Inflammation
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Correction – In the June print issue, in the feature ‘Guarding the granary’ by Peter van Esse the last line of the feature should have read: ‘With increased trade, movement of people, climate change and intensification of farming, the appearance of ash dieback is a taste of the challenges that lie ahead for farmers, government agencies and scientists in the UK’ not ‘Adaptation of the pathogen to common fungicide treatments and combination treatments’.
You’ve never had it so good (in the lab)

by Freddie Theodoulou, Science Editor

The first day that I was let loose in a real research lab to do an undergraduate project, I was fascinated to find a big folder filled with yellowing sheets of methods propped up against the freezer. Turning the first page, my eye was immediately drawn to ‘Preparation of ATP from autoclaved yeast’, filed under ‘A’. I never made it through to ‘Z’ because the idea of having to isolate what seemed to me to be a very everyday reagent, before even thinking of doing an experiment, was quite a shock. Now we just flick through the Sigma catalogue for this sort of thing, but of course someone, somewhere has spent time extracting or synthesizing it.

This anecdote calls to mind what has become an informal scientific sport, where seasoned members of our lab compete to celebrate ‘old-school’ methodology and alarm students with stories of alleged hardship at the bench. A newbie moaning that Stores has run out of DNA miniprep kits will inevitably be regaled with colourfully exaggerated tales of caesium chloride gradients that had to be run for days, industrial quantities of ethidium bromide and – for the more gullible audience – recklessly unbalanced centrifuge rotors careening across the room. References to time-saving technology often provoke a Pythonesque monologue: “In my day (assume gritty Northern accent at this point) we had to pour our own thin-layer chromatography plates/make our own dideoxy/prepare acrylamide/synthesize fifty millicuries-worth of radiotracers, all before breakfast!” (delete as applicable). I didn’t have to pour my own thin-layer chromatography plates or prepare acrylamide/synthesize fifty millicuries-worth of radiotracers, all before breakfast! This is all good clean fun but these anecdotes imply not only that toiling at the coalface of biochemistry is an honourable thing (which of course it is), but also that we somehow look down on those who for whom advances in technology has removed much of the laboratory drudgery that dominated our early research experiences. But making life easier in the lab is a good thing! Moreover, we now expect much more of our PhD students in the three years that they spend at the bench, especially if they hope to publish in good journals. Almost every issue of The Biochemist features examples of how new methods and machines have allowed us to push the boundaries of resolution, to progress more rapidly, to ask different, bigger questions and (maybe) spend less time in the cold room. I, for one, am all for that.
Resolution in an ‘over-inflamed’ era

Mauro Perretti and Trinidad Montero-Melendez (Queen Mary
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Unlike other pathologies, inflammation is a condition that all individuals experience in their lives. Toothache, sunburn, a twisted ankle or cutting your hand while slicing bread, they all evoke what we call an acute inflammatory response. This type of response normally displays the cardinal signs of inflammation originally described by Aulus Cornelius Celsus: redness, swelling, heat and pain. Acute inflammation does not normally require any therapeutic intervention other than perhaps a painkiller, as it resolves, with the damage being naturally repaired. Inflammation is also at the root of many other diseases in a more ‘silent’ way as the cardinal signs of inflammation are not so evident. It is now appreciated that inflammatory mechanisms and processes contribute to the pathogenesis of a number of conditions including obesity, cancer, rheumatoid arthritis, atherosclerosis and diabetes. These are examples of chronic inflammation, arising either by the persistence of the injurious element causing it, or by a defect in our endogenous natural protective mechanisms grouped under the terminology of pro-resolving mechanisms.

A common perception, likely to have been enhanced by the large variety of nonprescription anti-inflammatory drugs available to anyone experiencing mild-to-moderate pain, is that inflammation is something harmful that must be stopped. In the next sections we will discuss on the protective life-saving role of the inflammatory response, the existence of our own body’s resolutive mechanisms that regulate it and on when and why we need a pharmacological intervention to treat inflammation.

Inflammation: good or bad?

Although Celsus’ observation on the signs of inflammation dates back more than 2000 years, it was not until the end of the 19th century when a ‘defensive function’ of the inflammatory response was firstly proposed by Elie Metchnikoff in his work on phagocytes. Among scientists today, it is unquestionable that inflammation plays a physiological rather than exclusively pathological function. Such an important role of inflammation could be inferred by the fact that inflammatory processes are highly conserved in nature. From an evolutionary perspective, a mechanism that has been preserved and optimized along millions of years of natural selection is a strong indication that it confers a survival advantage to the individual. The ‘survival of the fittest’, as explained by Charles Darwin when he founded the basis of evolutionary biology, will lead to the accumulation in the gene pool of those genes/mechanisms that favour survival and hence increase the chances of reproduction. Those individuals that cannot cope with diseases and die early in life, will have less probability of reproducing and hence their genes will not be passed on to future generations. Then, the inflammatory response still exists and becomes operative in our body because it makes us stronger from a survival point of view. As such, inflammation is essential for the preservation of the organism’s integrity.

The inflammatory response may help deal with a bacterial infection or damaged tissue and its timely intervention and regulation allows tissue allows tissue to be ready for wound repair. A plethora of locally generated mediators which include autacoids like histamine or serotonin, lipid mediators like eicosanoids, proteins like cytokines and chemokines and plasma-derived factors such as complement, all operate to widen the blood vessels (leading to blood stasis, hence redness) and promote permeability of the vessel walls (leading to plasma protein extravasation and oedema formation, hence swelling). These initial changes are a prerequisite for white blood cells – which otherwise would move at high speed in the bloodstream – to slow down and then stop on the vessel wall. Such a process of adhesion is necessary to lead to immune cell movement outside the vasculature into the damaged tissue, in order to reach the cause of the inflammatory reaction, an example being a specific bacterium or virus. At the site of inflammation, white blood cells like the granulocytes, which are the fastest to arrive, will release toxic products including reactive species (oxygen species but also chloride and nitrogen species) to damage the bacterial wall and promote death of the causative agent. This is followed by a process of cleaning, with phagocytosis of the bacteria. During non-infective inflammation, white blood cells will phagocytose (engulf) the cause of tissue injury and destroy it in their cytoplasm.

The importance of the fundamental processes that characterize inflammation is evident by the consequences that arise when inflammation does not work. For instance, patients suffering from leukocyte adhesion deficiency (LAD), a rare genetic disorder affecting any of the genes ITGB2, SLC35C1 or FERMT3, undergo recurrent serious infections because their leukocytes are unable to move into the surrounding area and mount an appropriate response. These patients present with defective wound healing and their life expectancy is...
severely shortened to just a few years (when deficiency is marked, >80%) or up to 20–30 years of age (when deficiency is intermediate, ~40–50%).

**Regulation of the inflammatory response**

We explained earlier the basic major components of the inflammatory response, leading to immune cell infiltration into the damaged tissue and the production of ‘pro-inflammatory’ mediators necessary to protect the host. However, there is another crucial component of the inflammatory response, conceptually conceived more recently, designated as the ‘pro-resolving’ response. The pro-inflammatory mechanisms that confer protection are naturally regulated and terminated actively, rather than passively as previously believed. Those mechanisms are now very intensively studied worldwide under the field known as Resolution of Inflammation, a scientific field officially born in 2007 with a manifesto-like review article (see reference 3 - Serhan et al). For example, after a fight between neutrophils and bacteria in an infected tissue, those cells killed in the battle do not passively disappear from the tissue, but are actively cleared by macrophages which eat them to clean and restore tissue homeostasis. Hence, the process of phagocytosis is designated as a pro-resolving mechanism. The white blood cells that have migrated to the site of inflammation and do not die must enter into a resolving-reparative mode instructing and regulating stromal cells to enact an efficient repair and return to tissue homeostasis, before finally leaving. An example here is the macrophage, known to be highly plastic, which changes its phenotype and cross-talk with stromal cells (e.g. fibroblasts in the arthritic joint).

