twice as many of those – in just the few pounds of saliva, nasal mucus, lung fluids, and digestive juices, in a healthy human – as all of the internal antibodies in the entire remaining weight and bulk of that person's body. Our bodies would not devote so many resources to creating huge numbers of mucosal antibodies, unless they were truly important, and immunologists know, full well, that if vaccines could be created which could safely and reliably create mucosal AND internal antibodies, those "balanced, bi-functional vaccines" would be better and more effective (especially against "upper respiratory tract" infections) than vaccines which can only create internal antibodies. However, scientists and vaccine companies have never previously been able to make vaccines which can trigger BOTH internal AND mucosal antibodies, without using truly nasty chemicals which are not acceptable for livestock or pets, let alone humans. MALT-targeting mucosal vaccines have the potential to change that, dramatically.

7. WE "SCREENED" A BILLION DIFFERENT PHAGES, FROM A "PHAGE LIBRARY", TO IDENTIFY AND ISOLATE ABOUT 100 THAT WERE CARRYING POTENT MALT-TARGETING SEQUENCES.

"Phages" (originally called "bacteriophages") are the smallest viruses ever discovered. Each type can infect only a small set of bacteria, and none can infect plants or animals, so they are treated as harmless and non-pathogenic.

Phage "libraries" (aka phage display libraries) contain billions of different phage particles, and each particle carries a different, randomly-created "foreign peptide insert" on an exposed

“coat protein.” They took decades to develop, but now, a top-quality library with a trillion different phage particles can be purchased for less than $800.

“Screening tests” are clever ways, thought up by scientists, to subject millions of phage particles to a fair competition, usually involving something like, “Uptake and processing, by a specific and unusual type of cell”, so that they can isolate and then analyze those few phages which happened to be carrying an inserted peptide sequence which caused those cells to perform that activity.

So, we thought up and used a new type of screening test which isolated about 100 phage particles (out of a billion candidates/contestants) which happened to be carrying peptide inserts which made those particles appear to be so dangerous, and important, to the M cells and dendritic cells in mice, that those specific particles needed to be taken in and processed – as quickly as possible – in a way that would have led to antibody formation, if we had allowed the cells to continue. Instead, we extracted a mixed batch of mucosal surface cells, and used a clever screening method, to isolate only those dendritic cells which had become transformed and activated, by their contact with the specific phages they had taken in. We then broke those activated dendritic cells open, and analyzed the foreign inserts in the phages they had taken in.

8. TESTS WHICH USED THE “FIRST TESTABLE PHAGE CONSTRUCTS” SHOWED THAT A SINGLE NASAL INFUSION TRIGGERED ROBUST PRODUCTION OF BOTH IgG ANTIBODIES IN BLOOD, AND SECRETED IgA ANTIBODY DIMERS IN SALIVA. THOSE RESULTS WERE SO GOOD THAT WE MOVED ON TO “PATHOGEN CHALLENGE” TESTS, USING A DIFFERENT AND BETTER TYPE OF PHAGE.

Once we knew the DNA and amino acid sequences for the phage inserts that functioned as “MALT-targeting” peptides, we hired a phage assembly lab to create “the first testable constructs”, carrying 15 copies of the best-performing MALT-targeting sequences; and hundreds of copies of a well-known antigen that is easy to test for. In both mice and pigs, a single nasal infusion of those particles (with no adjuvants) caused “robust” formation of BOTH: (i) internal IgG antibodies, in blood, AND, (ii) secreted IgA antibody dimers, in saliva, as shown by both ELISA and SDS-PAGE/Western assays. Those results were so good that we shifted over to a different type of phage that is better suited for pathogen challenge tests.

9. THE FIRST “PATHOGEN CHALLENGE TESTS” WILL USE T7 PHAGES, CARRYING THE FI-6 ANTIGEN FROM INFLUENZA. HOPEFULLY, THEY WILL BE COMPLETED BY MID-MARCH 2026.

10. OUR HOPE AND PLAN IS TO BECOME A LICENSING COMPANY, AND NOTHING MORE. SO, WE WILL OFFER MALT-TARGETING PHAGES, AT LOW COST, CARRYING ANY ANTIGEN A REQUESTER SPECIFIES, IF THAT REQUESTER WILL COMMIT TO TESTING THEM IN ANIMALS; AND, WE WILL OFFER EXCLUSIVE LICENSES TO SPECIFIC VACCINES FOR LISTED ANIMAL TYPES, TO THE FIRST COMPANIES THAT GATHER ENOUGH SOLID DATA TO SUPPORT GOVERNMENT REVIEWS AND APPROVALS.

As described on the home page, we do not want to compete against any vaccine companies; we do not want to become a manufacturing company; and, we do not want to build,

or learn how to use, biosafety equipment, to be able to do pathogen testing on animals. Other people are already experts in all those things, and we truly and genuinely respect their expertise, and hope to work with them, in ways that will let them use their talents and skills to make the best vaccines that can possibly be made, both for humans (some day), and for non-human animals (as soon as possible).

Therefore, our goal is: (i) to become a licensing company, and (ii) to find good and effective ways to encourage, motivate, and incentivize experts in animal testing, to begin doing the work that needs to be done, in order to create the best possible government-approved and publicly-available vaccines.

IF YOU WANT TO GET IN TOUCH WITH US . . .

If you want to contact us, click on the red heading directly above, and write a short message in the box on the page that will open, to let us know who you are, what you want us to know, and how we can get in touch with you.

NEXT PAGE: www.tetraheed.com/t1-mucosal-membranes-pathogens

TOPIC 1. BRIEF INTRODUCTION TO MUCOSAL MEMBRANES, AND MUCOSAL PATHOGENS

With only a few exceptions (mainly for "blood-borne diseases", such as malaria and Lyme disease, which are spread by insect bites), almost every disease-causing microbe on this planet evolved in ways that caused them to become "mucosal pathogens", which infect animals by penetrating into cells on mucosal membranes. Even if their main damage occurs in other types of tissues, they must establish an initial infection, to establish a "foothold" (or beachhead, or similar terms) where they can begin reproducing, so that their progeny can then try to spread into other parts of the body.

Two major aspects of animal anatomy and physiology drove pathogens to mutate and evolve in that direction:

1. "Dry skin" is covered by "epidermal" cells, and those are not really "cells" at all; instead, they are empty-bag "pseudo-cells" which are dead from the moment they are created. Instead of being formed by cell division, they are formed by a "budding" process, in which precursor cells (located about 6-8 layers deep, in most areas of dry skin) rapidly enlarge to nearly twice their normal size, and then "pinch off" something which is basically an empty bag – with a normal outer membrane, having surface proteins which microbes can latch on to, but with almost nothing but slightly salty water inside that membrane. That makes epidermal cells ideal as "decoys" or "bait" for pathogens, which use their tricks and tools to break into those cells, only to discover that they have broken into an empty shell of a building, with none of the things they need, to reproduce, and none of the things they need, to escape.

2. By contrast, mucous membranes are covered by an entirely different class of cells, called "epithelial" cells. Those are full and complete cells, with all of the biochemical machinery and supplies that pathogens need, to reproduce. And unlike cuts, nicks, or wounds, which disappear fairly rapidly as they heal, mucous membranes are always available, and accessible, at all times, in any animal. Therefore, they are very tempting targets, for any type of pathogen which has evolved with some type of mechanism for grabbing hold of one or more types of epithelial cells, and then invading those cells (and, different types of microbes use a wide variety of such mechanisms).

3. Although most people are startled when they hear this number, the total area of the mucous membranes, in an adult human, is estimated to be about 200 times larger than the total area of dry skin, which covers the body. While that ratio might seem exaggerated or even preposterous, two factors help explain it:

(i) it includes all of the microscopically small sacs inside both lungs, when fully inflated to bursting pressure. That surface area, by itself, is (and must be) quite large, to provide enough gas transfer, across those membranes, to supply all of the muscles, organs, brain, and other tissues of the body with enough oxygen to keep them fully functioning, even under periods of strenuous exertion; and,

(ii) most mucosal surfaces have very large numbers of folds, ripples, invaginations, and other irregularities. Some are visible, but many more are microscopic in size. Those folds, ripples, etc., provide mucous membranes with numerous advantages, including remarkable flexibility and stretchability, as well as greatly expanded surface areas, which allow very large numbers of surface-mounted cells (and even specific types of cell surfaces) to perform specialized functions.

In addition, another huge advantage helped promote and enlarge the class of mucosal pathogens. If a pathogen can infect a surface mucosal cell and begin reproducing in that cell, it almost certainly will be able to begin forcing that host cell to either: (i) die and split open, thereby releasing dozens, hundreds, and in some cases thousands of new copies of that pathogen; or, (ii) become "leaky", and begin secreting pathogens, in ways that keep the host cell alive, so that it will keep making even more copies of that pathogen. Since nearly all types of viruses and bacteria can reproduce very rapidly after they "set up shop" inside a host cell, the pathogens will be able to reproduce, and release multiple copies of themselves, long before all of the necessary steps can be completed to: (i) enable the infected cell to begin creating and moving distress-signaling cytokine molecules to its basal surface; (ii) enable a "killer T cell" to find that infected cell; and, (iii) wait for the killer T cell to engulf and destroy the infected cell. By the time all those steps can be completed, the infected cell is likely to be already dead, or actively spewing out copies of that pathogen. This is a major part of the reason why, even if someone has been vaccinated against COVID viruses, and has even been previously infected by COVID, if that person gets COVID again, s/he is likely to have very high numbers of active and infective COVID viruses, in his/her saliva.

With those and other factors actively encouraging the development of mucosal pathogens, animal immune systems had to develop a "first line of defense" against mucosal pathogens; and, "MALT patches", and a special type of secreted antibodies – with a completely different structure and function than classic Y-shaped internal antibodies – became two of the main components of that "first line of defense" against mucosal pathogens. Those topics are described on the next two pages.

NEXT PAGE: www.tetraheed.com/t2-malt-patches-m-cells

TOPIC 2. MALT PATCHES, AND HOW "M CELLS" SAMPLE AND PULL IN APPARENT PATHOGENS

The phrase "lymph node" refers to specialized tissue sites where T and B cells work cooperatively with each other, to create antibodies that will bind to chunks of proteins that are brought into the lymph nodes, by mobile "antigen-presenting cells." For historical reasons (described in the downloadable Background section of a patent application which can be found

HERE), any cells which can create antibodies are called “B cells.” T cells (which were given that name, because they must pass through the thymus, which is inside the chest, and which is at a halfway point between an organ, and a gland) are the cells which manage, supervise, and control B cells which are trying to create the best possible antibodies. Among other roles, T cells give essential stimulatory molecules to B cells which are creating promising antibody candidates; and, they refuse to give those vital signaling molecules to B cells which are not making promising antibodies, so that the non-promising B cells will die off, and simplify the competition.

