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New Approach to Thwarting Inflammation

Howard Hughes Medical Institute (HHMI) researchers have discovered a way to shut down the inflammatory response in cells that spares related mechanisms that cells need in order to function properly. Their experiments have demonstrated that such treatment relieves inflammation in mice with surprising effectiveness.

In an article published in the September 1, 2000, issue of *Science*, HHMI investigator Sankar Ghosh and his colleagues at Yale University report that they have found a way to short circuit NF- κ B, a central coordinator of the cell's inflammatory response. When NF- κ B is triggered by an external chemical signal, NF- κ B sets in motion the gene expression machinery that drives inflammation.

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— Sankar Ghosh

"It has become clear over the years that NF- κ B plays a crucial, evolutionarily conserved role in the cell's response for getting rid of pathogens," said Ghosh. "As a byproduct of this immune response, inflammation can get out of control and cause pathogenic states. We now know that many diseases that at first glance don't seem to have much in common actually have inflammation as an underlying reason for their pathology." Alzheimer's disease, for example, might well involve inflammatory responses that damage brain tissue, Ghosh noted.

The most widely used anti-inflammatory drugs, including salicylates such as aspirin, and steroids, inhibit NF- κ B to some extent, but they can also have severe side effects. The great need for more precise inhibitors of inflammation prompted Ghosh and his colleagues to look at ways to block inflammation by interfering with NF- κ B.

The scientists knew that in unstimulated cells NF- κ B remains in the cell's cytoplasm attached to inhibitory proteins known as I κ Bs. When an external inflammatory signal affects the cell, the I κ B-kinase (IKK) complex which consists of a pair of catalytic enzymes is activated and phosphorylates the I κ B proteins. The phosphorylated I κ B is then rapidly degraded, thus freeing NF- κ B to trigger the inflammatory process.

While the majority of research on blocking the inflammatory process has focused on blocking the catalytic activity of the IKK complex itself, Ghosh and his colleagues chose another route. "By themselves, the enzymes of the IKK complex don't respond to signals," explained Ghosh. "Each enzyme needs a regulatory subunit protein called NEMO, and it has been shown that when NEMO is knocked out in mice, NF- κ B becomes unresponsive to signaling."

In a series of biochemical experiments, Ghosh and his colleagues sought to identify the minimal region of the IKK enzymes that interacted with NEMO. "We were quite surprised to find that a very small region of the IKK enzymes seemed to be completely responsible for interacting with NEMO," said Ghosh. The scientists called the region the NEMO-binding domain (NBD).

"We reasoned that if the NBD was such a small region, then maybe we could use it as a way to disrupt the whole IKK complex and prevent it from forming," said Ghosh. Sure enough, when the scientists synthesized a small peptide that mimicked the NBD and put it into cells, they discovered that NF- κ B activation was blocked significantly.

"Then we raised the stakes," said Ghosh. "We decided to see if this blocking method would work *in vivo* in animals." The scientists used the NBD peptide to attempt to block inflammation in two mouse models where chemicals are used to induce inflammation artificially.

"In both models, when we injected our peptide, we observed quite a dramatic amelioration of the inflammatory process," said Ghosh. "I think that the remarkable anti-inflammatory effect that we achieved validates the approach of relieving inflammation by interfering with the activation of NF- κ B alone. This approach should become a major focus of future research."

Ghosh also said that blocking the activation of NF- κ B by external inflammatory factors quite likely spares the basal activity of the NF- κ B machinery that operates via a different pathway, and which cells need for normal function.

"One of the concerns that has been raised about inhibiting NF- κ B, is that NF- κ B also has some beneficial effects, such as helping cells survive apoptosis, or programmed cell death," said Ghosh.

Ghosh and his colleagues are planning further studies to investigate the NEMO-IKK interaction. They are going to test the NBD peptide on mouse models of asthma and other diseases that involve inflammation. They also hope to initiate collaborations with pharmaceutical companies to develop

mimics of the NBD peptide that can be used as precise inhibitors of inflammation.