The concept of the biological nature and role of the resolution of inflammation has immediate implications for our understanding of the pathogenesis of chronic inflammation as well as for the development of new approaches to target inflammation. The definition of this process made us realize that chronic inflammatory diseases may arise, not only due to a persistence of the injurious stimulus, as believed during the ‘pre-resolution’ era, but also, and at least in part, due to a defective internal regulation of it. A direct downstream consequence of this understanding is the development of pharmacological strategies to activate and promote endogenous pro-resolving mechanisms within the patient, rather than merely reduce and deactivate the pro-inflammatory component.

**Inflammation: to treat or not to treat?**

To the question whether it is necessary to treat inflammation or not, the answer is yes, but only following a full analysis to understand the situations when an *a priori* protective mechanism may turn detrimental. These situations are explained in Figure 1. During mild-to-moderate inflammatory responses, our endogenous defensive mechanisms are sufficient to prevent any tissue damage and restore homeostasis without further pharmaceutical aid. However, the response is exacerbated in more serious situations, risking tissue integrity and function, making the use of anti-inflammatory therapies necessary.

The case of chronic inflammation deserves deeper explanation as it is behind the diseases ranked at the top of the major causes of mortality in modern societies. Heart disease, cancer, diabetes and Alzheimer’s disease are among the top 10 and they all are characterized by underlying chronic inflammation. The higher incidence of these conditions in recent times may also be explained from an evolutionary point of view. In general, these are pathologies that appear during late adulthood. If we consider Peter Medawar’s hypothesis, ageing and hence...
the diseases associated with it, do not exert any evolutionary pressure and will not be affected by natural selection. Genes or mechanisms that exert detrimental effects after reproductive age will not be gradually ‘removed’ from the gene pool along successive generations as the individuals have already reproduced before those negative effects manifested. As mentioned earlier, genes with detrimental effects on individual survival before reproduction age, will not be passed on and will eventually disappear by natural selection. Medawar’s hypothesis then suggests that ageing represents an accumulation of late-acting genes or mechanisms.

By having a look at the life expectancy records one can quickly observe that something is going on. Life expectancy at birth in the Paleolithic has been estimated to be 33 years old. In 1950, world life expectancy raised to 48, i.e. 15 years extra in about 10,000 years of human history. That means that our life expectancy has been increasing at a rate of 0.55 days per calendar year since the end of the Stone Age. However, since 1950 the increase in life expectancy has been 23.4 extra years (i.e. 71.4 years according to 2015 WHO Global Health Estimates), representing an astonishing increase for life expectancy of 131.4 days per year in only the last 65 years of human existence. This unprecedented improvement in survival of Homo sapiens in such a negligible period of time can by no means be attributed to evolution by natural selection. What indeed has changed so rapidly has been our environment including improvements in sanitary conditions, better access to food and clean water, and obviously, medical advances. Medicine allows us to live longer and healthier lives, but also allows survival (and reproduction) of individuals that may have died early in life (e.g. type-I diabetes patients) before such treatments existed. However, a side effect of this rapid environmental change is that our organism did not have time to adapt. We are not optimized for an environment with an excess of food (hence the increase in obesity) and our cells may not be ready to divide for so long without making mistakes (hence the increase in cancer). From a strictly biological point of view, modern chronic inflammatory diseases may actually be a by-product of the success of modern medicine. Our bodies have not been optimized in the last 65 years to adapt to the environment and consequently we need to intervene and design new drugs to treat our over-inflamed population.

**The Resolution Pharmacology approach**

Similar to the pro-inflammatory phase of the inflammatory response, the resolution phase is set in motion by a variety of mediators, these being bioactive lipid mediators, autacoids, gases, proteins and peptides. Pro-resolving mediators act on specific receptor targets to enable resolution, that is the series of molecular and cellular responses (e.g. apoptosis, phagocytosis, phenotype switch of the macrophage) that ensure the functional engagement of resolution and, as mentioned above, which enable return to physiological tissue function. As such, there are several ways by which these mechanisms can be harnessed for innovative pharmacological approaches. At the same time, we propose that resolution-based therapies provide a fresh way to treat or at least control chronic inflammatory conditions, perhaps imposing a turning point in the disease progression towards normal tissue functionality. As an example, in the arthritic joint, pro-resolving-based therapies can reduce synovial hyperplasia (increased cells in the synovium) through modulation of macrophage phenotype and their cross-talk with the fibroblasts, or equally possible, by acting directly on the fibroblast. An important aspect of this philosophy to innovative drug discovery is that pro-resolving-based therapies will act as agonists enabling downstream long-lasting effects through the release of other mediators and gene reprogramming, in essence changing the course of the inflammatory cascade operating within the inflamed tissue of the patient. This reliance on the patient’s own mechanisms represents a profound philosophical switch from current anti-inflammatory therapies, where the drugs available to the clinician act by blocking specific pro-inflammatory mediators and pathways.

How can we harness the biology of resolution for the development of novel anti-inflammatory therapies? At present there are at least three distinct ways in which this opportunity is being assessed, in some cases also through clinical trials.

The first possibility is based on potentiating endogenous pathways of resolution through the delivery of precursors, the classical example being the nutraceutical use of omega-3 fatty acids that should increment levels of resolvins (autacoids) in the body. Similar strategies are based on the delivery of other nutraceutical supplementation that would ‘push forward’ patients’ protective mechanisms either in the vasculature (e.g. beetroot juice to increase nitric oxide) or in the tissues (e.g. vitamin D or B12 supplementation to improve the fight against bacterial and viral infection).
The second approach is based on the synthesis of compounds that mimic endogenous pro-resolving mediators, often with the aim of increasing stability and improving pharmacokinetics. Examples that are in clinical development include adenosine analogues and analogues of resolvins and melanocortin stable peptides. Many more examples of such compounds have been described in preclinical settings, including models of arthritis as well as atherosclerosis and heart failure. In all cases, the pharmaceutical opportunity here is to develop an agonist better than the endogenous mediator to exploit their specific biology. The third way is more conventional and entails the targeting of pro-resolving mediator receptors to develop small molecule agonists. This involves the application of high-throughput screening assays and the use of a library of scaffold chemical structures and similar, ultimately leading to new chemical entities to yield new medicines.

**Future expectations for Resolution Pharmacology**

At present, with the exception of the adrenocorticotropic hormone (though its main action is through the release of cortisol from the adrenal glands) and other melanocortin-based compounds, there are no resolution-based medicines on the market. We predict that these anti-inflammatory medicines of the future would be burdened by a lower degree of side effects, since they will be working through the patients' physiological pathways of tissue protection, while at the same time providing a completely different opportunity to correct ongoing inflammatory processes typical of several chronic pathologies that affect Western societies.

In our recent literature we have used the terminology ‘Resolution Pharmacology’ to characterize this wealth of biological and pharmacological research.

