Study reveals current multi-component vaccines may need reworking

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Current strategies for designing vaccines against HIV and cancers, for instance, may enable some components in multi-component vaccines to cancel the effect of others on the immune system, eliminating their ability to provide protection, according to an article to be published shortly in the *Proceedings of the National Academy of Sciences* (*PNAS*). The authors also suggest, and successfully test, techniques that offer a solution to newly revealed mechanisms that enable some vaccine components to outcompete others.

Recognizing molecules as part one's self versus foreign invaders is the responsibility of the immune system. The adaptive component of that system creates a great variety of immune cells on the hope that one will be the right shape to become activated by any invader encountered. When one of those immune cells recognizes an invader, it expands into an army of clones specifically selected to attack that organism. In a landmark discovery, researchers discovered that part of the immune system selects certain, small pieces of each disease-causing entity (or pathogen) to trigger immune cell expansion, while ignoring the rest. Those triggering protein fragments, or peptides, are termed "immunodominant." For many years, the field has faced three questions. How does this selectivity evolve? Does the process always focus on the peptid fragments that will provide the strongest immune response/protection? If not, can we make changes to useful peptides that make them the center of the immune system's attention?

One workhorse of the adaptive system is the helper T cell, a white blood

cell that partners with dendritic cells to make careful decisions about which disease-causing peptides attract attention. Upon encountering an invader, a dendritic cell will "swallow it," cut it up, and carry the pieces to the nearest lymph node. Once there, major histocompatibility complex (MHC} class II proteins inside the dendritic cells present peptides on the cell's surface for consideration by T cells gathered there. Activated T cells begin dividing, which greatly increase their number, until they become capable of the destroying the pathogen in question when exposed to high enough levels of the peptide representing it.

Andrea Sant, Ph.D., professor within the David H. Smith Center for Vaccine Biology and Immunology at the University of Rochester Medical Center, published a July 2005 article in the journal Immunity which revealed the quality that confers immunodominance on a peptide to be the strength and lifespan (kinetic stability) of its bond to the MHC class II protein. Kinetic stability determines whether, in the face of competing reactions, a peptide:MHC class II complex can accumulate at the surface of the dendritic cell, and then remain intact long enough to sustain T cell expansion. Dr. Sant's team found that immunodominant peptides held onto to MHC molecules ten to one hundred times longer than nondominant, or "cryptic," peptides because they fit together better. In the years since, Sant and colleagues have determined how the kinetic stability of the MHC:peptide bond has its effect.

In 2006, she published a study in the *Journal of Experimental Medicine* that found an enzyme called human leukocyte antigen-DM opens pockets on the MHC class II molecule for a given peptide to fit into it before the complex heads to the cell's surface to recruit T cells. Within dendritic cells (and related cell types that present peptides to immune cells), DM was found to favor peptides with highly stable MHC interactions, a process dubbed "DM editing." Sant's team found kinetically stable peptide-MHC complexes arrived at the cell surface as much as 100 times more often than cryptic peptides, and thought DM

editing was solely responsible for determining immunodominance.

The current paper found, however, that the persistence of peptide-MHC Class II bonds also determines which peptides cause a T cell expansion after the complexes have arrived at the cell surface, as well as through DM editing inside the cell. A peptide's persistence at the cell surface also controls the ability of CD4 T cells to continue to expand. Importantly, this additional level of control over immunodominance by stability was only detected when many peptides were present, and were "competing" to see which would trigger a T cell response.

The team also found that low-stability peptide:class II complexes support the initial priming and expansion of CD4 T cells, but the expansion is "strikingly aborted" in the presence of competitive T cell responses to immunodominant peptides. Peptides that fall off of MHC class II molecules too quickly fail to sustain the expansion.

"What we saw is that if a dendritic cell offers weak or unstable peptides alone, T cell responses move forward," said Sant, the study's lead author. "But if we offered weak peptides in the presence of immunodominant ones, the weak peptides trigger an expansion at first, but then the response stalls. This competitive aspect is a new and has profound implications for vaccine design."