With that as the definition of “lymph node”, the specialized immune system tissue patches that are exposed and accessible, on the surfaces of mucosal membranes (called MALT patches, for “mucosal associated lymphoid tissues”) fully qualify as “lymph nodes”, since they do indeed contain B and T cells which work together, to create antibodies that will bind to alien/invading/non-self peptides that are presented to them by mobile “antigen-presenting cells”.

However, MALT patches usually are not called “lymph nodes”. Instead, they belong in a special category, which deserves its own special name, because they have additional roles, capabilities, and functions that internal lymph nodes cannot match.

And, as brief asides:

(i) MALT patches that occur in the nasal cavity or mouth are also called NALT patches, where the N refers to “naso-pharyngeal”, to include not just the nasal cavity, but also the pharynx (i.e., the place where the nasal cavity, mouth, and throat all come together and intersect); and,

(ii) MALT patches in the intestines are sometimes called GALT patches (where G stands for “gut”), but they are more commonly called Peyer’s patches.

The crucial difference between surface-exposed MALT patches, versus “internal” lymph nodes, arises from the presence and activities of highly specialized cells, called “M cells” (from either “membrane” or “microvilli”, depending on what sources you read).

The “lumenal” (also spelled luminal) surface of an M cell (i.e., the surface which is exposed to food and/or air, in the mouth, nasal cavity, and lungs, and to food which is being digested, in the intestines) will have surface-mounted receptor proteins that are constantly looking for “pathogen patterns”, on particles which contact those receptors; and, when one of those cell receptors recognizes a “pathogen pattern”, on a particle which has contacted the cell, the cell will pull that particle inside the cell, for processing.

Summarized briefly, “pathogen patterns” (their full scientific name is “pathogen-associated molecular patterns”, or PAMPs) are protein sequences (i.e., specific sequences of the 20 “primary amino acids” which are strung together to make all proteins, in all forms of life on this planet) which appear on numerous different types of microbes. Those “pathogen pattern” sequences became “highly conserved”, and appear on numerous different types of microbes, because they mutated and evolved to a point where they reached a “sweet spot” of truly optimal functionality and efficiency, to a point where any other microbes, carrying different mutated versions of those “highly conserved regions”, will not be able to function as well, and will not be able to compete effectively against microbes having the optimal, highly-conserved sequences.

Accordingly, if an M cell, on the surface of a MALT patch, detects that a particle which has been inhaled or swallowed has one or more “pathogen patterns” on its surface, the M cell will pull that particle into the cell, using a process called “endocytosis”, or “phagocytosis.” As another brief aside, endocytosis is a broader term, because it also includes a second process called “pinocytosis”, which involves taking in tiny droplets of liquid; however, if intact particles are being pulled in by a cell, the term “phagocytosis” is more specific, and preferred.

When “phagocytosis” occurs, the cell encloses an incoming particle inside a bubble of

membrane material, called a "phagosome", partly to isolate the cell against the risk of being attacked by the particle, and in many cases, to begin the process of digesting and breaking apart that particle. In nearly all cases involving cells other than M cells, intake of a particle, inside a phagosome, leads to digestion of the particle, by a two-step process: (i) special enzymes will begin pumping acid into the phagosome, to begin softening, prying apart, and loosening up whatever is in the particle, in a manner comparable to way stomach acidity gets chewed food more ready to be digested; then, (ii) a "lysosome" will merge with the phagosome, and that merger will add more acidity, and aggressive digestive enzymes, to the mixture, so that the particle can effectively be "cooked and dissolved" inside a bubble which protects the rest of the cell components from those very harsh conditions.

However, if phagocytosis ***by an M cell*** was triggered by a "pathogen pattern" on a particle which was pulled inside the cell, the M cell will shift into a very different mode, which is believed to be unique to M cells, and only M cells. Using a combination of microtubules and energy-donating enzymes, an M cell with a phagosome containing an apparently dangerous pathogen will actively push, pull, and hustle that phagosome, as rapidly as possible, toward the "bottom surface" membrane (usually called the "basal" membrane) of the M cell. And, when the moving bubble of membrane material (which encloses the phagosome) gets pressed against the basal membrane of the cell, those two membranes will merge together with each other, since they are made of exactly the same types of molecules. When that happens, the "apparently dangerous particle" will be ejected out of the M cell, in "naked form" again, with no membrane surrounding it. That unique M cell process is called "trans-cytosis", which translates into "through the cell", or "across the cell".

And, that process will eject the particle directly into a special and unusual type of open and roughly U-shaped cavity, on the "underside" (or "basal" side) of the M cell.

Those cavities are called "docking sites", because they are functionally similar to the "docking sites" that big trucks will back up into, on the back sides of retail stores, to both: (i) deliver new products to those stores; and, (ii) carry away, for delivery, anything which is too large or heavy for customers in that store to carry away on their own.

And, those docking sites are extra-special, because that is exactly where large numbers of immature dendritic cells go to, in order to wait for a "pathogen delivery" by an M cell. They do that, because there is a specific "chemo-attractant" cytokine molecule which is slowly and constantly released, by M cells, into the docking sites on their basal surfaces. That chemo-attractant actively attracts *immature* dendritic cells, to those docking sites.

And, that leads into a discussion of "dendritic cells", on the next page.

NEXT PAGE: www.tetraheed.com/t3-dendritic-cells

TOPIC 3. DENDRITIC CELLS ARE THE "GENIUS" CELLS WHICH CONTROL ANTIBODY-FORMING RESPONSES, TO PATHOGENS THAT ARE EITHER IMPORTANT, OR NOT. THEY ARE THE PERFECT TARGETS, FOR "TARGETED TRANSPORT" VACCINE PARTICLES HAVING "PATHOGEN PATTERNS" WHICH MAKE THOSE VACCINE PARTICLES LOOK EXTREMELY DANGEROUS.

As an opening statement, dendritic cells rank in the "genius" league, when it comes to individual cells. *THEY* are the immune cells which must figure out which invaders are important, and which are not; and, when *THEY* determine that some particular (and apparently foreign, invading, non-self) particle is important, *THEY* are the cells which begin traveling toward a

lymph node, to deliver that package to the B and T cells in a lymph node. While traveling, they semi-digest the surface proteins on that particle, and place the resulting "chunks" of the foreign protein on specialized "mounting plaque" proteins (called MHC proteins) . And, dendritic cells even determine and control whether the chunks of foreign protein, from a foreign particle, will be mounted on MHC-1 proteins (which will trigger the creation of "killer T cells" that can engulf and destroy any particles having those peptide sequences on their surfaces), or whether the chunks of foreign protein will be mounted on MHC-2 proteins (which will trigger the creation of antibodies that will bind to those proteins).

Therefore, in a very real sense, the T cells and B cells in a lymph node merely respond to whatever chunks of proteins have already been selected, processed, and presented to the T and B cells, by dendritic cells. It is ***dendritic cells***, rather than B cells or T cells, which perform the ***absolutely essential, crucial, central role in determining what an immune system will respond to, and what it will not respond to***.

Since that process is absolutely crucial, in an immune response to a vaccine, it merits stating in alternate words. Dendritic cells are the crucial types of immune cells which launch, and ultimately control, antibody-forming responses to pathogens – and, to vaccines, as well. They "determine" (since individual cells do not have brains, scientists do not use the word "decide" to refer to what cells do; they can "determine," and they can "commit", but they do not "decide" anything) which pathogens are dangerous and important – and, therefore, worth responding to – and, which ones are not. Therefore, if some new type of vaccine can get the vaccine particles delivered directly to dendritic cells – and, if it can somehow "persuade" those dendritic cells, rapidly and reliably, that *THESE* vaccine particles are dangerous, and important, and merit a fast-as-possible launch of an antibody-forming response – that would be a remarkable accomplishment, and something worth serious attention, and careful study.

And, that is exactly what MALT-targeting vaccines can accomplish.

Backing up a bit, dendritic cells deserve a more comprehensive description, to help people better understand what they do, and how they do it.

They do not have eyes, and they cannot see anything; and, they do not have noses, or "olfactory receptor neurons", so they cannot "smell" things, in the way that animals with noses can smell things. What they use – instead of sight or smell – to find (and then travel toward) whatever they are looking for, at any given moment, is a cellular process called "chemotaxis". That type of cellular travel uses multiple surface receptors which will be triggered and activated by "chemo-attractant" signaling molecules; and, those surface receptors are positioned at numerous spaced-apart locations, around the entire outer surface of each dendritic cell.

"Chemotactic surface receptors" are specialized proteins which "straddle" a cell membrane, with one portion in an exposed outer location – where it can be contacted by "chemo-attractant" signaling molecules – while another portion is inside the cell, so it can send signals to the biochemical "machinery" inside the cell. In nearly all cases, a dendritic cell (or any other type of "mobile" immune cell) will move in the direction of the highest apparent concentration of "chemo-attractant" molecules, as indicated by signals the cell is getting from the chemotactic surface receptors on whichever side of the cell is getting the most signals, at any given moment.

So, large numbers of newly-created "immature" dendritic cells use chemotaxis to help them locate, and settle into, "docking sites" on the "basal" surfaces of M cells, in MALT patches.

Why do they do that?

Because that is exactly where "immature" dendritic cells need to be, in order to be available, equipped, and ready to "spring into action", when an "apparently dangerous particle"

suddenly pops out of the basal membrane of an M cell, enters that "docking site", and directly encounters the "waiting arms" of an "immature" dendritic cell.

The word "immature", when applied to dendritic cells, needs to be explained, lest anyone assume they are not yet full-grown, or mature enough, or strong enough for the tasks they must perform. None of those things are true; instead, "immature" dendritic cells are full-sized, fully-grown, and fully ready to pick up arms, and begin marching off into battle; however, but they have not yet encountered the pathogen particle which will change and transform their lives, forever. They are simply waiting (patiently) to contact what they will determine to be an "apparently important and dangerous" pathogen. Accordingly, rather than calling them "immature" – which suggests negative things, or a lack of some necessary skill, in most contexts – they could more accurately be called "pre-transformed", or "pre-committed", or similar terms.