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Trinidad Montero-Melendez graduated in Pharmacy at the University of Granada, Spain, where she also earned her PhD in Molecular Biology. She joined Professor Perretti’s lab in 2008 where she has been working on the concept of Resolution Pharmacology and leading the section on melanocortin biology and therapeutics. Her interests focus on translating key scientific findings on the physiopathology of inflammation into medicines for patients by working on the development and characterization of activity and modes of actions of novel molecules, involving a strong interaction with industry. Email: t.monteromelendez@qmul.ac.uk.

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**References**


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**Additional reading**

Inflammation at the inception of cancer: using fish and fly to tell the story

Yi Feng and Lisa Kelly
(University of Edinburgh, UK)

The use of fruit fly (*Drosophila melanogaster*) and zebrafish (*Danio rerio*) as models in cancer biology has recently led to the discovery that the earliest pre-cancerous cell induces an inflammatory response, which was later found to be important for pre-neoplastic cell (PNC) early progression by providing trophic support for PNC growth. Therefore, an understanding of the mechanisms that regulate the trophic inflammatory response toward PNCs might help us develop cancer prevention strategies. The use of fly and fish models for live imaging studies of the initiation of cancer and what we know so far about the signalling mechanisms involved during the earliest interactions between PNC and its host is discussed here, as well as some future perspectives.

The development of cancer is a multi-step process whereby a single cell changes and gains a growth advantage over its neighbours to become a pre-neoplastic cell (PNC). These changes arise as a result of genetic mutations in key genes and lead to uncontrolled growth of the mutant PNC, ultimately giving rise to a mass of transformed cells referred to as a tumour. While tumour progression is dependent on the accumulation of these changes over time, extrinsic factors derived from the host tissue also play an important role in tumour development. Cells within the tumour microenvironment including fibroblasts, endothelial cells, adipocytes and various immune cells, provide a vast array of signals to the developing transformed cell. Interestingly, these signals can have conflicting roles, as both pro- and anti-tumour signals are present in the tumour microenvironment. The balance of these conflicting extrinsic signals plays a vital role in determining the eventual progression or elimination of the tumour.

Immune cells such as lymphocytes and natural killer cells can sense a mutant, ‘non-self’ cell and generate an anti-tumour immune response, which eradicates transformed cells, preventing the development of tumours. However, transformed cells are capable of evading this anti-tumour response by exploiting immune-suppressive pro-inflammatory signals for tumour promotion. These pro-inflammatory signals establish a non-resolving inflammatory environment similar to that seen in chronic inflammatory conditions such as inflammatory bowel disease. For this reason, cancer has been referred to as ‘a wound that does not heal’. This chronic inflammatory environment is thought to contribute to cancer progression in a number of ways. In particular, tumour-associated macrophages and neutrophils that are conditioned in the inflammatory tumour microenvironment can suppress the anti-tumour immunity of the host. In addition, tumour-associated macrophages and neutrophils provide growth factors and inflammatory mediators to stimulate proliferation of transformed cells, angiogenesis and tumour spread. Neutrophils can also provide reactive oxygen and nitrogen species that can contribute to DNA damage and genomic instability. Recent data suggest that the inflammatory response toward tumours is elicited during the earliest initiation stages of tumourigenesis, namely the pre-neoplastic stage and its role in tumour development has become an exciting area of research.

**New animal models for studying tumour initiation**

Whilst uncontrolled chronic inflammation is considered to be a hallmark of cancer and the role of the inflammatory microenvironment in tumour promotion has been well established, there is very little empirical data on how the inflammatory response is initiated during tumourigenesis or the cells and signals involved in igniting the chronic inflammatory environment in cancer. This is largely due to difficulties in visualizing the initiation of a tumour from the pre-neoplastic stage *in vivo* using traditional murine cancer models. Recently, new cancer models have been developed using other model organisms, namely *drosophila* (*Drosophila melanogaster*) and zebrafish (*Danio rerio*). In contrast to mice, these organisms can be genetically manipulated with ease, allowing the generation of complex transgenic strains. This, combined with the optical transparency of these organisms, provides
new opportunities to visualize tumour initiation from its inception so as to monitor the development of the tumour microenvironment at the cellular and molecular level using advanced imaging techniques.

The zebrafish is an attractive model organism for studies of inflammatory responses during tumour initiation. It has a similar repertoire of innate inflammatory cells as humans, and in zebrafish the functional innate immune cells are present from very early developmental stages, from 22 hours post-fertilization (hpf) onward. However, in zebrafish, cells of the adaptive immune system are functional much later, at 2 weeks post-fertilization. This provides a time-window whereby the influence of inflammatory responses during tumour initiation can be investigated without the functional complexity of adaptive immunity.

Drosophila has been established as a model organism for decades. While it shows less genomic conservation with humans than the zebrafish, it provides a further in vivo system for modelling human disease conditions. Combining these established models with modern imaging techniques can provide important insights into the role of inflammatory responses in the developing tumour microenvironment.

In these organisms, cancer models have been generated by the overexpression of oncogenes such as oncogenic HRAS or vSRC in a variety of cell lineages and/or clonal mutation of tumour suppressor genes; these give rise to PNCs, which have the potential to establish a tumour. Inducible systems have been used to give greater control over oncogene overexpression. Such systems grant spatial and temporal control of PNC induction, which, when combined with transgenic inflammatory cell reporter lines, allows developing PNCs and cells within their microenvironment to be monitored from the time of initiation throughout tumour development.

**Inflammatory responses during tumour initiation**

Recent work has shown that PNCs induce an inflammatory response early on during initiation. In a larval zebrafish model where oncogenic forms of human HRAS or vSRC are constitutively expressed in larval skin, neutrophils and macrophages are recruited to the developing PNC at very early stages. The innate immune cells dynamically interact with the PNCs, actively making physical contact and investigating individual PNCs before moving on. As PNCs progress and the number of PNCs increases, these pro-inflammatory interactions fail to resolve and the innate immune cells remain in the developing tumour microenvironment, establishing a chronic inflammatory environment. Recruitment of these early immune cells occurs as a result of attractants released from PNCs. One of the cues required for innate immune cell recruitment is hydrogen peroxide (H$_2$O$_2$), which is both directly chemoattractive to innate immune cells and modifies the extracellular environment thereby indirectly enhancing innate recruitment. Cytokines such as CXCL8 (also referred to as IL-8) and TGFβ are also involved in the recruitment of neutrophils to the developing PNCs. We envisage more factors will be uncovered that promote the early innate immune sensing of PNCs.

*Drosophila*, on the other hand, has a single lineage of immune cells called haemocytes. These cells are macrophage-like in nature and are the main cellular mediators of innate inflammation. Interestingly, expression of mutant HRAS alone induces activation and recruitment of haemocytes toward PNCs, but this seems to be stronger in specific tissues. In addition, multiple mutations can induce a stronger inflammatory response in this model, which suggests that various mutations in PNCs have distinct contributions toward the host inflammatory response and tissue-specific signals may be involved in modulating the inflammatory response induced by PNCs.

