Anadys Uses Toll-like Receptors to Switch on Immunity

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In the last several years, researchers have increasingly focused on using the innate immune system to fight disease. Considered the body's first line of defense, the innate immune system is stimulated by a broad spectrum of molecules usually found on or in pathogens, and it triggers an inflammatory response and spurs the adaptive immune system to generate lasting immunity. The innate immune system features "toll-like receptors" (TLR) that are expressed on the surfaces of antigen-presenting, dendritic, and other cells. Ten human TLRs that respond to a variety of pathogens have been identified so far. Most TLRs interact with the proteins, liposaccharides, and lipopeptides expressed on cell surfaces, but some are able to detect intracellular elements such as nucleic acids.

A number of biotech companies, in collaboration with pharma, are now exploring the possibilities for manipulating immunity by developing TLR-based therapeutic agents for ailments such as hepatitis B and C, HIV, certain cancers, allergies, asthma, and autoimmune diseases. However, elucidating the path by which TLRs stimulate and direct innate immunity's "master switchboard" is tricky, as it is such a pervasive system.

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Funny-Looking Fruit flies

Toll-like receptors acquired their name because in 1988 a German researcher, future Nobel laureate Christiane Nusslein-Volhard, stumbled across peculiarly misshapen fruit fly embryos. A link to the immune system was established when it became apparent that these fruit flies, missing a functional protein, were more susceptible to fungal infection. Nusslein-Volhard termed the gene that caused this "toll," for strange or wondrous. The attributes of the toll genes took a while to be appreciated; certain drugs, like 3M's topical cream Aldara (imiquimod) for basal cell carcinoma, HPV, and genital warts, were marketed before researchers knew they worked via TLRs.

Flu Symptoms for a Year

Chronic hepatitis C (HCV), a viral disease that destroys the liver, affects 170 million people worldwide, according to the World Health Organization. The current treatment is to inject pegylated interferon in conjunction with ribavirin for close to a year to stimulate and maintain an antiviral immune response. "It is a difficult regime to tolerate," says Devron Averett, Ph.D., chief scientific officer of San Diego-based Anadys (http://www.anadys.com). According to Averett, the treatment produces nasty flu-like symptoms and typically works about 50%–60% of the time. "We thought it would be possible to stimulate innate immunity through toll-like receptor-7," said Averett. "It would be an oral, rather than injectable, therapy and have fewer side effects. That is the basis for our partnership with Novartis around ANA975." This isatoribine-based compound for hepatitis B and C stimulates TLR7 to cause release of interferon-α and other cytokines important to the immune cascade into the bloodstream. The company's preclinical pipeline also includes ANA773, an oral TLR7 prodrug for certain types of cancer, and ANA380 for chronic hepatitis B, which has completed Phase II clinical trials. Anadys is also pursuing preclinical antivirals for human immunodeficiency virus (HIV) infection.

"Toll-like receptors are a fairly recent scientific understanding," said Averett. "What we did know is that isatoribine stimulated innate immunity." Publications by researchers including immunologist Charles Janeway at Yale as well as other data on lipopolysaccharide-resistant mice from Bruce Beutler at Scripps gave the company a better understanding of how their compound worked.

The body has many TLR7 receptors in the gut, but swallowing a slug of isatoribine will cause symptoms similar to food poisoning. Through medicinal chemistry sleight-of-hand, Anadys developed a "prodrug," a masked oral form of isatoribine which can be absorbed in the gut without causing nausea, and which becomes active only in the bloodstream.

The Financial Picture

Anadys is a publicly traded company, has 94 employees, and was established in its present incarnation in 2000 by Kleanthis G. Xanthopoulos, Ph.D., and Devron Averett. The company garnered a $20 million licensing payment in 2005 from Novartis, and another $10 million milestone payment upon the acceptance of the IND application by the FDA. A potential $540 million in milestone payments rides on getting ANA975 approved as a drug. Anadys has additional collaborations with LG Life Sciences and Aphoenix, Inc.

Back-up at the Toll Plaza

Immunologists are mindful of the case of TeGenero's TGN1412, a CD28 agonist, which caused the volunteers who received it in a Phase 1b trial during the spring of 2006 to develop massive inflammation and a sepsis-like response due to overproduction of cytokines.

In June 2006, ANA975 was undergoing a 28 day Phase 1b trial in HCV patients when the preclinical toxicology data in a 13 week trial of rats and monkeys showed an unusually high level of lymphocyte proliferation and enlarged lymph nodes. Although the drug was seemingly well tolerated in the human study, Anadys halted dosing and notified the FDA, which put the brakes on the company's IND application. Anadys is now conducting a new animal toxicity study using a crystalline form of the drug supplied by Novartis. While ANA975 targets a different area of immune response than TGN1412, the symptoms of immune system overstimulation were similar enough to warrant a deeper look.