The reference to "waiting arms of a dendritic cell" also merits a brief digression, to explain and defend it. Despite the absolutely crucial role they play, in launching antibody-forming responses, and in being in charge of telling the B and T cells exactly what antigen sequences they must respond to, dendritic cells were not even discovered, or known to exist, until 1973, when a fellow named Ralph Steinman recognized that a specific cell type that no one had paid attention to, previously, was much more active, and important, than anyone had previously realized. He won a Nobel Prize for that discovery, but not until almost 40 years later, in 2011, and as he remains, to this day, the only person who has ever been awarded a Nobel Prize posthumously.

As the discoverer, Steinman was entitled to name them, and he chose the name "dendritic", from the Greek root that refers to tree branches, and other "branching"-type extensions that become smaller, as they get farther from their source, and which tend to extend outward, rather than having an appearance like a batch of stirred limp noodles.

However, "dendritic cells" turned out to be an unfortunate name, for several reasons. One problem was that other types of cells (especially neurons) also contain branch-like projections that are also called "dendrites"; and, that overlap and conflict apparently blocked or prevented the emergence of a single-word name (such as dendricytes, or dendrocytes). Accordingly, the term "dendritic cells" became and remains the standard term, and whenever a physician or researcher hears that phrase, they must do a quick but distracting mental check to ask, "Are we talking about neurons, or immune cells, at this moment in time, and in this context?"

In addition, subsequent research (after Steinman had already assigned that less-than-ideal name to them) revealed that their "dendrites" are not actually tubular, and do not resemble the branches of trees. Instead, they have substantial width and flatness, and are more similar in shape to petals on a flower (or leaves on a "succulent" plant), than to branches on a tree. The wider, flatter shape provides the projections with more surface area, which is needed for large numbers of surface receptors, and for surface-mediated activities that are carried out by the cells. However, it also is worth mentioning that any comparison to the shapes of petals on a flower, or leaves on a "succulent" plant, requires yet another qualification. Rather than being firm, engorged, and "reluctant to yield or bend", they can shrink and collapse, if and when a need arises, and any liquid inside those protrusions apparently can be retracted, into the main cell body, if a need arises; and, that is yet another important trait and capability of dendritic cells, because that capacity is a crucial part of how they travel, or "migrate", if and when a need arises. They are capable of using a type of motion called "pseudopod migration", which is used by amoebas (and also by octopuses, if they are challenged to squeeze through a small hole or gap, to get to a fish or crab). That type of travel enables dendritic cells to squeeze through the lymph-filled gaps between neighboring cells, in soft tissues.

Returning to the main subject, large numbers of newly-created "immature" dendritic cells are actively recruited (via chemo-attractant signaling molecules) to find, and settle into, the "docking sites" on the "basal" surfaces of M cells in MALT patches. Those "docking sites" are the perfect locations for "pre-committed" dendritic cells to go to, so that they will be ready to respond, quickly and directly, when an M cell pulls in an apparently dangerous particle (i.e., having a "pathogen pattern" on its surface), and rapidly transports that particle (isolated inside a phagosomal bubble) through the cell. When the M cell ejects that particle into its docking site (in "naked" form again, after the phagosome holding the particle merges with the "basal" membrane of the M cell), that particle will be delivered directly to the surface of a dendritic cell, which has been patiently waiting for exactly that type of "pathogen delivery," by the M cell which created (and which controls) that docking site.

When a dendritic cell receives such a particle, it will use a complex and sophisticated set of numerous surface receptors to analyze that particle, and the cell will then "commit" to either of two very different options:

OPTION 1: If the particle appears to be "not really important", the dendritic cell can simply take it in, break it apart (i.e., digest it), and release its "biochemical building blocks," so that other cells can use those building blocks for their own nutrition; and then, that "unchanged, not-yet-activated, unmoving, still-immature" dendritic cell can simply wait, without further ado or commotion, in that same docking site, for the next "pathogen delivery" from that M cell;

– OR –

OPTION 2: if the particle appears to be a dangerous and important pathogen which merits a full-scale "antibody forming" response . . . then . . . that dendritic cell will "commit" to an "activation/maturing/transforming" event, which will become a major "life-changing" event, for that "previously immature" dendritic cell.

Accordingly, when a dendritic cell finishes analyzing a particle which has been handed to it by an M cell, the dendritic cell must choose between the two options described above. There are no other, alternate, "partial" or "halfway" options available. A dendritic cell must commit, fully and completely, to either activating, maturing, and leaving that docking site; or, it must remain in place, without making that transition. By way of analogy, when a train leaves a station, a person is either on that train, or not on that train; there are no halfway or partial options available (at least, not for people who are still alive, and have all their limbs still attached).

Therefore, if vaccine particles can be made to APPEAR to be extremely pathogenic, dangerous, and important – by placing one or more peptide sequences on them which, in nature, are up at the very highest levels of appearing to be both dangerous, and important – then those peptide sequences can trick and fool dendritic cells into "believing" that those vaccine particles are indeed extremely dangerous and important. And, if that can be accomplished, then those dendritic cells which receive those types of vaccine particles, will rapidly and irrevocably commit to activation, maturing, and leaving that docking site, to go find the germinal center of a lymph node while beginning the process called "antigen presentation."

And, that is exactly what an effective "MALT-targeting" sequence (as described herein) can do. A good and potent MALT-targeting sequence can (and will) cause surface-exposed M cells, in MALT patches, to actively pull in those apparently dangerous and important particles, rapidly push them through the cell, and eject the particle (in naked form again), directly into their docking sites. Once that has been accomplished, those same MALT-targeting sequences can (and will) cause immature dendritic cells (which are waiting for pathogen deliveries, in those docking sites) to interpret those "pathogen pattern" danger signals in ways that will rapidly drive those

dendritic cells into a full and irreversible commitment, to convert into an activated/maturing dendritic cell, which will leave that docking site, and go off in search of a "germinal center" in a lymph node (i.e., a place where T and B cells wait for "antigen-presenting cells" to bring them new challenges).

Those are exactly the types of responses, by exactly the right types of immune cells, that will trigger, launch, and drive the type of antibody-forming response that vaccines particles are designed, and intended, to create.

And, it gets even better than that, for three reasons that can be summarized as:

(i) The "newly activated" dendritic cell will not need to spend hours, or sometimes days, slowly using amoeba-like "pseudopod" travel to squeeze through the narrow lymph-filled gaps between cells, trying to find the inlet to a "lymphatic drainage channel", which will then (slowly) carry that activated cell to a lymph node. Why not? Because MALT patches are already lymph nodes, which happen to be mounted on the surfaces of mucosal membranes. A newly-activated dendritic cell, when ready to leave a docking site behind an M cell, is already fully inside a lymph node.

(ii) The use of "MALT-targeting" peptides can completely eliminate any need for using the types of irritating, inflammatory, and toxic additives that are called "adjuvants", which are necessary to make injected vaccine more effective. That topic is discussed in more detail, **HERE** [*link to www.tetraheed.com/t5-irritating-vaccine-adjuvants*].

(iii) When these types of ***MUCOSAL*** vaccines are used, they will trigger and drive the formation, not just of the standard, typical, Y-shaped "internal" (IgG) antibodies that are triggered by injected vaccines, but also of an entirely different type and class of "secreted mucosal (IgA) antibody dimers", which have very different structures, and very different functions, than internal antibodies. That topic is discussed in more detail, **HERE** [*link to next page*]

NEXT PAGE: www.tetraheed.com/t4-secreted-vs-internal-antibodies

TOPIC 4. ANIMALS USE "SECRETED MUCOSAL ANTIBODIES" TO HELP THEM FIGHT OFF MUCOSAL PATHOGENS. HOWEVER, MUCOSAL ANTIBODIES HAVE ENTIRELY DIFFERENT STRUCTURES AND FUNCTIONS THAN INTERNAL ANTIBODIES, AND THEY ARE NOT TRIGGERED AND CREATED IN RESPONSE TO INJECTED VACCINES.

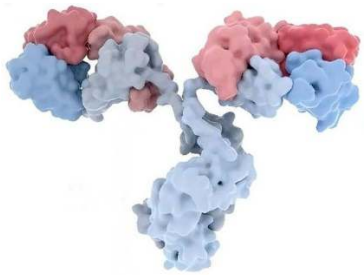
Vertebrate animals have two entirely different types of antibodies, to help them fight off pathogens and diseases.

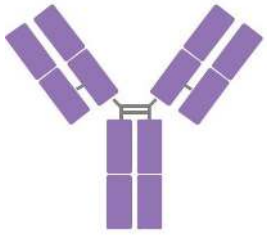
Actually, vertebrates have six distinct type of antibodies, but most of them are not important, in actually helping fight off pathogens. So-called "IgE" antibodies are generally unwanted, and are more involved in allergic reactions, than in defending against diseases. In a classic goof-up, the "IgM" name applies to two very different types of antibodies: (i) an extra-large ring structure, formed by coupling together 5 Y-shaped antibodies; and, (ii) a "small, inexpensive, trial-sized" antibody, created only inside lymph nodes, when B cells are creating new candidate antibodies in response to a newly-presented antigen sequence (those extra-small "testing" versions do not leave a lymph node, and are eliminated once a T cell chooses a "winning" B cell and signals it to begin making the full-sized versions). And, a rare type of

antibody is called IgD, because it has a “delta” chain. Anyone who wants more info on (or pictures of) any of those can find that info easily, in Wikipedia or via an internet search.

So, that leave two main types of antibodies which actively help fight off pathogens and diseases. The easiest way to grasp a huge and crucially important difference between them, is to realize that:

(i) the “famous” type, usually called IgG (“immunoglobulin Gamma”), which essentially all educated people already know about, have shapes that roughly resemble the letter Y, because they have two “sticky arm” components, attached to a single “stem” component. Here are two ways to illustrate an IgG antibody:





ILLUSTRATIONS OF Y-SHAPED IgG ANTIBODIES, WHICH CAN ONLY FUNCTION INTERNALLY. THE TWO UPPER COMPONENTS CAN BE CALLED “STICKY ARMS”, OR Fv (“fragment variable”), OR Fab (“fragment antigen-binding”). THE “STEM” COMPONENT WAS INITIALLY CALLED Fc (“fragment constant”), BUT WHEN SCIENTISTS REALIZED IT CHANGES SHAPE INTO A SIGNALING MODE, WHEN THE ANTIBODY BINDS TO A PARTICLE, THEY CHANGED THE Fc NAME TO “fragment crystallizable” (TO KEEP THE SAME Fc LABEL)

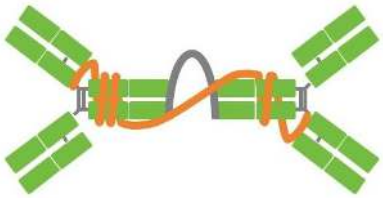
These Y-shaped antibodies can work effectively, ***only INSIDE the body***, because they do not carry any toxins, or defensive weapons, and they are much too small (as a single molecule) to be able to seriously entangle, slow down, or hinder even a virus, let alone a bacterial cell. So, they function by changing the shapes of their “stem portions”, when either of the two “sticky arms” binds to something that looks dangerous.

