Inflammation: the cancer connection

A truly collaborative research initiative led by **Professor Steven Tannenbaum** seeks to understand the relationship between inflammation and cancer by shedding new light on the chemical mechanisms responsible. Here, members of the programme discuss their passion for the field and future research plans

When did you become interested in the causal relationship between inflammation and cancer?

Bevin Engelward: I became interested in this relationship when I joined the Massachusetts Institute of Technology (MIT) Nitric Oxide Program Project Grant (NO PPG) in 1997. Through interactions with members, I came to appreciate how input from chemists and engineers synergises with that of biologists to generate an integrated understanding of inflammation. For my own work, the NO PPG was important in developing approaches to study large-scale sequence rearrangements driven by inflammatory chemicals. My recent work has used *in vivo* models to study the inflammatory processes that drive carcinogenesis.

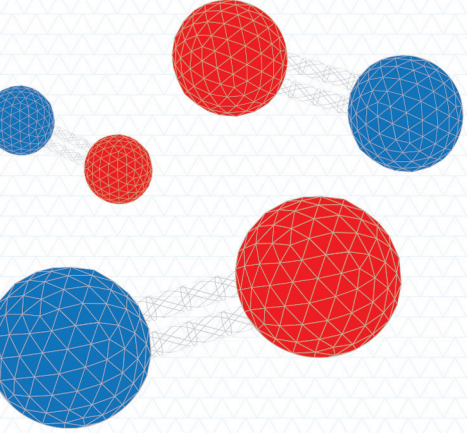
Jim Fox: As a veterinarian, I have a background in infectious diseases, studying many that also infect humans. When I joined MIT I continued my studies in infectious diseases, focusing on an enteric microaerobic bacterium, *Campylobacter jejuni*, that causes disease in humans. My group discovered that ferrets were naturally infected with the same pathogenic bacteria. This, combined with our knowledge that the ferret stomach is similar to that of humans, convinced Professor Steven Tannenbaum to use the ferret to model nitrosamine formation in the stomach. Around the same time, *Helicobacter pylori* (another microaerobic bacterium) was found in patients with gastritis and peptic ulcer disease. We cultured gastric tissue from a ferret with a peptic ulcer, and were the first to isolate a gastric *Helicobacter sp*. from a non-human mammalian stomach, which we named *Helicobacter mustelae*. We were able to link *H. mustelae* to gastric cancer in the ferret, similar to *H. pylori*-induced inflammation leading to gastric cancer in humans. While studying *Helicobacter*-associated gastric inflammation in mice, we were asked by the National Cancer Institute (NCI) to investigate chronic inflammation and hepatic tumours in a group of control mice in a carcinogenicity study. We isolated an enterohepatic *Helicobacter*, which we named *H. hepaticus*, and demonstrated its causal association with liver and bowel cancer in mice. This model has since been used extensively by the NO PPG to dissect the chemistry of inflammation-induced malignancy.