Importantly, the interactions between developing PNCs and innate immune cells appear to be trophic in nature, aiding survival and proliferation of PNCs. Zebrafish models suggest that prostaglandins, including prostaglandin E2 (PGE$_2$), released by inflammatory cells

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**Figure 1:** (a) In fruit fly larvae, tumours often grow in the epithelium of the larval imaginal disc which needs to be dissected out, so the imaging time for live interaction between haemocytes (red) and tumour cells (green) is short or the sample is often imaged after fixing. (b) Zebrafish larvae are translucent and accessible, pre-neoplastic cells (PNC, green) can be induced in skin epithelium, the in vivo live imaging of immune cell (red) and PNC interaction can last for hours or even days.
are important for aiding PNC growth, while drosophila models indicate that cytokines, such as TNFa, are involved. To date, efforts are continuing to uncover additional trophic factors produced by innate immune cells during tumour initiation.

**Signalling pathways involved in regulating tumour initiation**

How the trophic inflammatory response is regulated during tumour initiation has become a burning question, and answers to this question may pave the way to the development of novel cancer prevention therapies. Studies in flies have revealed the importance of Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) and c-Jun N-terminal kinases (JNK) signals in regulating PNC and host cell interaction. PNC intrinsic JAK/STAT appears to determine the outcome of PNC and host cell competition. JNK activation in PNC leads to their elimination whilst JNK activation in host cells is required for their growth-promoting function. The cell-type-specific functions make these pathways difficult to target without finding cell-type-specific effectors for their activation. Using various transgenic signalling reporters one could start screening for effector molecules that are specifically required in certain cell types. In flies, various signalling reporters such as JAK/STAT, JNK, ERK and Imd (NF-kB) have been used to study PNC-host interaction and inflammatory response during tissue damage.

In zebrafish models, several signalling reporters have recently been generated, which have enabled live imaging of the TGFβ, Notch, Bmp and Shh activities during zebrafish cancer development. Again, cell-specific activation of these signals was observed either in PNCs or their neighbours. Interestingly, in zebrafish models, most transformed cells up-regulate TGFβ signalling in the developed tumour, suggesting an immune-suppressive environment.

One key regulatory pathway that has been implicated in regulating both tumour cell survival and host inflammatory response is the NF-kB signalling pathway. NF-kB is aberrantly activated in a wide range of human cancers. As a consequence, it promotes cell survival through up-regulation of anti-apoptosis proteins and growth factors; it induces innate immune cell activation in the tumour microenvironment through up-regulation of pro-inflammatory cytokines; and NF-kB is key to propagating a chronic inflammatory state, which promotes metastasis and tumour spread. Using a NF-kB reporter fish we have seen up-regulation of the pathway within PNCs as well as recruited immune cells suggesting its involvement in the inflammatory response during tumour initiation.

Given its extensive role in a wide variety of cellular processes, it is a challenge to pharmacologically target NF-kB for cancer treatment. Therefore, it is important to further dissect more cell-specific effectors of the pathway so as to guide the design of targeted therapy and prevention.

**Future Directions**

We have come a long way in our understanding of the inflammatory microenvironment during tumour initiation and development. New models in fish and fly have allowed unprecedented in vivo imaging capabilities, allowing us to observe transformed cells from their inception and to monitor their interactions with surrounding cells, particularly inflammatory...
cells. However, much remains to be done. The intricate signalling interactions between developing transformed cells and early visiting inflammatory cells can now be imaged in vivo thanks to genetic engineering of models for signalling activities in pathways of interest. Elucidation of these complex signalling interactions will help us further understand the trophic inflammatory response that appears to be vital for tumour initiation and progression. This could lead to the identification of novel therapeutic targets for cancer prevention. Methodologies for high-throughput compound screening in drosophila and zebrafish continue to be improved. Therefore, these models could be used not only to characterize the early interactions in the tumour microenvironment, but also for identification of pharmacological compounds which may be suitable as therapeutic agents. Taking a forward look, these models will continue to evolve and will provide deeper insight into cellular interactions and signalling changes that occur in the tumour microenvironment.

Further Reading

References


Additional reading

Inflammation

From the inside out: the gut microbiome and inflammatory bowel disease

Monica Viladomiu
(Jill Roberts Institute for Research in IBD, USA) and Randy S. Longman (Jill Roberts Institute for Research in IBD, USA and Jill Roberts Center for IBD, USA)

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal (GI) tract with two main clinical types: Crohn’s disease, which can affect any part of the GI tract, and ulcerative colitis (UC), which is limited to the colon. While early research focused primarily on the immune dysregulation and genetic susceptibilities associated with IBD, recent groundbreaking technological advances have allowed the investigation of additional factors, including diet and microbial exposures, in the onset and severity of disease. Advances in high-throughput microbial sequencing, anaerobic bacterial culturing techniques and generating germ-free mouse models have revolutionized our understanding of the microbial species associated with inflammation. While the long-standing clinical efficacy of antibiotics or surgery for Crohn’s disease highlights the potential contribution of the so-called ‘IBD microbiome’ to inflammation, recent seminal studies revealed the impact of IBD-derived gut microbiota on host mucosal and systemic inflammation. This mechanistic understanding of how our ‘microbial organ’ functionally impacts both mucosal and systemic inflammatory pathways will help drive novel diagnostic and therapeutic approaches for IBD.

The altered microbiome in IBD

Over a century ago, Elie Metchnikoff recognized the contributions of the gut microbiome to immunity. Specifically, he hypothesized that alterations in the normal microbiota disrupted the balance of pro- and anti-inflammatory response, which consequently resulted in disease. Although it took nearly a century to develop the necessary tools, advances in sequencing technologies over the last decade have enabled scientists to further evaluate Metchnikoff’s hypothesis. These advances allow the specific taxa and strain level identification of bacteria within complex, anaerobic microbial communities, which are difficult to characterize by standard culture-based tests. Faecal microbiome analyses using these novel tools have revealed characteristic changes in the microbiome of IBD patients. Collectively, these studies reveal a contraction in IBD microbial diversity (particularly in Crohn’s disease) compared to healthy individuals, which frequently parallels an expansion within the phylum Proteobacteria and a concomitant decrease in certain Firmicutes such as *Faecalibacterium prausnitzii*.

While these findings support an association between microbial dysregulation and IBD, is there any data to suggest a direct contribution of these changes to inflammation? In a study evaluating disease recurrence following surgery for Crohn’s disease, the abundance of *F. prausnitzii* inversely correlated with endoscopic inflammation after 6 months. In line with the anti-inflammatory properties of these bacteria, *F. prausnitzii* and its secreted factors were able to counterbalance intestinal inflammation in mouse models of colitis. Furthermore, analysis of the microbiome in a recent inception cohort of paediatric-onset Crohn’s disease showed that microbial differences (including an increase in Enterobacteriaceae, Pasteurellaceae, Veillonellaceae and Fusobacteriaceae) diagnostically define disease activity before any treatment is initiated.

Although IBD is primarily characterized by GI tract inflammation, about 30% of IBD patients present with extra-intestinal manifestations including liver, skin, eye and most commonly, joint inflammation. Clinical evidence linking intestinal inflammation with extra-intestinal joint inflammation or spondyloarthritis (SpA) has implicated the gut microbiota as the antigenic stimulus of aberrant systemic inflammation. Supporting this possibility, we recently discovered the enrichment of a unique *Escherichia coli* pathotype called Adherent-invasive *E. coli* (AIEC) in patients with Crohn’s disease-associated SpA. AIEC closely associate with the epithelial cell layer and are sufficient to induce colitis and arthritis in mouse models. Collectively, these data support both a diagnostic and functional role for the microbiome in IBD.
Minding the borders

The GI tract harbours the largest microbial community of the body. A single-cell epithelial cell barrier, covered by a thick mucus layer establishes a ‘demilitarized zone’ that keeps intestinal bacteria physically separated from the host immune system. This highly evolved intestinal architecture reinforces compartmentalization and avoids unrestrained inflammation under normal conditions. How do the microbiota impact both mucosal and systemic inflammation?