We call that process a “tag and flag” process; the antibody effectively tells an apparent pathogen, “Aha! I found you, and now I've caught you! And so, **TAG!** You are now **IT!**” The sticky arm(s) of the IgG will latch onto the particle, and when the stem portion of that antibody changes shape, it effectively becomes a “flag”, attached to that particle, which begins the process of alerting the immune system that an invading foreign particle has been identified, latched onto by an antibody, and marked.

Next, a special class of proteins (called “complement proteins”) will recognize and bind to a “shape-shifted” stem on an internal IgG antibody. That binding reaction will trigger an “activation cascade” which will cause the complement proteins to have a “protective cap portion” removed, to expose an active portion. When complement proteins become “activated” – by binding to an IgG antibody which has bound to something – they perform the equivalent of attaching a loud electronic alerting device, with a loud siren and a flashing light, to the shape-shifted stem of an IgG antibody. Any nearby immune cells will recognize and respond to those signals, and they will go to where the shape-shifted antibody, and the complement proteins, have attached to the alien/hostile/non-self/intruding particle.

However, in “secreted mucosal fluids” which are completely outside of any cells or tissues (those fluids include saliva, nasal mucus, digestive juices, lung fluids, fluids in the vaginal cavity and urethra, and fluids that keep the eyes lubricated), there simply are not enough immune cells (or complement proteins) present, in those fluids, to be able to offer any significant help, to any “shape-shifted” antibodies.

Therefore, in a beautiful, brilliant, and elegant feat of evolution, the immune systems of vertebrates somehow figured out (or “evolved with”) a way to strap two antibodies to each other, via their stem components, to create elongated antibody “dimers”, with each “dimer” containing two Y-shaped antibodies coupled to each other, so that each and every dimer contains not just two, but four “sticky arms” (with two at each end of a double-length molecule). Those dimers are called “secreted IgA antibodies” (or related phrases, such as “sIgA dimers”). Here is an illustration of an IgA dimer:



IgA “DIMERS” ARE FORMED BY ATTACHING TWO Y-SHAPED ANTIBODIES TO A PEPTIDE CALLED A “J CHAIN” (SHOWN IN GRAY), INSIDE A “B CELL” WHICH MAKES ALL THREE COMPONENTS. THAT “LIGHTLY ATTACHED” DIMER IS SECRETED BY THE B CELL, THEN IT IS PULLED IN BY A RECEPTOR PROTEIN ON THE BOTTOM (“BASAL SURFACE” OF AN EPITHELIAL CELL. PART OF THAT RECEPTOR (SHOWN IN ORANGE) WRAPS AROUND THE STEM COMPONENTS AND J CHAIN, TO CREATE A STRONGER DIMER, WHICH IS THEN SECRETED BY THAT EPITHELIAL CELL INTO SALIVA OR MUCUS. THE “DOUBLE-STICKY DIMER” THEN PERFORMS A “GRAB AND DRAG” PROCESS, ON PATHOGENS.

Since a “tag ang flag” function is useless, in secreted mucosal fluids with no immune cells around, the **MUCOSAL** immune system developed a “grab and drag” process instead. If even a single sticky arm segment can latch onto some foreign particle, the antibody will effectively block and prevent that particle from burrowing, tunneling, or otherwise penetrating into any mucosal cells or membranes. Instead, the IgA antibody will forcibly drag that particle down into the stomach acids (which will kill nearly all microbes), if they connected inside the mouth or nasal cavity; or, the antibody will keep that particle suspended in the liquefied and then semi-solid mass of food that is passing through the intestines, until the particle and the antibody get “pooped out” and eliminated from the body; or, if they connected inside the windpipe, bronchial tubes, or lungs, they will be coughed up, and either spit out, or swallowed.

Most people have never even heard of “secreted mucosal antibody dimers”, and they have no idea that they even exist. However, here is an absolutely crucial fact, which can help people better understand how important they are:

In just the 3 to 5 pounds of secreted mucosal fluids that a typical human adult is carrying, at any given moment, there are MORE THAN TWICE AS MANY SECRETED IgA ANTIBODIES, THAN ALL OF THE Y-SHAPED IgG ANTIBODIES IN THE ENTIRE REMAINING BULK AND WEIGHT, OF THAT PERSON.

In summary, because of the factors described in Topic 1, the number of pathogens that infect animals by penetrating into a “mucosal membrane” is hundreds of times larger and more populous than the second-largest category of pathogens (i.e., “blood-borne” pathogens, usually

transmitted by insect bites). So, driven by constant and pressing needs, mammals developed a specialized "mucosal" immune system, which operates in ways that are almost entirely separate from, and independent of, the internal immune system. The immune system figured out how to create IgA dimer antibodies, and how to secrete them into the mucosal fluids that are no longer inside any cells or tissues (for anyone interested in more details about HOW they are made and secreted, in ways that go deeper than the figure caption above, download the patent application section available from this website, and search for the words "J chain" or "secretory component"). Then, once those sIgA antibody dimers have been created and secreted, they are entirely on their own, and they will get no more help from any other immune cells or components.

However, there are two unhappy and difficult facts, about injectable vaccines, which everyone should know about.

The first unhappy fact is that injected vaccines do ***NOT*** trigger ***mucosal*** antibody formation, except perhaps as rare, sporadic, and unreliable side effects. As described on the next page, there simply has not been any good way, before now, to create vaccines that can safely and reliably trigger ***mucosal*** antibody responses, against any antigen sequences that are "loaded onto" those vaccine particles.

The second unhappy fact is this: in order to make injected vaccines more potent and effective, the complete injectable formulations must include harsh and unpleasant chemical additives, called "adjuvants". The reason why adjuvants must be "necessarily nasty" compounds, which are used to deliberately irritate and inflame the cells and tissues at the site of an injection, are described on the next page. And, it is hoped and believed that the "MALT-targeting" approach to creating mucosal vaccines can entirely bypass, and eliminate, any need to include any harsh, inflammatory "adjuvants" in the final formulations. If that can be accomplished, it will provide a major and much-appreciated step forward, to a point where MALT-targeting vaccines might earn and deserve the label of "Post-Adjuvant Vaccines".

NEXT PAGE: www.tetraheed.com/t5-irritating-vaccine-adjuvants

TOPIC 5. WHAT ARE "VACCINE ADJUVANTS"? HOW DO THEY WORK? ARE THEY INTENTIONALLY INFLAMMATORY, AND IRRITATING? ON BALANCE, ARE THEY GOOD, OR BAD? HOW AND WHY DO "MALT-TARGETING" VACCINES AVOID ANY NEED FOR ADJUVANTS? IS IT REALISTIC TO HOPE FOR "POST-ADJUVANT VACCINES"?

As a general definition, "adjuvants" are things that are added to vaccine formulations, to make them more effective and potent; and, to clarify that definition, they generally must rise above the level of being mere "excipients" (i.e., the types of pharmaceutical additives, such as diluting, liquefying, anti-caking, preservative, or other agents that help make the active ingredients of a drug function better), and must rise to a level that experts refer to as "immuno-stimulatory". And, anyone interested in this topic also should realize that there are a number of "gray areas" where it isn't clear whether some particular compound should be regarded as a mere excipient, or as an actual adjuvant. As an example, if a topical mucosal vaccine contains an agent called a "muco-adherent", which will cause vaccine particles in that formulation to cling more tightly (and/or for a longer period of time) to a mucous membrane, that agent might be called

either an adjuvant, or an excipient; and, various phrases like “adjuvant-like activity” also are used, to deal with those types of “grey areas”.

However, rather than becoming entangled in or distracted by those “grey areas”, a clear focus needs to be placed on a very unpleasant truth, which lurks behind the “immuno-stimulatory” label that is commonly used to describe and defend adjuvants.

That highly unpleasant fact is this: essentially all adjuvants that are added to injected vaccines are, in truth, **deliberately and intentionally irritating, inflammatory, distress-causing additives**. Stated in other words, they are harsh, unpleasant, and “necessarily nasty” chemicals. Why? Because of a reason that no one has been able to overcome, or avoid, or eliminate, before now. Their goal, their role, their job, and their assigned task, is to rapidly and seriously irritate the muscle cells, at the site of an injection. Why? So that those distressed muscle cells will rapidly begin sending out “distress signals” (in the form of messenger molecules called “cytokines”), which will attract and recruit mobile immune cells to hustle over, as quickly as possible, to the site of the injection, so that the immune cells will be able to begin processing the vaccine particles, as quickly as possible, before those particles (and the antigen sequences they are carrying) can be seriously diluted, diffused, or degraded, by the normal cell and tissue repair processes at the site where the vaccine was injected.

A huge portion of the opposition to vaccines, among people who are often labelled “anti-vaxxers”, actually arises from the unpleasantness of the adjuvants which must be added to injected vaccines, in order to protect as many people as possible, whenever a large population is inoculated.

However, because of how “MALT-targeting” vaccines are designed, and because of how they function, we believe that they can and will completely eliminate any need to add any harsh and unpleasant “adjuvants” to them, to make them effective; and, if that goal can be accomplished, it will be so important, and so widely welcomed and appreciated, that these new classes of vaccines may well be called “Post-Adjuvant Vaccines.”

So, in answer to the question posed above – “On balance, are they good, or bad?” – the best answer would seem to be, "Well, they were good, when (and because) they were necessary, to help protect more animals and more people, against diseases. However, if and when they become no longer necessary, because of some scientific and medical discovery or advance that offers a *BETTER* way to reach that good result, then they will move into the 'Bad, on balance' category."