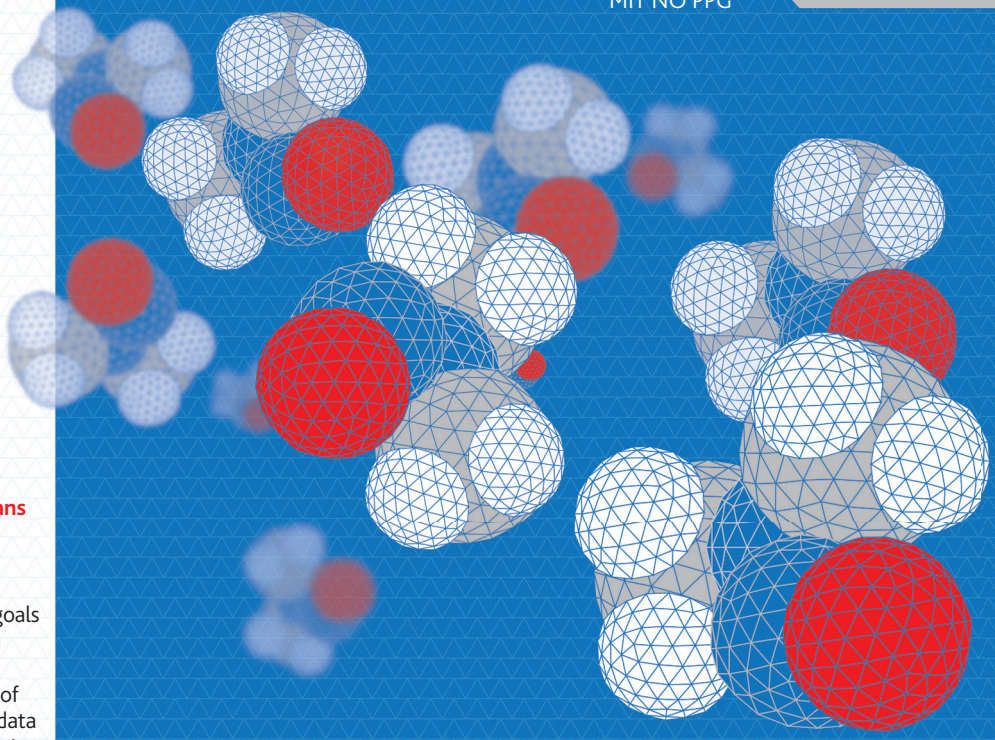
Gerald Wogan: My interest arose from a previous collaboration with Pelayo Correa, Steve Tannenbaum and Jim Fox. We showed that chronic gastritis associated with *Helicobacter* infection progressed through a series of stages leading to stomach cancer. Jim's subsequent development of mouse models of colon carcinoma induced by *Helicobacter* allowed us to confirm the original findings of genotoxic events that play an important role in inflammation-induced cancer. Through collaboration with Drs Dedon, Tannenbaum, Essigmann, Engelward and Fox, using a variety of experimental models, we were able to develop and test hypotheses concerning mechanisms of action of reactive oxygen and nitrogen species (ROS and RNS) in tumour development.

Peter Dedon: My interest in inflammation and cancer was piqued when I joined the MIT faculty in 1991 and learned of the ongoing NO PPG research programme. It was a natural segue from my graduate and postdoctoral research, which focused on drug-induced DNA damage in cancer chemotherapy, to the chemistry of DNA damage produced by the endogenous processes of inflammation. This shift in research questions was facilitated by the acquisition of novel bioanalytical technologies developed by researchers in the NO PPG, which fuelled numerous collaborative interactions with programme researchers and further development of the technologies. The resulting synergy led to the discovery of novel endogenous DNA lesions and unexpected chemical mechanisms underlying inflammation-induced cellular damage *in vitro* and in tissues from the NO PPG mouse models of colitis and colon cancer.

John Essigmann: I joined MIT in 1981. Before that time, I worked with Gerald Wogan on a human liver carcinogen. Through that work, we devised a way to study how DNA lesions caused by inflammation produce mutations. This led us to join the NO PPG, where my group linked the chemical formation of lesions with specific genetic changes. Those DNA lesions could act as functional biomarkers to predict risk of oxidative stress, and evaluate future chemo-prevention strategies.

John Wishnok: I joined the Department of Nutrition and Food Science in 1974 to measure nitrosamines in food by gas-chromatography-mass spectrometry. Owing to my background in physical organic chemistry, I became interested in the carcinogenic behaviour of these compounds. Nitrosamine projects evolved into studies on endogenous nitrosation and endogenous nitrate formation, which in turn led to a focus on nitric oxide biochemistry, ultimately leading to current studies of the mechanisms and biomarkers of inflammation. As the bioanalytical chemist of the group, I've had a continuing interest in inflammation-related biochemistry and identifying and measuring the molecules involved.





Looking ahead, what are your research plans for the coming five to 10 years?

JW: The objectives of the analytical lab have followed, and in some cases prompted, the goals of the programme's projects. As the research expands further into inflammation-related mechanisms, we'll enhance the exploitation of quantitative proteomics and metabolomics data by increasing the data mining and data analysis abilities of the lab to probe more deeply into these mechanisms.

JF: We plan to continue studying the pathogenesis of inflammation-induced gastrointestinal cancers, particularly those caused by a variety of *Helicobacter* species identified and characterised in our lab.

GW: My future research interests focus on the mechanisms through which ROS and RNS alter signalling pathways involved in carcinogenesis. Of particular interest are post-translational modifications of signalling proteins important in cell growth.

BE: Our recent studies point to inflammation as a driver of sequence rearrangements, but do not shed light on the underlying cell types that experience those changes. Our future work is aimed at exploiting new approaches to understand the impact of inflammation on sequence changes in somatic stem cells – a relevant cell type in the initiation of cancer.