One of these mechanisms is the production of soluble factors, particularly short chain fatty acids (SCFAs), which are end products of bacterial fermentation of dietary fibre including butyrate, succinate and propionate. These molecules act on the host epithelium through a specific G-protein coupled receptor and serve as a trophic factor for the epithelial border. In humans, surgical diversion of the intestine can commonly result in ‘diversion colitis’, which is thought to be secondary to the lack of bacteria-derived SCFAs and can be treated with butyrate enemas.

Despite the epithelial barrier, interactions between the immune system and the microbiota still occur under non-inflammatory conditions. Two factors contribute to this regulated interaction. First, some microbiota have the ability to penetrate the mucus layer and associate with the epithelial cell border. This subsequent close interaction and cell adherence is essential for the productive impact on mucosal and systemic immunity. Second, macrophages, which act as sentinel cells within the lamina propria of the mucosa, closely associate with the epithelial cell layer. These macrophages can extend dendrites across the tight junctions of epithelial cells and capture bacteria (or pieces of bacteria). This sampling provides a regulated portal for the immune system to integrate signals from key microbiota.

Microbial regulation of T lymphocytes in inflammatory disease

A cell type of particular interest to immunologists who study inflammatory disease is the T helper (or CD4+) T cell. These T cells play a critical role in enforcing barrier immunity, but are also the foot soldiers supporting inflammation. Initially, two types of T helper (Th) cells were described. Th1 cells were considered to be primary mediators of inflammation and autoimmunity while Th2 cells were found to promote allergic responses. Over the last 10 years, two additional types of Th cells that play a central role in intestinal immunity and inflammatory disease have been discovered—regulatory T (T_{reg}) and Th17 cells. T_{reg} cells were found to play an essential role in suppressing aberrant inflammation in various tissues including the intestine. In contrast, Th17 cells, named after their production of cytokine IL-17, are thought to play a pathogenic role in autoimmunity. Genome-wide association studies identified genetic variants in Th17-related genes in rheumatoid arthritis, ankylosing spondylitis and IBD. However, Th17 cell activation may act as a double-edged sword in mucosal immunity by promoting neutrophil recruitment and anti-microbial protein production to protect against pathogenic bacteria and fungi.

While considerable attention has been given to the molecular elements controlling T_{reg} and Th17 differentiation, recent studies have begun to detail the mechanisms by which gut microbiota shape the balance between Treg and Th17. Natural Treg arise early on during thymic selection mainly from the CD4-single positive cells, while inducible T_{reg} cells are generated and regulated by commensal microbiota. Pioneering work using mice colonized with human microbiota identified 17 strains of bacteria within the Clostridia cluster IV, XIVa and XVIII (including F. prausnitzii) which can effectively induce T_{reg} cells in the colon. Parallel work in mice identified commensal bacteria, which exert a disproportionately large effect in generating mucosal Th17 cells. This serendipitous finding that mice from one vendor (Taconic Farms) had significantly more Th17 cells than mice from another vendor (Jackson Laboratories) led to the identification of a particular commensal bacteria called segmented...
filamentous bacterium (SFB), responsible for inducing Th17 cells in the intestine. More recently, AIEC derived from patients with Crohn's disease were also found to specifically induce Th17 cells in the gut and throughout the body.

The inflammatory effects of Th17 cells are not one-dimensional. In both mice and humans, microbial induction of Th17 cells can protect the intestine against infection. Surprisingly, treatment of Crohn's disease patients with secukinumab (anti-IL-17 therapy) not only fails to improve, but may even precipitate intestinal inflammation. However, in both mice and humans with genetic susceptibility to inflammatory disease, including IBD and arthritis, microbial triggers of Th17 cell activation can be sufficient to induce inflammatory disease.

There are several models used to describe the activation of autoimmunity:

1. **Antigen-specific molecular mimicry**: this model suggests that antigens supplied by the microbiota mimic a self-antigen and this allows for self- or cross-reactive inflammation.

2. **Epitope spreading**: this model describes antigens exposed by collateral damage of normal tissue, which then feed continued self-reactive priming.

3. **Inflammatory micro-environment**: this model allows for the generation of self-reactivity in a context-dependent fashion.

The specificity of the inflammatory Th17 cell response remains an active area of research.

**Therapeutically targeting microbial inflammation in IBD**

How can we disrupt the pro-inflammatory effects of microbiota on intestinal and systemic autoimmunity? There are several access points that can be envisioned. First, we can actively change the microbial community. Faecal microbiota transplantation (FMT) or transplanting faecal microbiota from a healthy donor to a recipient with IBD is an emerging therapeutic option. FMT is a highly successful therapeutic strategy for recurrent *Clostridium difficile* infection, which works by blocking niche colonization to prevent re-infection (a process called colonization resistance). In IBD, however, there is no overt pathogenic infection, so the potential efficacy of FMT for IBD may result from the direct or indirect effects of the microbiota on the mucosal immune system. Recent placebo-controlled trial data supports the potential efficacy of FMT in treating ulcerative colitis. The initial data suggest that the donor microbiota may hold the key for understanding the effects, but the particular immune-modulating bacteria remain elusive.

Another critical target for regulating inflammation in IBD is T cell trafficking to the intestine. Surface proteins including chemokine receptors, integrins and sphingolipids can all serve as critical mediators of T cell homing to the intestine. Microbial activation of dendritic cells draining to secondary lymphoid organs activate the production of retinoic acid, which subsequently serves to imprint T cells with the homing receptors CCR9 and α4β7. Blockade of α4β7 with vedolizumab is clinically effective in improving mucosal inflammation in ulcerative colitis. This paradigm-shifting therapeutic approach to IBD supports additional targets of T cell egress from the lymph nodes such as sphingosine-1-phosphate blockade, which has already shown efficacy in multiple sclerosis and ulcerative colitis.

Finally, extra-intestinal inflammatory manifestations associated with IBD may reveal shared pathways allowing for more precise anti-inflammatory therapeutics. For example, we have recently shown that Crohn's disease patients with systemic manifestations of spondyloarthritis have increased Th17 cells in both the mucosa and...
the peripheral circulation. Anti-IL-23 therapy (ustekinumab) was recently approved for the treatment of Crohn’s disease. It will be exciting to see if these extra-intestinal manifestations identify an IBD phenotype that will more favourably respond to this therapy.

Future directions

The gut microbiome plays a critical symbiotic role for the host, participating in energy-nutrient extraction and blocking pathogenic infection. Although a reinforced mucosal barrier keeps the bacteria and the host separated, these recent advances have shown a definitive role for microbiota and certain keystone species in shaping homeostatic immunity and inflammation. These mechanistic discoveries highlight key therapeutic targets for microbial-induced inflammatory disease that will help guide the use of more precise medical therapy.