NEXT PAGE: www.tetraheed.com/t6-mucosal-vaccine-failures

TOPIC 6. (A) THERE ARE MORE THAN TWICE AS MANY SECRETED MUCOSAL ANTIBODIES, IN JUST A FEW POUNDS OF MUCOSAL FLUIDS IN A HEALTHY ADULT, THAN ALL OF THE INTERNAL ANTIBODIES IN THE ENTIRE REMAINING WEIGHT AND BULK OF THAT PERSON. THAT IS A POWERFUL INDICATOR THAT MUCOSAL ANTIBODIES ARE VERY, *VERY* IMPORTANT, IN FIGHTING MUCOSAL PATHOGENS.
(B) HOWEVER, BEFORE NOW, THERE WERE NO RELIABLE, NON-TOXIC WAYS TO USE VACCINES TO GET THE MUCOSAL PORTION OF AN IMMUNE SYSTEM, TO CREATE SECRETED MUCOSAL ANTIBODIES.
(C) MALT-TARGETING VACCINES APPEAR TO BE FULLY CAPABLE OF

CHANGING THAT, IN WAYS THAT WILL CREATE NOT JUST ONE, OR TWO, BUT *THREE MAJOR ADVANCES* IN VACCINES

The previous pages in this website describe a number of specific points which now need to be assembled into a larger, cohesive structure or system:

(1) Nearly all pathogens have evolved in ways that enable them to attack and invade mucosal membranes, and they are (by far) the most important and numerous class of pathogens on this planet;

(2) The internal immune system simply is not designed, equipped, or suited, to deal with pathogens that can reproduce – usually, very rapidly – entirely within a single cell, in the outermost surface layer of a mucous membrane;

(3) Therefore, to help fight off such pathogens, vertebrate animals evolved with "mucosal immune systems" that function independently of the internal defenses, and use secreted mucosal IgA antibody dimers which are very different from internal IgG antibodies, in both shape and function;

(4) There are more than twice as many mucosal antibodies, in just the few pounds of secreted mucosal fluids in a healthy adult, than all of the internal antibodies in the entire remaining weight and bulk of that person; and,

(5) The huge number of secreted mucosal antibodies is powerful and even compelling evidence that mucosal antibodies are hugely important, and valuable, in fighting off mucosal pathogens.

All five of those points directly support an entirely logical conclusion: if any types of vaccines could trigger the creation of good and effective ***secreted mucosal antibodies***, those vaccines (and the antibodies they would help create) could be very useful, and helpful, against numerous different types of pathogens (especially pathogens that attack the "upper respiratory tract", such as influenza, COVID, and "common cold" viruses).

However, the sad and troubling fact is that, under the "prior art" (i.e., before the scientific community becomes aware of "MALT-targeting" vaccines), there are almost no "really good" mucosal vaccines; and, there is almost no research currently being done on mucosal vaccines, because the only types of "adjuvants" which can make them work potently, in test animals, are too dangerous, and too toxic, to allow the results of any such research to be transferred over, to possible use in humans.

Here are four facts that can help illustrate, and explain, the limited and inadequate status of mucosal vaccines, prior to any announcements about MALT-targeting vaccines.

1. Even though there are hundreds (or thousands, depending on how different strains and variants are classified) of different mucosal pathogens, a recent review article dedicated entirely to mucosal vaccines (Rhee et al, "Mucosal vaccine adjuvants update," Clin Exp Vaccine Res 1: 50-63 (2012)) listed all of the commercially available human mucosal vaccines that the authors could find information about. However, that listing took less than a single paragraph, and it didn't even merit a table, to list them.

2. The so-called "FluMist" vaccine, which is administered via a nasal spray, usually reaches only about 30 to 40% efficacy, each year. Those numbers mean that 60 to 70% of the people who get that vaccine, still become infected by serious cases of influenza, each year, despite being vaccinated by it.

3. The most recent attempt to create a new nasally-delivered vaccine occurred in Switzerland, where a mucosal influenza vaccine was put into human use. However, even though

it had performed well in animal tests, it ended up causing some of the human recipients to suffer a neurologic affliction called "Bell's palsy," which creates a partial paralysis of the facial muscles, usually on either the left or the right side of the face. As such, it can lead to strange, unsettling, sometimes bizarre, and in some cases grotesque and genuinely frightening facial expressions, among sufferers. That vaccine had to be withdrawn from the market when those cases began to appear, and the manufacturer was driven out of business, by the costs of the liabilities it was facing. That event is well and widely known, among vaccine experts, and it powerfully discourages any companies or researchers that might otherwise be tempted to launch an expensive research project into any other potential mucosal vaccine, no matter how promising it might be.

4. It appears that the only mucosal vaccine which has actually been commercialized for human use, since the year 2000, is an orally-ingested vaccine against "rotaviruses" (which create severe digestive problems among infants, in tropical regions). However, the first attempt to release that vaccine led to major problems, and that first version had to be withdrawn, after it began causing a severe and potentially fatal intestinal problem called "intussusception" – which must be corrected surgically, as quickly as possible – in some of the infants who received that vaccine. The sponsoring company re-designed and re-tooled that vaccine, by reducing the number of rotavirus antigens the virus particles carried (which presumably made it somewhat less effective). It is now being sold under the trademark ROTA-TEQ (TM).

More information to support and explain the facts listed above (and which further discusses the severe inadequacies of currently-available mucosal vaccines) is contained in the "Background Section" of a pending (but not yet published) patent application, which can be downloaded via a button at the bottom of the Home page.

Accordingly, mucosal vaccines simply are not being actively and successfully developed, for human use; and, vaccines which are injected almost never lead to mucosal antibody formation. That has created a huge and unmet need, for better methods for creating, not just vaccines that can be APPLIED topically to mucous membranes, but which will actively trigger the formation of secreted mucosal antibodies, when applied in that manner.

However, based on everything we have seen so far, in the tests we have done to date, and based on what we know about how M cells, dendritic cells, and MALT patches function, we believe and assert that mucosal vaccines with large numbers of a selected antigen, and small numbers of potent MALT-targeting sequences, can trigger and drive the formation of ***BOTH***: (i) ***internal*** IgG antibodies, ***AND***, (ii) ***secreted mucosal*** IgA dimers, which will bind – tightly, and selectively – to pathogens which have those antigen sequences on their surfaces.

THAT claim (i.e., as stated directly above) is being made with a fairly high level of confidence, based on "antibody production tests" that have been completed to date. In absolutely every animal tested to date – in which their saliva and blood samples were tested for both internal IgG antibodies in blood, and secreted IgA antibodies in saliva, using both ELISA tests, and SDS/PAGE-Western blot tests – a single nasal infusion of droplets containing (filamentous *Inovirus*) phage particles carrying both a MALT-targeting transport sequence (in low numbers), and a well-known and easily tested antigen sequence (the HA-tag epitope sequence, in large numbers), triggered the "robust" formation of both secreted IgA dimers in saliva, and internal IgG antibodies in blood serum.

As this is being written, we do not yet know how they will perform, in "pathogen challenge tests". The first such tests (now underway) will use engineered T7 phages, carrying about 400 copies/particle of the FI-6 antigen from influenza (described in Corti et al 2011; also

see the NIH Epitope Database at www.iedb.org/epitope/162644) and carrying about 40 copies of the MALT-targeting sequences we selected for the first "antibody production" tests. They will be performed in mice, using "mouse-adapted" influenza viruses (which were "passaged" 9 times through mice, to select viruses which can potently infect mice). The results should be available by mid-March 2026.

Even though we do not yet know the results of the pathogen challenge tests, we feel fully justified in stating that every immunologist, every vaccine research team, every vaccine company, the faculty and students at every veterinary school, and every federal or state agency that has any active interest in vaccines, should at least become aware of the MALT-targeting approach to designing vaccines, and should begin learning, now, about the science, the logic, the reasoning, and the immune cell activities that have supported and guided this work so far. Regardless of whether the results from the first sets of pathogen challenge tests show positive results, or whether they point out how many problems still need to be solved before MALT-targeting vaccines can become practical and approved for use in animals such as poultry, pets, or livestock, the simple fact is that if more people, and more companies, become interested and involved in this research, sooner rather than later, the benefits and cost savings will begin appearing sooner, rather than later.

So, we do not make the following three predictions as confident claims, but as reasons why people who are already working with vaccines should become interested in, and aware of, the "MALT-targeting transport peptide" options that can be used to improve mucosal vaccines. Based on everything we have seen, learned, and shown to date, we hope and believe that the first two predictions below will be proven in animal usage, and will become reliably "do-able", within the next five years:

1. Mucosal vaccines carrying MALT-targeting sequences will be shown to be fully capable of triggering and driving the formation of, not just internal IgG antibodies, but also, "secreted mucosal IgA antibody dimers"; and, since they can provide a balanced, bi-functional, "two-handed" immune response – both internally, and in mucosal secretions – these vaccines will be able to provide better protection than any other vaccines that are available as this is being written . . . especially against upper respiratory tract infections.

2. Mucosal vaccines carrying MALT-targeting sequences will be shown to be capable of eliminating any need for using the types of inflammatory and irritating "adjuvants" which, today, are used to make injected vaccines more effective. As mentioned on a prior page, most "adjuvants" work by causing muscle cells, at the site of an injection, to rapidly begin sending out distress signals (cytokines), which will recruit nearby mobile immune cells to come to the site of the injection as quickly as possible, before the vaccine particles can be diluted, diffused, or degraded. Accordingly, we believe MALT-targeting mucosal vaccines will end up creating a new generation, and a new era, of vaccines and vaccine technology, which might be called "Post-Adjuvant Vaccines", and/or "Precision-Guided Vaccines".

3. In addition to the two predictions above, which can be established by vaccines in non-human animals, we also predict that, when MALT-targeting mucosal vaccines become available for human use, they will be able to eliminate any need for injections, needles, and the types of hazardous and dangerous medical wastes that are created when needles are used. Instead, "preferred" modes of inoculation are likely to become social events, in which bowls of lollipops (with vaccine particles embedded in a hard-candy matrix) are passed around, by nurses or even just administrators, at places like senior centers, community centers, schools, churches, walk-in clinics, drugstores, offices and other workplaces, etc.; and, we also predict that usage and

inoculation rates will increase, by substantial and possibly large ("very large"?) percentages, when compared to COVID vaccination levels in the early 2020s.