PD: My research programme is evolving from a focus on the chemistry of inflammation-induced molecular damage to a broader analysis of cellular responses to chemical mediators of inflammation, from the perspective of the human host and microbial pathogens.

JE: Looking down the road, I see two clear goals. The first is to use biomarkers to evaluate chemo-prevention strategies, based on collaborations with the Chulabhorn Research Institute in Thailand. Thailand has a wealth of natural products and an outstanding chemical biology programme, which is orientating its activities increasingly to the arena of chemo-prevention. Our second goal is to shift focus to damage of the nucleotide pool as a source of genetic changes from inflammation. This work will be done initially with Peter Dedon and Gerald Wogan, but will likely draw in the others if the initial results are encouraging.

Cancer chemistry

A group of researchers at **Massachusetts Institute of Technology** is working together to reveal the chemical basis of the link between inflammation and cancer. One of the few chemistry-based projects to do so, this programme has the potential to reveal new cancer biomarkers

ALTHOUGH THERE IS now insurmountable evidence that inflammation can cause cancer, quite how it does so is not known. The mechanisms by which inflammation initiates or influences the progression of cancer remains a mystery to modern science and, as a result, it is difficult to see a clear path toward the design agents to prevent or treat such cancers.

There are many inflammatory mediators, but one particularly noxious agent is nitric oxide (NO). A seminal report published over 50 years ago revealed the carcinogenicity of a NO-containing molecule. The study showed the formation of liver tumours in mice and encouraged a surge of research efforts to determine the risk of these compounds to humans.

Professor Steven Tannenbaum and his colleagues at the Massachusetts Institute of Technology (MIT) Department of Biological Engineering have made major contributions to this effort with their ever-evolving research programme. Beginning with the analysis of nitrosamines in food, the team eventually found that nitrate can be produced inside the body as activated immune cells convert an amino acid called arginine to NO. Progressing to characterise the subsequent DNA damage, the current focus of the team is on the chemical mechanisms of inflammation-induced cancer in humans. This research, spanning over 30 years, has been supported by a Program Project Grant (PPG) from the National Cancer Institute (NCI), involved over 40 scientists from seven research groups, and yielded over 350 publications.

But beyond this prolific publication rate, the project ultimately aims to provide a robust scientific understanding of how inflammatory processes contribute to cancer. This could engender a rational approach to chemoprevention, and reveal new biomarkers to analyse the chemical origins of mutagenic lesions, and perhaps even diagnose and characterise the progression of cancer.

BIOCHEMICAL INTERPLAY

Though chronic inflammation is a well-documented risk-factor for cancer, the goal of the team's research is to elucidate the molecular mechanisms underlying this; exactly how inflammatory chemicals generate genetic changes that lead to cancer. A team with such a broad range of expertise means many research threads can be explored, including the roles of immune cells in exposing the native cells of tissues to reactive chemical species, and the damage they cause to proteins, DNA and whole cells.

The group's primary interest is NO, but the researchers also study the reactive species formed alongside it. Depending on the dose

INTELLIGENCE

ENDOGENOUS NITRITE CARCINOGENESIS IN MAN

OBJECTIVE

To understand the mechanisms linking chronic inflammation to colon cancer.

KEY COLLABORATORS

Over the years there have been many key investigators involved in this research at MIT. Their names appear as multiple combinations in the authorship of papers emerging from this research, and their accomplishments are so entwined that they are impossible to unravel. In alphabetical order they include:

Peter Dedon; **William Deen**; **Bevin Engelward**; **John Essigmann**; **James Fox**; **Michael Marletta**; **David Schauer**; **Steven Tannenbaum**; **John Wishnok**; **Gerald Wogan**

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THE MIT GROUP ON NITRIC OXIDE AND INFLAMMATION's early research focused on the potential toxicity and carcinogenicity of nitrite and nitrate, and the formation of a class of compounds called nitrosamines, many of which were potent carcinogens in animal models. Early on, the researchers discovered that nitrate, a common constituent of vegetables and a preservative in cured meats, was reduced in human saliva to nitrite, which could then react with amines in the acid conditions of the stomach to form nitrosamines. This observation led to an interest in the overall fate of nitrate in the human body, and in a series of papers in the late 70s and early 80s to the discovery that nitrate was made in the human body from some reduced nitrogen compound, and that the biosynthesis of nitrate could be greatly stimulated by infection and inflammation. Subsequently, when he was at MIT Michael Marletta discovered the enzymatic mechanism of nitrate formation as a novel oxidation of arginine in macrophages to nitric oxide (NO), and the race was on. NO was now intimately connected to inflammation and innate immunity, and many types of cells could be induced to make NO, which became a major player in the chemistry and chemical damage to all types of biological molecules.