Further reading

Inflammation

The microbiome and food that fuels the fire of inflammation

Silvia Melgar (University College Cork, Ireland)

Inflammatory bowel disease (IBD), encompassing two main conditions – Crohn’s disease (CD) and ulcerative colitis (UC), are multifactorial chronic intestinal inflammatory diseases with increasing incidence worldwide, especially in countries adopting a westernized lifestyle. Recent findings point towards a major impact of diet on human health since it can affect both the gut microbiota and the host response. Epidemiological studies have identified that the consumption of processed food, red meat, saturated fat and low fibre/vegetables are high-risk factors for IBD. While the consumption of fatty fish, fermentable fibres and vegetables are lower risk for IBD. Experimental studies have supported these findings. Animals fed certain specific diets demonstrate alterations in host immune responses and microbiota composition including the blooming of pathobionts as a result of diet treatment. Recent seminal studies have also provided evidence on the role of food additives such as emulsifiers in IBD and metabolic diseases. In the future, controlled trials and mechanistic studies will identify diet-induced beneficial or triggering mechanisms which will lead to the development of new treatment strategies for these debilitating diseases.

Contribution of inflammation, genetics and microbiota to IBD

The aetiology of IBD is still unknown despite its description in 1859 by Wilks and Moxon (for UC) and in 1932 by Crohn and colleagues (for CD). The collected evidence to date indicates that environmental factors such as smoking, stress and particularly the microbiota can trigger and/or sustain a tissue-damaging immune response in genetically susceptible individuals. A reduction in microbial diversity with an increase in Proteobacteria and a reduction in beneficial commensals in the Firmicutes phylum have been identified in both conditions. Among these, *Escherichia coli* strains, so-called adherence and invasive *E. coli* (AIEC) have been isolated from CD mucosa and shown to have pro-inflammatory properties in experimental studies. Other commensal strains such as, *Faecalibacterium prausnitzii* has shown anti-inflammatory potential, which is why strategies to increase its presence in IBD patients are under investigation. Large genome-wide analysis studies (GWAS) have identified over 160 susceptibility genes, several of which are associated with the innate immune response (e.g. NOD2), the adaptive immune response (IL-23R) and barrier function (HNF4A). Crohn’s disease can affect any part of the gastrointestinal tract but it is generally localized in the distal ileum and proximal colon. The inflammation is patchy and often transmural resulting in fibrosis, fistulas, fissures, strictures etc. Typical features of CD include the presence of dense infiltrations with macrophages and lymphocytes which can form granulomas in the submucosa of the intestinal wall. Patients with CD present a T helper (Th)1 and Th17 profile and high levels of innate cytokines such as TNFa and IL-1β. Ulcerative colitis, on the other hand, is restricted to the colonic mucosa and the inflammation often originates in the rectum extending proximally. Typical features of UC include crypt abscesses, ulcerations and goblet cell loss. The mucosa in patients with UC presents an atypical Th2 cytokine profile accompanied by high levels of IL-5, IL-10, IL-13 and transforming growth factor beta (TGFβ).

Diet as a trigger of inflammation and microbial changes

In the last four decades, there has been an increased incidence of autoimmune disease such as asthma, allergy and IBD. These conditions are all multifactorial and although a genetic predisposition exists, the emerging incidence cannot be solely attributed to genetic modifications. Over the last decade, it has become evident that the microbiota is highly involved in the regulation of several aspects of life, including the development of the immune system as well as being a major factor in fighting invading pathogens. More recent evidence has also uncovered that dietary nutrients can shape the intestinal environment by having a crucial impact on the intestinal microbiota and on mucosal immune responses. For example, the consumption of a westernized diet, characterized by excessive amounts of refined and processed foods, red meats and sugary beverages, and a low consumption of fibres, fruits and vegetables, is associated with
the increased manifestation of metabolic diseases such as diabetes and obesity and systemic low-grade inflammation, supported by pro-inflammatory cytokines IL-6 and TNFα. Interestingly, germ-free mice (mice devoid of a microbiota) fed a high fat diet do not develop these features, indicating an important role for the microbiota on the host response and subsequent disease development. The immune response in obese mice is highly associated with change in the macrophage population from anti-inflammatory M2 cells in lean individuals to a pro-inflammatory M1 profile in obese individuals. Similarly, a reduction in T regulatory cells (T\(_{reg}\)) is associated with the obese phenotype. The consumption of red and/or processed meat, dietary fat (especially n-6 poly unsaturated fatty acids [PUFAs]), low levels of vitamin D and alterations in gut microbiota are associated with an increased risk of IBD. Omega 6 (n-6) PUFAs, found in dietary fat from meat, have been generally attributed to be pro-inflammatory as they give rise to arachidonic acid which can result in the production of both pro- and anti-inflammatory eicosanoids such as prostaglandins and leukotrienes. Several similarities exist between individuals with obesity and IBD, e.g. induction of pro-inflammatory cytokine IL-6 and TNF, reduction in Tregs, etc. However, to date, a correlation between obesity (measured as body mass index, BMI) and IBD morbidity has not been identified. Recent evidence also indicates that the type and quality of fat can affect the subsequent host response differently. For example, a diet rich in milk fat led to an aggravation of intestinal inflammation due to an exacerbated pro-inflammatory Th1 response and blooming of a pathobiont, *Bilophila wadsworthia* in IL-10-deficient mice (IL-10\(^{-/-}\)). In contrast, other types of fat such as a lard-based fat diet or medium-chain triglycerides resulted in either no effect, worsening or amelioration of inflammation. As alluded to earlier, processed foods have been identified as a risk factor for IBD and a hypothesis launched by Roberts and colleagues suggested that the increased incidence in CD was the result of a higher consumption of emulsifiers present in processed foods. This hypothesis was elegantly proven by Chassaing and colleagues who reported aggravation of metabolic syndrome in mice deficient in Toll-like receptor (TLR)-5 and an aggravation of colitis in IL-10\(^{-/-}\) mice when exposed to two common emulsifiers, carboxymethylcellulose (CMC) and polysorbate-80 (P80). The aggravated colitis was accompanied by increased gut permeability and reduced mucus thickness, which promoted higher
penetration of intestinal bacteria, elevated levels of myeloperoxidase (MPO) and pro-inflammatory mediators as well as an altered microbial composition, particularly the enrichment in *Bilophila* spp. The effects of emulsifiers on other systems such as intestinal epithelial cells and an *ex vivo* faecal culture system are supportive of these findings. The relevance of these findings on the host immune response in human IBD and in other chronic conditions is yet to be addressed.

**Dietary nutrients for the management of IBD**

In contrast to dietary fats, diets rich in fish (containing n-3 PUFAs), fermentable fibres and vegetables appear to lower the risk for IBD. The gut microbiota play a vital role in the fermentation of undigested food components such as complex carbohydrates into the short chain fatty acids (SCFA) acetate, propionate and butyrate. Butyrate is the principal energy source of the colonic epithelium and modulates enterocyte differentiation, proliferation and restitution. Several immune-modifying properties have been attributed to butyrate including the reduction of pro-inflammatory cytokines, the induction of Tregs differentiation and the regulation of epigenetic gene transcription by inhibiting histone deacetylases (HDACs). SCFAs can bind to different G-coupled protein receptors (GPCRs), including GPR41, GPR43 and GPR109a, which can be found in the intestinal mucosa on enteroendocrine L cells, mast cells and leukocytes (GPR41 and GPR43), and on colonocytes/enterocytes and immune cells (GPR109a). Binding of SCFAs to GPCRs can regulate several pathways including mitogen-activated protein kinase signalling (MAPK) and nuclear factor κB (NF-κB). To date, much of the anti-inflammatory potential attributed to SCFAs has emerged from experimental models and although there are some reports attributing a beneficial effect of SCFAs in the treatment of IBD, especially in UC, placebo-controlled trials are scarce.