Among other advantages, candy-flavored lollipops, on "sticks", can be sucked until halfway dissolved, and then placed in some type of small, simple, convenient storage device for several hours, to provide enough time for newly-activated dendritic cells get "saddled up" and leave their M cell docking sites, and to then allow a "new crop" of immature dendritic cells to locate and settle into the M-cell docking sites that were vacated when the "first wave" of dendritic cells became activated, and left those docking sites. In other words, a simple "lollipop or lozenge" delivery system can provide a "sustained release" mode of administration, which can be substantially more effective than a "one-time, single-shot" dosage.

NEXT PAGE: www.tetraheed.com/t7-phages-phage-libraries

TOPIC 7. WHAT ARE PHAGES, AND "PHAGE LIBRARIES"? AND, HOW DID WE "SCREEN" LITERALLY A BILLION DIFFERENT PHAGES, TO IDENTIFY AND ISOLATE LESS THAN 100 WHICH WERE CARRYING POTENT MALT-TARGETING SEQUENCES?

This topic is included, to reassure any vaccine companies or researchers who might be interested in MALT-targeting mucosal vaccines that we did indeed do the work we claim to have done, and that this isn't some effort to defraud or swindle anyone. It requires, first, a brief summary of phages, and what they are (and, that requires a bit of history). That is followed by a brief summary of what "phage display libraries" are, and of how "screening tests" can be thought-up, and then used, to identify which particular phage particles, out of millions of "candidate" or "contestant" particles, happen to be carrying a foreign insert peptide which will cause those particular particles to be treated and processed in some particular way, by some particular type of cell, or tissue type, or animal.

Then, we will describe the specific type of screening test we created, and used, to identify those particular phages, from a phage library, which happened to be carrying foreign insert peptides which triggered and drove both M cells, and "immature dendritic cells", in MALT patches in the nasal airways of mice, to "determine" that those particular phages were dangerous and important pathogens . . . which, therefore, needed to be pulled in and processed, as quickly as possible, so that an antibody-forming response – which would help fight off those "apparently dangerous and important pathogens" – could be commenced, as quickly as possible.

WHAT ARE "BACTERIOPHAGES" (WHICH ARE NOW CALLED JUST "PHAGES")?

As a very brief introduction to "phages":

1. People had been experimenting for hundreds of years with various types of lenses, including "magnifying lenses", when the Dutch fabric merchant Van Leeuwenhoek became interested in trying to make them better, in the 1670s, so that he could more closely examine the thinnest, tiniest threads in the fabrics he handled. Once he got started, he kept refining and improving his magnifying lenses, until he could clearly see (in samples of water, rather than fabrics) microbes that actively moved, which people initially called "animalcules".

2. Within a few decades, after seeing and categorizing numerous types of bacteria and other microbial cells, scientists realized that there was an entire category of microbes that were

infective, somehow, but which were too small to be seen by even the best light microscopes of that era. Those came to be called "viruses", after the Greek root word for "virulent". Until the 1930s, when electron microscopes were invented and scientists could actually "see" and begin to seriously study viruses, no one knew what viruses were, or how they could reproduce.

3. In the 1890s, scientists realized that there was some type of "virus", in some of the rivers in India, which could kill and inactivate the bacteria which caused cholera; and not long afterward, a different scientist discovered a similar "virus" that could kill and inactivate the bacteria which caused dysentery. When World War I began, the French armies were the first to develop liquid drinks carrying those viruses, which they fed to their troops, to "immunize" those troops against cholera and dysentery.

4. As scientists began looking for and finding other viruses that could attack and destroy other types of bacteria that caused other diseases, they realized that each such virus could attack only a very specific and limited class of bacteria. So, they named that entire category of viruses "bacteriophages", from the Greek root "phage", which translates into "eating", and which implies an aggressive form of eating (rather than just "nibbling"), comparable to the English work "devour". Later, the name "bacteriophages" was shortened to just "phages".

5. Accordingly, the noun "phage" has come to refer to any virus which: (i) can infect only some limited group, type, or class of bacterial cells; and, (ii) is classified as "non-pathogenic", and not dangerous, since phages cannot infect plants or animals, in any way. The search for new and additional types of phages became very active, and motivated, because "phage therapy" grew into a major and crucial branch of medicine, before the advent of sulfa drugs and then penicillin. If someone was infected by some particular type of bacterial pathogen, the scientists and physicians of that era could usually figure out what type of bacteria it was, and they would administer, directly to the infected site, a batch of phages which could kill that type of bacteria. That approach has recently come back into favor, to help fight certain types of antibiotic-resistant microbes.

PHAGE LIBRARIES (aka PHAGE DISPLAY LIBRARIES)

In the 1970s and 1980s, a group of scientists (led by Prof. George Smith, at the University of Missouri, who later won a Nobel Prize for that work) began developing new and clever ways to work with a specific class of phages called "Inoviridae" (aka Inoviruses), which are "filamentous" phages" that have extremely small genomes, and which infect E. coli cells. They created new mixtures called "phage display libraries" (now also called "phage libraries"). Summarized briefly, all of the billion or more phage particles, in a "phage display library", are exactly the same, *EXCEPT FOR* a short "foreign insert" DNA sequence, at a known and specific location, in a specific gene of that phage. Today, those short inserts are created by "almost entirely random" chemical synthesis (i.e., the chemical methods are entirely random, except for some non-random steps used to avoid unwanted "stop codons"). The foreign DNA inserts are inserted into a precise location in a gene called the "coat protein 3" (cp3) gene, so that the foreign peptide sequence will appear at the outer tips of all five copies of the long tentacle-like cp3 proteins, which Inovirus phages use to latch onto (and infect) new E. coli cells.

The work required to create really good phage libraries took decades; but, now that that work has been completed, anyone can buy a really good phage library, with about a trillion different "candidate" particles, all in a single small tube, for less than $800 (e.g., www.neb.com,

catalog number E8210S, which is a “kit” that also includes monoclonal antibodies and magnetic beads, all for $719 as this is being written).

The “trick” to using any phage display library comes in thinking up some new and useful type of “screening test”, which will somehow identify which particular phages, out of a few million candidates in a small “aliquot” of a liquid suspension of phage particles (in this context, “aliquot” refers to a small quantity of liquid having a known and specific volume, which will contain some known number or portion of the molecules or particles from a larger batch of that liquid suspension), will be taken in and/or processed, in some particular way that is of interest, when all of the particles in that aliquot are treated in a certain way. Almost all “screening tests” will create some type of “fair competition” between the particles, such as by contacting all of the candidate/contestant particles with a specific type of cell or tissue, and seeing which particles are pulled inside those cells (or, as alternate examples, by passing an aliquot of particles through an “affinity column” or other device; or, by infusing or injecting them into a lab animal, and then looking to see which ones reach some particular targeted cell or tissue type).

The basic rule of “screening tests” is that no one can predict, in advance, which particular particles will be able to do “the XYZ trick”. So, a scientist who hopes to isolate those particles which can, indeed, perform “the XYZ trick”, will need to figure out two things: (i) how to pit millions of phages against each other, as “candidates” or “contestants” in a fair competition; and, (ii) what type of isolation or purification process the scientist can then use, to identify (and, usually, to isolate, preferably in a still viable and reproductive form) those specific phages which happened to be carrying a foreign insert which enabled them to become “the winners” in that competition.

THE SCREENING TESTS FOR “MALT-TARGETING” PHAGES

This summary lists several of the major steps that were used, to screen a phage library in a way that isolated only those phage particles which happened to be carrying forteign insert peptides which were able to trigger and drive each and all of the following steps, by M cells and then dendritic cells, in MALT patches:

(i) intake (inside a phagosome) of a phage particle carrying a “winning” foreign insert peptide, into an M cell on the surface of a MALT patch;

(ii) rapid trans-cytosis of the phagosome, through the M cell;

(iii) ejection of the particle, in exposed and naked form again, into a docking site holding an immature dendritic cell, on the “basal” side of the M cell; and,

(iv) analysis of the particle, by the immature dendritic cell, leading to a “determination” that the foreign peptide insert sequence, on that particular particle, showed that that particle was a truly dangerous and important pathogen, to a level and extent which triggered an irreversible commitment, by the dendritic cell, to “activation” (aka “maturation”), which would turn that dendritic cell into an “antigen-presenting cell” which would leave that docking site, and begin searching for a “germinal center” of a lymph node, so that it could present a set of semi-digested “chunks” of surface proteins, from that particle, to the B and T cells in that lymph node.

With the goal of creating and using a new type of screening test to isolate and identify those phages which were carrying foreign insert peptides which could potently drive all four of those steps all the way to completion, here are the steps we used:

STEP 1: We purchased a high-quality “phage display library” from New England Biolabs, having roughly a trillion total phages from the filamentous Inovirus class, with randomly-

generated foreign inserts (12 amino acids long) at the outer tips of their long tentacle-like CP3 proteins;

STEP 2: A small “aliquot” of liquid, carrying about 20 million phages, was “infused” into the nostrils of a sedated mouse (using 50 mice, in that stage, for a total of a billion candidate particles), via a micro-pipette. That allowed the particles, in a liquid suspension, to enter the nasal passages, and contact the MALT patches (which are in a well-known location, in those nasal airways).

STEP 3: After giving the mucosal cells enough time to take in and process any particles they chose to take in, but not enough time for the dendritic cells to begin breaking apart and digesting the phage particles, the mouse was painlessly euthanized, and ice-cold saline was infused into its vasculature, to slow down any digestion of the phages by the cells, but without killing the cells. A “transverse skull section” was created, which exposed the nasal airways in the location where the MALT patches are known to be, in mice. Surface cells from those MALT surface areas were harvested, using very gentle pressure and a very thin brush tool.

STEP 4: The harvested cells from the nasal lining were treated by using a “first screening method”, which selected for any and all dendritic cells, regardless of whether they contained any phages. That first screening method selected for cells which had a specific known receptor protein on their surfaces (dendritic cells have that receptor, on their surfaces). The cellular selection and purification process involved using tiny magnetic beads, with molecules which bind to the dendritic cell receptors, coupled to the magnetic beads. Cells which became coupled to the magnetic beads (because their surface receptors became bound to the molecules that were coupled to the magnetic beads) were purified, by using a small but strong (neodymium) magnet to pull the beads into a cluster, located halfway up a vertical column of liquid and pressing against the inside wall of the tube holding the liquid. All of the liquid below that clump (and any unwanted cell debris and other particles) were suctioned out of the tube, and the magnet was then pulled away, to release the beads, which were then resuspended in a fresh batch of liquid cell medium. That “washing” process was repeated three more times, to obtain purified dendritic cells. Their membranes were then broken apart, using a special detergent which will attack cell membranes, but not proteins, which cover and enclose the phages. That released any phages which had been pulled inside of any dendritic cells, or which were clinging to the surfaces of the dendritic cells. The selected phages were then “plated” at low density, on a “lawn” of fresh host cells, on top of a semi-solid gel nutrient called agar, in a shallow dish. That allowed “clonal colonies” of the “First Round Winner Phages” to be isolated, and reproduced in fresh batches of host bacteria.