and cell type affected, these compounds can programme cells to die (apoptosis), or act in sharp contrast to inhibit this process. This increases the mutation rate by damaging the bases in DNA and causing breaks or small loci of miscoding potential in the information-containing strands. Tannenbaum's team studies these processes at all levels, from the individual chemical reactions to their effects in cells, tissues, organs and finally whole organisms.

Under the umbrella of describing the biological and chemical interplay of inflammation, the researchers are conducting four interlinked projects to translate fundamental understanding of *in vitro* chemistry to cell and animal models of inflammation, mutagenesis and cancer. In turn, they hope to shed new light on the inflammatory mechanisms of cancer in humans.

INTEGRATED INVESTIGATIONS

The first element of the programme aims to develop, validate and implement analytical and omic approaches to identify and quantify the molecular changes taking place during inflammation. The chemical changes identified here will be applied to cell and mouse models, and eventually human tissues. Following this first step, the identified DNA lesions will be examined in a second part of the programme, which characterises the properties of the lesions, and examines whether inflammation can work in ways not related to the coding sequence of DNA – via epigenetics.

Members of the team have offered new insight on the role of inflammation in colon cancer, the third most common form of cancer worldwide

S-Nitrosation, the addition of NO to cysteine residues on proteins or small peptides, is an important signalling mechanism, and the third sub-project characterises its role in DNA repair, growth and survival pathways. The final project takes a more microbiological perspective, aiming to define the impact of pathogens on DNA damage and repair processes, many of which are indeed mediated by NO and its reactive products.

COLON CARCINOGENESIS

Through these strands, members of the team have offered new insight on the role of inflammation in colon cancer, the third most common form of cancer worldwide. In a 2012 paper, they described an experimental animal model linking infection and inflammation to colon cancer. This model enabled them to provide sound evidence that the innate immune system is critical in driving the progression of this cancer.

The group showed that damage to epithelial cells caused by *Helicobacter* infection provides bacterial products with access to receptors on immune cells. Their binding to these receptors leads to the activation of transcription factors that regulate the production of chemicals that attract immune cells, like macrophages. Such characteristic inflammatory events are reinforced by the expression of potent inflammatory mediators, amplifying inflammatory gene expression and enhancing cell survival. If this process is not quenched, the immune response is not only maintained but intensified, by the activation of the second arm of immunity – adaptive immunity.

EXACERBATING THE DAMAGE

But what happens next is arguably most important. A growing body of evidence suggests that the activated macrophages (alongside neutrophils) produce a raft of highly reactive oxygen and nitrogen species (ROS and RNS, respectively). These can also be produced endogenously by endothelial cells, and can damage all biomolecules found in the body.

In an earlier study, the MIT team was able to pinpoint the importance of ROS in cancer development. And more recently, they showed precisely how neutrophils contribute to DNA damage, revealing a chemical pathway resulting in the formation of 5-chlorodeoxycytosine. These chemicals, and their reaction products, cause injury, death and mutation in cells, intensifying the damage caused by inflammation.

Further studies showed that damage to cells, and the genetic information they contain, is not only induced by chemicals produced by immune or epithelial cells, but enhanced by bacterial toxins, which both damage DNA and suppress its repair. The coincidence of DNA damage and compensatory increases in cell replication here creates a perfect storm for tumour development.

A NOVEL HYPOTHESIS

Through their ongoing studies and novel approaches, the group has developed an appealing model of the missing link between inflammation and cancer. Subgroups of inflammatory cells generate reactive chemicals that bombard the cell, create mutations in DNA, cause cellular damage, and alter key pathways for growth and survival. Together, and exacerbated by the action of bacterial toxins, Tannenbaum believes these cause cancer.

Inflammation is now acknowledged as a major factor in the pathogenesis of many different types of cancer, as well as other diseases such as lung fibrosis. The work of the NO PPG group will generate an unprecedented level of understanding on the role that chemical changes to proteins and DNA, derived from immune cells attracted to the inflammation site, has on the processes leading to cancer. This better fundamental scientific understanding will lead to the potential for improved characterisation and treatment of cancer.