While still a theory, Sant speculates that losses in some T cell responses can be explained in part by trogocytosis, a process where T cells "steal" key pieces of the dendritic cell each time they dock onto it as a step in their decision to start expanding. Stolen pieces may include the peptide at hand, the MHC class II protein and so called co-stimulatory molecules. If there are many more of immunodominant peptide-MHC class II complexes initially displayed by the dendritic cell due to DM editing, and if cryptic peptides are falling off the MHC class II complex at a faster rate, and if T cells poach from the peptide-bearing dendritic

cell with each pass, dominant peptides would continue activating T cells long after responses to cryptic peptides had petered out.

Without causing an actual infection, many modern vaccines introduce detoxified versions of disease-related molecules to the immune system, which remembers them for next time. Once researchers confirm the kind of immune response needed to achieve protection, they can choose for inclusion in a multi-component vaccine the key peptides that trigger the strongest immune response. The immune system reacts, not to the presence of a whole bacterium or virus, but instead to key fragments of those pathogens.

Peptide-based vaccines are already used clinically in cancer, and researchers have made progress determining the specific peptides on the surface of bacteria, viruses and tumors that trigger T cell responses. Oftentimes, vaccine designers will seek to determine several peptides that elicit T cell responses and mix them together to assure greater immune protection. The assumption was that mixing components, each known to elicit a strong T cell response, together would make for a stronger vaccine.

Working in Sant's Lab, Jason Weaver conducted experiments that revealed that including several pathogenic peptides, each known cause a T cell response in isolation, may not be additive when injected into the same dendritic cell. In fact, some of the peptides may cancel out the ability of others to cause T cell expansion because of immunodominance.

Vaccine designers in the future may have to inject the several peptides in multi-component vaccines into different regions of the body so that the strong peptides do not cancel out the weak, researchers said. A second solution may be to change cryptic or weak peptide vaccine components into immunodominant ones. Sant and members of her laboratory

including Francisco Chaves, Christopher Lazarski and Weaver have already shown that by switching out single amino acid building blocks, the team was able to drastically increase the potency of the T cell response to target peptides, including those that would otherwise fail to achieve a T cell response in the presence of other immunodominant peptides. Sant's team increased the ability of a cryptic peptide to hold onto MHC class II proteins by making changes to one or more of their four "anchor residues" responsible for binding into the pocket on the MHC Class II proteins, changes that sustained T cell responses.

In addition, peptide vaccines traditionally address just one half of the adaptive immunity system, the cell mediated/T cell system concerned with attacking pathogens once they have already infected their target cells. One of the principle roles of helper CD4+ T cells is to turn on CD8 "killer" T cells that sense infected cells and destroy them before a virus can use the cell as a virus factory. Peptide vaccines work by targeting only T cells, which explains why they have not been widely used against pathogens like the flu viruses, which spend part of their lifecycle in the space between cells. Those invaders must be addressed by antibodies, which directly glom onto invaders, and tag them for further immune attack.

Sant argues that understanding immunodominance has become even more important in light of recent publications that show strong T cell responses to a peptide have a role in the reactions that causes B cells to start mass producing antibodies as well. Activated T cells move into "the B cell area" of the lymph node and encourage B cells to start making antibodies. Recent studies have also shown that this process may be most efficient for stable peptide:MHC class II complexes. Kinetic stability may influence both the B and T cell aspects of the adaptive immune system.

The studies were performed within the Department of Microbiology and

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"Evolution may have chosen kinetic stability as the way in which it decides which pieces of disease-causing invaders activate a full T cell response because it may make it more likely that T cells can destroy cells infected with viruses, and because it may help B cells produce antibodies to viruses like influenza," Sant said. "It may also make infected cells more vulnerable to CD8+ T cells that kill an infected cell by releasing toxic chemicals before a virus can use the cell to copy itself. Furthermore, it may help keep alive memory T cells that remember a given pathogen and more quickly attack it upon their next encounter."

Source: University of Rochester Medical Center (news : web)