STEP 5. The work described above ended up isolating 145 different “First Round Winner” phages. However, while that work was being done, the scientist who created that screening process continued to study and learn more about what actually happens to dendritic cells, and how they change, when they shift from “immature” to “antigen-presenting” status; and, as a result of that work, he realized there was a way to design and run a better screening test, which would not select any and all dendritic cells, but which, instead, could select only those particular dendritic cells which had already irreversibly committed to the transition, from “immature” to “antigen-presenting”.

We are not going to disclose, in this website, the specific steps and methods the inventor used, to isolate and select those particular cells which had taken in the phages we wanted to isolate and identify, since those steps sit at the heart of an invention which has been described and claimed in a patent application which will be published before long. The details will be in

there, and once that application has been published, this website will be updated, to provide a complete downloadable copy, and a "summary guide" to help non-experts understand it.

However, in a spirit of playfulness, and in the hope of triggering some curiosity, and original thought, here is a hint, which should be regarded as a riddle, a tease, and a challenge. The method the inventor created, and used, involved two crucial numbers: 7, and 19.

We hope anyone who already knows what those two numbers refer to, will be thinking, "Well, if they knew about THOSE two things, they might have actually done what they say they did."

And, anyone who does not know what those two numbers refer to, is invited and encouraged to send copies of this text, to any experts who might be able to provide that information. And, if any experts are able to answer THAT question, they should be asked how THEY would try to design a screening test, to identify phages which can drive dendritic cells all the way through an irrevocable commitment to activate and mature into an "antigen-presenting" mode.

STEP 6: Rather than abandoning the results of the "First Round" screening test, and moving those 145 phages into deep storage in a freezer, the scientist who designed the tests realized that, if he handled them in a certain way, he could use those 145 "First Round Winner" phages to create a "potency ranking" which would indicate the best and most potent performers, from among those 145 phages. Therefore, he created two large mixed batches of phages, with one batch containing 72 of the 145 "First Round Winner" phages, and the other batch contining the other 73 "First Round Winner" phages, all in roughly equal numbers. The concentration of particles in each of the 145 starting batches were measured, and adjusted, to provide roughly equal numbers of each competing phage, by using a spectro-photometer to measure "light absorption" by each starting batch, at 280 nanometer wavelengths (a standard wavelength used to measure "total protein content" in liquid suspensions).

STEP 7: Using the same methods described above, aliquots of about 20 million phages, from either of the two mixed batches, were infused into the nostrils of sedated mice; after a controlled delay, the mice were sacrificed, and ice-cold water was infused into their vasculature; skull sections were created; surface cells were harvested from the nasal airways where MALT patches are located; and, the harvested cells were processed, using the "7 and 19" method.

The resulting "activated, maturing, antigen-presenting" dendritic cells were isolated, their cell membranes were dissolved, and the phages released from those cells were plated, at low density, to create clonal colonies. The phage DNA was processed, using "Polymerase Chain Reaction" (PCR) to create large numbers of copies of only the foreign DNA inserts, and those DNA preparations were sent to an outside lab, for sequencing.

The sequence listings were then sorted, using a computer, to determine which sequences appeared most frequently, among the "Second Round Winners".

To give the initial tests the best possible chance of success, we selected not just one, but the three "top performers" (or, more precisely, the top performers which did not contain any cysteine residues, to avoid possible complications involving "disulfide bonds" created by cysteine residues, which can seriously disrupt the three-dimensional shape of a protein). Since there is enough room, in the cp3 proteins of Inovirus phages, to add foreign inserts up to roughly 100 amino acids long, and since the total amino acid number in all three MALT-targeting sequences was less than 50, all three were placed together, in tandem, in a "triple" MALT-targeting sequence (with at least two glycine residues between each sequence, to create a "linker"

which would allow more flexibility and accessibility). Those phages became our "first testable constructs", carrying both MALT-targeting sequences and a "testable antigen", as described on the next page.

NEXT PAGE: www.tetraheed.com/t8-antibody-production-tests

TOPIC 8. THE FIRST ROUND OF TESTS WERE LIMITED TO "ANTIBODY PRODUCTION" TESTS, FOR BOTH: (i) IgG ANTIBODIES, IN BLOOD SERUM, AND (ii) SECRETED IgA ANTIBODY DIMERS, IN SALIVA. THE RESULTS WERE SO GOOD THAT WE HAVE MOVED ON TO "PATHOGEN CHALLENGE" TESTS, USING A DIFFERENT (i.e., BETTER AND MORE RELIABLE) PHAGE CONSTRUCT.

As mentioned on the prior page, the three "top performing" MALT-targeting sequences, from the Round 2 screening tests (or, more precisely, the top three performers which did not contain cysteine residues, to avoid unwanted complications) were selected for inclusion in a "tandem-triple" MALT-targeting sequence.

Since we were already accustomed to working with Inovirus phages, and since they are small and easy to engineer, we hired a contract company to assemble a set of "first testable phage constructs" suited for use in "antibody production tests", containing both:

(i) the "three-in-tandem" MALT targeting sequence, at the outer tips of all 5 copies of the long tentacle-like cp3 proteins on each particle; and,

(ii) a well-known antigen sequence (the "HA-tag epitope", which first appeared in a troublesome influenza strain about 60 years ago). It is widely used for testing, largely because monoclonal antibodies that will bind to it are readily available, at reasonably low "mass-manufactured" costs.

The HA-tag antigen sequence was placed in some, but not all, of the small brick-like cp8 proteins that are packed together to assemble the cylindrical capsid which encloses the phage DNA, in Inovirus phages. It was discovered, in the 1970s, that if a peptide sequence longer than about 6 amino acids was added to all of the cp8 proteins, the resulting viruses would have severe difficulty in assembling (or "packaging") themselves. Therefore, scientists began inserting a second engineered cp8 gene into the phage genome, controlled by a relatively weak or inducible gene promoter, so that a longer foreign peptide can be inserted into several hundred copies of the cp8 proteins, randomly distributed among nearly 3000 copies of the unmodified ("wild-type") protein.

Those constructs were tested in both mice, and pigs, and in both types of animals, testing via both ELISA, and SDS-PAGE/Western, clearly showed that a single nasal infusion of those phage particles, with no adjuvants, and no booster dosages, triggered "robust" formation of both secreted IgA dimers, in saliva, and internal IgG antibodies, in blood serum.

During the lead-up to those tests, several challenges were encountered with the Inovirus phage constructs, including a severely time-wasting episode of instability. When we looked into that problem, we learned that researchers have known, for decades, that the classic "fd-tet" construct – which contains not just a simple tetracycline resistance gene, but an entire "tetracycline resistance complex" which is self-regulating, and which is not expressed unless tetracycline is present – is inherently unstable, for not just one but two reasons:

(i) the tetracycline resistance complex was inserted into the "long inter-gene region" of the starting-point fd phages; and, Inovirus phages have a natural ability to spontaneously delete any

foreign DNA which has been inserted into that region; and, any phages which happen to delete any foreign DNA from that region can reproduce more rapidly than phages carrying that "unwanted baggage", and will soon overrun any subsequent batches of phages grown from those earlier batches; and,

(ii) the "tetracycline resistance complex" came from a transposon, and transposons (which often are called "jumping genes") are known to spontaneously jump from one genome, to another.

Therefore, when the first constructs designed to carry a currently-active and important antigen sequence were being planned, the initial plan was to shift over to a different Inovirus construct, using two important modifications developed by the Jonathan Gershoni group, as described in Enshell-Seijffers et al, "The rational design of a 'type 88' genetically stable peptide display vector ..." Nucleic Acids Res. 29(10): E50 (2001).

However, questions and concerns began to arise over how quickly, reliably, and consistently a "filamentous" phage can be taken in, by an immune cell. If scaled up to a 1/8-inch thickness (i.e., comparable to a strand of cooked spaghetti), an Inovirus phage would be nearly 2 feet long; and, while any human can happily imagine slurping in a strand of cooked spaghetti that long, if it is coated in butter and a tasty sauce, our ability to accomplish that feat depends heavily on having a tongue, teeth, and a pre-existing, long, generally tubular, and happily-receptive digestive system. However, immune cells have none of those things; instead, they need to form a special pouch, which will become a phagosomal bubble, from the same membrane material which makes up their outer membrane, any time they take in a particle. Therefore, simple logic suggests that a flexible filamentous phage will end up being "wadded up", in some haphazard rather than controlled way, as it gets stuffed into a phagosome that is trying to remain spherical.

To avoid that problem, it was decided to shift over to using a "lytic" phage (i.e., a phage with a roughly spherical "head" component, which can be grabbed and pulled in quickly and conveniently, by an immune cell, in a manner comparable to a hand grabbing a nugget, or a gem), as the starting point, when plans began to firm up for creating the first phage constructs that would be suited for the first "pathogen challenge tests".

That work is described on the next page.

NEXT PAGE: www.tetraheed.com/t9-pathogen-challenge-tests

TOPIC 9. THE FIRST "PATHOGEN CHALLENGE TESTS" WILL USE T7 PHAGES, CARRYING THE FI-6 ANTIGEN FROM INFLUENZA VIRUSES. HOPEFULLY, THEY WILL BE COMPLETED BY MID-MARCH 2026.

As described on the previous page, when it came time to move beyond "antibody production" tests and into "pathogen challenge" tests, we made two important changes, to create an entirely new type of engineered phage particle:

(i) we shifted out of using "filamentous" phages, which get "wadded up" in uncontrollable and unpredictable ways, when they get stuffed into a generally spherical phagosomal bubble, and we chose a type of "lytic" phage (the T7 class of phages), with a generally round "head" component, since that type of component can be easily and rapidly grabbed, and pulled in, by immune cells; and,

(ii) we stopped using an "easily tested" antigen (the HA-tag epitope, isolated from an influenza strain that was important 60 years ago), and obtained guidance from a specialist in influenza research, who recommended an influenza antigen called FI-6 (that is, capital F, and capital I, sometimes mistakenly called the F-16 antigen, and sometimes mistakenly interpreted as the F-lower-case-L-6 antigen). Its sequence, in single-letter code, is KESTQKAIDGVTNKVNS, and more information on it can be found in the NIH's epitope database, at www.iedb.org/epitope/162644. That antigen sequence was selected, because it is present on the surfaces of a *VERY* wide variety of different strains of influenza, as described in Corti et al, *Science* 333: 850-856 (2011).