Omega 3 and 6 (n-3 and n-6) PUFAs have been generally attributed to be anti-inflammatory and pro-inflammatory, respectively. Conflicting results have been observed in experimental murine models, although mice fed with fish oil showed a reduction in macrophage-derived TNFα, IL-1β and IL-6 upon lipopolysaccharide activation. Studies in patients with CD have also shown similar confounding results as in animal models, although a beneficial effect with fish oil was reported in patients with CD. In contrast, no effect of n-3 PUFA supplementation was observed in patients with UC.

Patients with IBD are generally deficient in vitamin D, which can regulate several inflammatory pathways including TLR- and NF-κB signalling, Th17/Tregs response, apoptosis, cell proliferation and differentiation, barrier function etc. Supplementation of vitamin D to mice with intestinal inflammation ameliorated disease by reducing pro-inflammatory cytokine secretion, improving barrier function and microbial composition. Therefore, vitamin D monitoring and supplementation in patients with IBD is of vital interest.
Diet as an induction and maintenance therapy has been mostly investigated in paediatric IBD. The most broadly researched dietary intervention is exclusive enteral nutrition (EEN), which has been used for the induction of remission of children, and, to a certain degree, adults with mild to moderate CD. Few mechanistic studies have addressed the effect of EEN on inflammatory responses, although reduction in pro-inflammatory mediators (IL-2 and IFNγ from immune cells and chemokine production from epithelial cells) and an induction of the anti-inflammatory cytokine (TGF-β) have been reported in the literature. Other mechanisms targeted by EEN include bowel rest, improvement of epithelial barrier and positive changes of the intestinal microbiota.

Other dietary interventions include partial enteral nutrition (PEN), which entails the use of a liquid enteral formula, in addition to consuming food, for maintenance of remission or treatment of active CD. In addition to EEN and PEN, the elimination of certain diets for disease management of IBD has also been addressed. Among these are the Specific Carbohydrate Diet (SCD), the Crohn’s Disease Exclusion Diet (CDED), the Anti-inflammatory diet (IBD-AID), the Allergen elimination diet (IgG), the Semi-vegetarian diet (SVD), the low Fermentable Oligo-saccharides, Di-saccharides, Mono-saccharides And Polyols diet (FODMAP), the Mediterranean Diet, etc. The findings to date using these diets are encouraging however, there is a lack of randomized controlled trials and mechanistic studies to evaluate their efficacy in patients with IBD.

Future directions

To date, epidemiological evidence from observational studies indicate that the intake of fibre-rich food, containing fruits and vegetables can protect against IBD while a westernized diet, rich in processed food, red meat and saturated fat can worsen IBD. Our current knowledge of diet as a trigger or as a treatment strategy for IBD emanates from experimental models. Therefore, more randomized controlled trials are necessary to identify potential benefits/trigger mechanisms affecting IBD pathology. In addition, mechanistic studies on how diets can modulate host immune and microbiota responses are necessary to better understand and design personalized treatments. Investigations into other food additives such as sweeteners, emulsifiers, thickeners, preservatives and food colourings are also necessary in light of the complex role that environmental factors have on the host response and microbial composition in these chronic inflammatory conditions.
Computational chemistry in the search for improved therapeutics for inflammatory and autoimmune diseases

Rheumatoid arthritis (RA), Crohn’s disease and multiple sclerosis are common diseases, which are directly related to interactions developed between a protein called the Tumour Necrosis Factor (TNF) and its receptors. Current drugs that effectively disrupt TNF–receptor associations also cause serious side effects. In this feature, we present a computational scheme that resulted in the discovery of two non-toxic compounds, which directly inhibit TNF function. Moreover, the polypharmacology of these compounds was demonstrated as they were also found to inhibit the function of RANKL, a member of the TNF protein family. Optimization of the compounds may lead to the development of new medications for inflammatory and autoimmune treatments.

Virtual screening accelerates anti-inflammatory drug discovery

Drug design directed at developing small-molecule TNF inhibitors can effectively overcome most of the above impediments, although it is far from being a trivial task in pharmaceutical research. Successful drug design requires the identification of compounds with low molecular weight, something extremely challenging, especially when attempting to block interactions between large molecules such as proteins. A very potent TNF inhibitor, SPD304, directly hinders TNF trimerization by blocking the formation of the biologically active complex; however, SPD304 appeared to be significantly toxic. Despite a few additional compounds that have recently been identified as direct TNF inhibitors, little progress has been made regarding the discovery of effective and harmless biological agents against inflammation.

Computational chemistry approaches can be particularly useful in the identification of compounds that match the aforementioned requirements for anti-inflammatory action and low toxicity. This can be achieved by applying a combination of molecular mechanics theory with simple energy terms (i.e., electrostatic, van der Waals, hydrogen bond and hydrophobic interactions) to virtual atoms that represent molecular structures (compounds and protein) in order to predict the strength of association between a compound and the TNF.
The process of collecting, retrieving, storing, combining and applying information about molecular structure, physico-chemical properties, docking sites and other relevant parameters is generally called cheminformatics. In the search for new anti-TNF compounds, various cheminformatics methods can be combined with molecular dynamics (a computer application that simulates the motion and evolution of atoms and molecules) to recognize substances as candidates for effective TNF inhibition.

To aid the discovery of improved drugs that effectively inhibit TNF action, a new computer-based platform for the virtual screening of large data sets of compounds has been developed. This platform uses a combination of several computational algorithms to predict the interactions and binding of small molecules to TNF. Thus, the chemical structures of over 14,000 diverse compounds with unidentified activities were virtually screened to estimate how effectively these molecules disrupt TNF trimerization. This procedure led to the 'filtering' of nine small molecules as potential candidate inhibitors. Importantly, these predictions were further validated through biological experiments that were conducted via traditional laboratory practices. The successful application of the above-mentioned computational protocol and the subsequent experimental validation resulted in the identification of two particularly effective TNF inhibitors, namely compounds T23 and T8. Toxicity indexes for both compounds were also estimated to be very low, thus suggesting they may be administered without severe side effects. A representative image of the most potent compound (T23) in TNF is shown in Figure 1. Experimental testing for thousands of compounds is unfeasible on a realistic timescale, and even for a much smaller number of molecules, their evaluation is a very slow and expensive procedure. The main advantage of computational approaches is that one may obtain reliable predictions in a very fast and low-cost way, thus greatly facilitating drug discovery.

**Polypharmacology could enhance drug action and reduce side effects**

Generally, pharmaceutical molecules interact with multiple target proteins, thus exerting secondary, often unwanted actions. Therefore, drug design procedures usually focus on the identification of substances that display major selectivity toward a particular target. However, in some cases, high binding affinity of a molecule to multiple target proteins may be desirable, considering that binding to several specific receptors may produce enhanced actions or compensate harmful effects. This approach is known as polypharmacology or multi-target drug design.