Taking Another Toll Road

Wellesley, MA-based Coley Pharmaceutical Group, Inc. (http://www.coleypharma.com), is named after Dr. William Coley, who in the late 1800's observed that patients with local infections had remissions in cancer. Dr. Arthur Krieg founded Coley in 1997. In his research at the University of Iowa and VA Medical Center, he discovered that synthetic oligodeoxynucleotides with the CpG (cytidine-phosphate-guanine) motif stimulate immune cells. This CpG sequence is methylated in humans (which avoids immune stimulation), but not in viruses and bacteria. Unknowingly, he discovered a TLR9 agonist. Coley's compound Actilon is now in Phase II trials for hepatitis C in patients unresponsive to conventional treatments.

Coley's TLR9 program for cancer is the company's most advanced. "When we inject the CpG or TLR agonist into someone who has cancer, it is like waking the immune system up and saying here is an infection," said Krieg. "At least theoretically if it is activated in the right way it should be able to kill tumors. We have been able to prove this in mice" [1]. According to Krieg, a problem with the classical approach of developing a tumor vaccine is knowing exactly what all the tumor antigens are. Stimulating the dendritic cells of the innate immune system could bypass that obstacle. "Dendritic cells are part of the innate immune system but stimulate adaptive immune responses, especially killer T cells," said Krieg.

Pfizer paid $60 million upfront and could make a potential $455 million in additional milestone payments for CpG 7909. PF-3512676, as Pfizer renamed it, is in Stage III clinical trials, administered in conjunction with standard chemotherapy drugs, and has shown promising results in non-small cell lung cancer (and Pfizer hopes for multiple oncology indications as well, including breast cancer).

Coley has licensed the know-how surrounding CpG 7909 to GlaxoSmithKline as an adjuvant for breast, lung, and prostate cancer vaccines. Coley is also jointly developing TLR9 agonists to treat asthma and rhinitis with Sanofi Aventis. Coley bases its allergy program on the "hygiene hypothesis" that the rising incidence of allergies in industrialized countries is due to insufficient stimulation of the immune system in childhood, and that it may be corrected via using CpG to mature the immune system.

Coley has focused on TLR9 because "[it] has the most narrow pattern of expression," Krieg said. If the immune system is activated too broadly, "One manifestation can be a cytokine storm, another one can be an autoimmune disease." According to Krieg, Coley and other companies working on TLR9 have treated thousands of people with TLR9 agonists with no major problems. Under a grant from NIAID, Coley is also investigating the possibility of stimulating TLRs 3, 7, and 8 with RNA from viruses and bacteria.

Boosting the Immune Response

One of the first uses for TLR agonists was as adjuvants for vaccines. Adjuvants are molecules that typically activate innate immune cells, particularly dendritic cells, and when used in conjunction with an antigen stimulate a stronger adaptive immune response, including antibodies and, in some cases, killer T cells. TLR agonists would seemingly make excellent candidates, because TLRs have the ability to stimulate some of the major regulatory lymphocytes and activate both the innate and the adaptive immune systems.

While Anadys and Coley are more concerned with developing drugs, companies like Berkeley-based Dynavax Technologies (http://www.dynavax.com) is involved in developing TLR9-based adjuvants for vaccines. Idera Pharmaceuticals (http://www.iderapharma.com), located in Cambridge, MA, just signed a licensing deal with Merck & Co. to use agonists of TLR9, TLR7, and TLR8 for vaccines for cancer, infectious diseases, and Alzheimer's disease.

Follow Those Mouse Tracks

While TLR-based programs look promising, all must contend with the fact that what works in animal studies may not map onto the human immune system closely enough for comfort. Current research may well indicate that the toll gates are not such straightforward passages to immune control. To wit: work by Jean-Laurent Casanova's research group at the Necker Medical School in Paris showed that a small group of unrelated patients who had inherited functional defects in a signaling pathway downstream of TLRs did not respond to eight of the nine receptor-specific ligands tested [2]. The patients suffered from pyogenic bacteria infections with minimum inflammatory responses. Another group of patients with impaired downstream signaling from TLRs 3, 7, 8, and 9 were only susceptible to herpes simplex virus encephalitis, but otherwise healthy [3]. These findings were surprising because mice with similar defects show gross susceptibility to microbial, viral, and fungal infections. These results suggest that TLR systems in humans may be redundant and may not necessarily play as crucial a role in the innate immune system as previously thought.

Drugs agonizing and antagonizing TLRs are being developed even as the rudimentary roles for the receptors in immunity are being uncovered.

Toll-like receptors harbor promise for treating an amazing spectrum of maladies, possibly with fewer side effects than current drugs. But in the case of the immune system, it is wise to proceed with caution. So in short: look both ways before taking the toll road.

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