That specialist steered us toward influenza, mainly because it can infect such a wide range of different animal types (including some types of mice, which can greatly reduce the costs of the initial tests), and because numerous labs already work with it. However, the choice of influenza, for the first pathogen challenge tests, will bring into the process a number of complicating factors, which require attention.

One complication arises from the mechanism that influenza viruses use, to infect cells. Most mucosal pathogens can penetrate into cells by binding to only a single specific type of protein, on the surfaces of the cells it targets. As three examples, HIV viruses (which cause AIDS) can only bind to CD4 receptors, which are present only on certain types of immune cells; the spike proteins on COVID viruses bind to a surface protein called "angiotensin-converting enzyme-2" (ACE2); and most "rhinoviruses" (which cause "common colds"; "rhino" is the Greek root for "nose") bind to a cell protein called "intercellular adhesion molecule-1" (ICAM-1).

However, influenza viruses use a very different mechanism. Each influenza particle carries several hundred copies of a protein called "hemagglutinin" (HA), and those proteins can grab hold of any "glycosylated protein" (i.e., any protein which has sugar groups attached to its strand of amino acids; roughly half of all animal proteins are in that category) which has a "sialic acid" group (i.e., a specific type of sugar molecule) at the tip of one of the sugar chains that are attached to the protein. Sialic acid groups are commonly used, to terminate the glycosylation process; therefore, they are present on lots of different proteins. That is why influenza viruses can infect so many different body parts, in a specific animal, and why it can infect so many different types of animals.

And, to make influenza viruses even more difficult and challenging, they are among the fastest-mutating viruses ever discovered.

And, to make the initial "MALT-targeting pathogen challenge tests" even more of a challenge, the engineered phage particles will be carrying only a single specific influenza antigen (i.e., the FI-6 antigen, as mentioned above). In direct contrast, the flu vaccines that are issued every year (actually, twice a year, for the northern and southern hemispheres), all contain a mixture of multiple different particles carrying different antigens, created by mixing together an assortment of different vaccine particles, created by separate manufacturing batches.

All those factors make it extremely difficult to create truly effective vaccines against influenza; so, it may turn out to be a mistake, to choose influenza as the pathogen that will be used in the very first round of pathogen challenge tests, to evaluate the efficacy of MALT-targeting vaccines. So, if it turns out to be a bad choice, subsequent tests will use a different antigen, from a different pathogen which infects animals via a more conventional specific-receptor pathway. However, if the decision to choose influenza for the first pathogen challenge

tests turns out to be a good decision, it will open more doors, and lay a better foundation for future work, more quickly, and more convincingly. So, time will tell.

To provide a bit more information to help readers understand T7 phages, they have a surface protein which appears in two different forms, which are called the 10A form (which appears in about 400 copies/particle), and the 10B form (which appears in about 40 copies/particle). The somewhat longer 10B form is created when the "translation" process (which occurs when a ribosome "reads" the codons on a strand of mRNA, and uses those codons as "instructions" to add another amino acid, as specified by each successive codon) "crashes through" a first stop codon, and continues translating a longer protein (the 10B version), until it reaches a second stop codon. Therefore, the FI-6 antigen sequence was positioned in the 10A segment (to provide about 400 copies of that antigen, per particle), and the MALT-targeting sequence was positioned in the 10B segment (to provide only about 40 copies/particle).

Additional details concerning the "genetic cassette" design of those T7 phage constructs will be disclosed in a patent application, rather than in this website. For now, it should be noted that the phrase, "genetic cassette," is used to indicate that a certain type of plasmid or phage construct has been designed and assembled in a certain way, to make it simple and easy to delete a specific DNA sequence, and replace that deleted sequence with a new and different sequence. Therefore, the "cassettes" that are being created, to create MALT-targeting vaccine particles, have been specifically designed to make it easy to "swap out" the antigen sequence, or the MALT-targeting sequence, and to either swap out, or delete, the selectable marker gene.

As a result, by using those cassettes as a starting reagent, we can provide custom-assembled MALT-targeting T7 phages, carrying ***any*** antigen sequence *that a qualified requesting company or research group specifies*, at a low cost, ***provided that*** the requesting company or group makes a firm commitment to actually test those particles, in pathogen challenge tests, in at least one type of animal. More information on that offer is available, ***HERE*** [*link to next page*]

NEXT PAGE: www.tetraheed.com/t10-next-steps-goals

TOPIC 10. WHAT ARE OUR NEXT STEPS, AND GOALS? WHAT ARE WE HOPING FOR, FROM VACCINE COMPANIES AND/OR RESEARCHERS THAT HEAR ABOUT OUR WORK?

Rather than "playing our cards close to our chest", we would prefer that every vaccine company, research group, and government agency know exactly what we are doing, why we are doing it that way, what we hope to do next, and what they can expect from us, during 2026.

That approach makes the most sense to us, in light of the following factors:

- We do NOT have any facilities, or expertise – or any desire, at any level – to begin testing actual pathogens, in any types of animals. Instead, all of the work we performed directly was in a "startup incubator lab," which only allowed mice and rats to be tested, and which did not allow any actual pathogens to be tested, used, or brought into the labs; and, we aren't even renting that lab space any more, since the remaining work needs to be done in different types of facilities.
- We have absolutely no desire to ever become a company which manufactures or sells any type of vaccine. Instead, we hope to become a licensing company, and a company that promotes and enables as much research as possible, as quickly as possible, into as many types of mucosal vaccines as possible, for as many different types of animals as possible.

And, those goals and desires are indeed affected by, and consistent with, a set of entirely humanitarian, altruistic, and benevolent hopes and wishes. We want this technology to begin helping people find ways to minimize or completely avoid sickness, suffering, and disease, among animals as well as humans, and we want to do all we can, to help reduce and control healthcare costs.

- Although it is not a pressing goal at the moment, we also hope to eventually help lay a foundation for better, more useful, and more productive exchanges, between anti-vaxxers, and the scientific and medical communities. If these new types of mucosal vaccines can eliminate any need for the harsh and nasty adjuvants that injectable vaccines require, and can provide other important advantages as well, they may end up creating a "middle ground" where people on both sides of the pro-vax and anti-vax arguments can meet, and talk, and actually communicate with each other, rather than pointing fingers, making accusations, and trying to defend against and deny anything and everything "the other side" is doing, to "try to score points".

So . . . instead of wanting to compete against anyone, we hope to become a licensing company, which can:

- create a structure and system that will incentivize those who are already experts – in testing vaccines against actual pathogens, in one or more types of animals – to do those types of tests. How can we offer that encouragement and incentive? By both: (a) offering, at low cost (our current goal is $3000 for a custom-assembled phage, for at least the first 20 phages, and we'll see what happens, after that), custom-engineered MALT-targeting phage constructs, carrying any antigen sequence that a qualified requester will commit to actually testing, in "pathogen challenge tests", in one or more types of animals; and, (b) openly offering a worldwide exclusive license, to any and all use of our MALT-targeting delivery system, for any and all vaccines against a specific pathogen or disease, in one or more types of animals, to the first animal vaccine company, vet school research group, or other qualified group which generates "proof of efficacy" that is sufficiently solid and detailed to enable "registration" and authorization, by the U.S. Department of Agriculture, for the requesting company to sell that type of vaccine; and,
- create an advisory board – if the research in animal vaccines looks strong and promising – to begin evaluating suggestions and proposals from vaccine manufacturers, for how they would suggest moving forward to begin testing and making MALT-targeting ***human*** vaccines against various diseases. In other words, we don't want to get ahead of ourselves, and we have made no decisions or commitments, of any sort, concerning human vaccines. Instead, if the animal work looks promising, we will begin talking with experienced people who have worked in or with the human vaccine industry, to get their advice, and possibly their support and/or participation.

Stated in alternate words, we hope and intend to create a large number of licensing opportunities, for a substantial number of animal vaccine companies, in ways that (we hope) will end up creating a network of friends, allies, and partners, rather than creating enemies, adversaries, and opponents.

NEXT PAGE: www.tetraheed.com/contact-us

Please use this form to contact us, if you wish to open a line of communication between us. Tell us whatever you would like us to know (preferably, including the company, agency, university, or whoever/whatever you work for or with, or, at least some sort of background info about yourself).

If you send us a text, using this page, feel free to include 1 or more links to web pages, but do ***NOT*** try to attach any documents, pictures, videos, or anything else, to your initial text message. Please understand that if we visit any links you designate, we will do so on an old backup computer, and if we encounter any malware on your link, we can wipe that computer clean and restore it, with no difficulties; and, we will post whatever we can find out about you, and your attempted malware, on various boards, to warn good people to avoid you.

Before you send a message, please at least try to understand, and accept, that we cannot be pushed or shoved into either a pro-vax, or anti-vax, category. Instead, our only goal is ***BETTER*** vaccines. If you are opposed to vaccines, and if you chose to not read anything else on this website, then please, at least read the page about "adjuvants", which is **HERE** [*link to www.tetraheed.com/t5-irritating-vaccine-adjuvants*]. In total sincerity, we are trying to help ***EVERYONE***, by ***ELIMINATING*** the types of ***inflammatory, toxic, and nasty adjuvant compounds*** that are necessary, to make ***injected*** vaccines work properly, today.

And, beyond that potentially major advantage, we also hope to show companies and researchers how they can make new types of vaccines which can provide ***BETTER*** protection against literally hundreds of different mucosal pathogens, than any currently available vaccines. How? By triggering the formation of ***BOTH OF TWO ENTIRELY DIFFERENT*** types of antibodies: (1) the standard Y-shaped internal antibodies that injectable vaccines can create (we can do it without requiring any injections, or nasty adjuvants); ***AND***, (2) by ***ALSO*** triggering the creation and secretion of mucosal antibody ***DIMERS***, as well (which work by a totally different mechanism, and which ***INJECTED*** vaccines can***NOT*** create).

[CONTACT BOX]