Inhibition of another protein, RANKL (Receptor activator of nuclear factor kappa-B ligand), has been shown to significantly diminish fracture incidents in postmenopausal women and a recent RANKL inhibitor has been recognized for its profound therapeutic effects against osteoporosis. RANKL belongs to the same protein family as TNF and constitutes an ideal target to evaluate the polypharmacologic potential of...
T23 and T8. Initial computational considerations were subsequently confirmed through biological assessments and demonstrated the inhibitory action of the two compounds against RANKL as well. Similar to the TNF system, RANKL inhibition was not accompanied by any significant toxic effects; therefore, it was demonstrated that T23 and T8 act as dual inhibitors of TNF and RANKL. This finding denotes the advanced pharmacological actions T23 and T8 may exert, thus further strengthening their role as potent anti-inflammatory agents.

**Compound screening against inflammation made easy**

The above computational scheme may significantly aid the development of new medications for the treatment of inflammation and autoimmune diseases, however, the successful application of such approaches is a complicated task since it requires specialized knowledge and a solid background in cheminformatics and molecular simulation techniques. To make the model predictions of drug candidates accessible to any interested user, the proposed model was built based on open source algorithms and was made publicly available online through the Enalos Cloud Platform (http://enalos.insilicotox.com/TNFPubChem/). The web service does not require special computational skills and can be easily operated by diverse groups of scientists like chemists, biologists, engineers, physicists or even non-experts involved or interested in effective TNF inhibition by new chemical substances. In this way, the complex computational algorithms are incorporated in the platform, which operates in an automated fashion by simply feeding molecular structures for further inhibition evaluation. Next, the platform quickly generates output predictions regarding the inhibitory potency of any drug candidate against TNF. The Enalos Cloud Platform is a web service that incorporates a fully validated, predictive model that can be used in small molecule anti-inflammatory drug design.

**Future perspectives**

Combating inflammatory and autoimmune diseases requires the ever-increasing efforts of multidisciplinary areas of science toward the development of potent and risk-free medications. In this direction, the use of computational chemistry may greatly facilitate the discovery of new compounds, which prevent specific interactions between TNF or RANKL and other proteins without inducing unwanted side effects (for instance, computationally identify and exclude from further study, pan-assay interference compounds (PAINS)). Then, newly discovered anti-inflammatory compounds, such as T23 and T8, may be used as direct
protein inhibitors or they could serve as molecular templates for further computational optimization, in search of optimal treatments against autoimmune conditions and inflammation. The development and public dissemination of a web-based platform (Enalos Cloud) for the fast and accurate virtual screening of small molecules can rapidly advance the identification of other (possibly improved) compounds as potent TNF or RANKL inhibitors. Importantly, the Enalos platform can be easily expanded to include other target proteins, thus also enabling the reliable virtual screening of compounds related to a wide range of additional diseases.

Further reading

References


Additional reading

Development of NOSH-NSAIDs: a new class of anti-inflammatory pharmaceuticals for the treatment of cancer

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Non-steroidal anti-inflammatory drugs (NSAIDs) have anti-cancer (chemopreventive) properties; however, side effects preclude their long-term use. NOSH-NSAIDs, designed as safer alternatives, are novel hybrid chimaeras that release nitric oxide (NO) and hydrogen sulfide (H2S). NOSH-NSAIDs are gastrointestinally safe yet retain all the pharmacological properties of their native NSAID. NOSH-NSAIDs are orders of magnitude more potent than their conventional counterparts in inhibiting the growth of various human cancer cell lines of different tissue origins, adenomatous, epithelial and lymphocytic. This growth inhibition is a result of a reduction in cell proliferation and cell cycle arrest, leading to increased apoptosis. In xenograft mouse models of cancer, NOSH-aspirin was better than normal aspirin as a chemopreventive agent; it dose-dependently inhibited tumour growth and tumour mass. NOSH-naproxen was significantly more efficacious than normal naproxen in reducing the growth of established tumours.

Evidence that NSAIDs protect against cancer

NSAIDs are primarily used: (i) as analgesics to relieve the most common forms of pain, (ii) as anti-pyretics to reduce fever, (iii) as anti-inflammatory agents and (iv) as anti-thrombolytics – the so-called four ‘As’. A key piece of evidence that links cancer and inflammation is that the use of NSAIDs such as aspirin reduces the risk and mortality from many cancers. Over 30 epidemiological studies, collectively describing results on more than 1 million individuals, have established NSAIDs as the prototypical chemopreventive agents against many forms of cancer. In particular, three well-designed randomized, double-blind trials of aspirin as a chemopreventive agent against colorectal adenomas established its chemopreventive effect. These studies have expanded to underscore the chemopreventive effects of NSAIDs in general against colon, breast, pancreas, bladder, head and neck, oesophageal, ovarian, prostate, hepatocellular and skin cancers. Of significance are two relatively recent reports indicating that daily aspirin use, whether regular strength or low dose, not only resulted in reductions in cancer incidence and mortality, but also prevented distant metastasis.

The general concept of cancer chemoprevention by NSAIDs cannot be overstated. However, all studies to date have failed to provide details of the big picture. For example, which one of the nearly 30 NSAIDs is the most effective? What is the optimal dose and what is the ideal...
schedule of administration? Even if such information existed, it would probably be of limited or no practical value. The reason is that, although we have unassailable proof-of-principle for chemoprevention, current NSAIDs cannot overcome two prohibitive limitations concerning their safety and efficacy. For example, for colon cancer, the one most thoroughly studied, NSAIDs can prevent at best 50% of the cases; all NSAIDs eventually cause some degree of gastrointestinal (GI) erosion that may eventually lead to ulcers, with most having cardiovascular and renal side effects.

**Side effects of NSAIDs limits their long-term use**

Although the use of NSAIDs in general, and aspirin in particular, as a chemopreventive agent is highly convincing, the use of these drugs is limited by their significant toxicity, which largely fall into three areas: GI, ranging from dyspepsia to GI bleeding, obstruction and perforation, and renal and cardiovascular. It is estimated that about 16,500 NSAID-related deaths occur among patients with rheumatoid arthritis or osteoarthritis every year in the United States. This figure is greater than the number of deaths from multiple myeloma, asthma, cervical cancer or Hodgkin’s disease. If deaths from GI toxic effects from NSAIDs were tabulated separately in the National Vital Statistics reports, these effects would constitute the 15th most common cause of death in the United States. Yet these toxic effects remain largely a ‘silent epidemic’, with many physicians and most patients unaware of the magnitude of the problem.

**The search for better NSAIDs**

Many different approaches have been attempted in this area, the most notable being the generation of selective cyclooxygenase-2 (COX-2) inhibitors or coxibs. In patients with a low risk for GI damage, coxibs have been successful in the short term in limiting the upper GI ulceration associated with traditional NSAIDs. However, in patients with co-morbidities or other factors that increase the risk of GI ulceration, the benefits of coxibs over traditional NSAIDs is significantly reduced. Several large-scale clinical trials have shown that long-term use of coxibs and even traditional NSAIDs is associated with an increased risk of adverse myocardial events. Also, COX-2 inhibition in the kidneys could lead to increases in blood pressure and hence increases in myocardial infarctions and stroke. These major side effects have resulted in a number of coxibs being withdrawn from the marketplace and some have suggested that there might be a ‘class effect’ associated with coxibs. Currently, all coxibs and traditional NSAIDs are required by the FDA (Food and Drug Administration) to carry a black-box warning.
Inflammation

cardiovascular and renal safety profiles because NO and H$_2$S have protective roles in the cardiovascular and renal systems. We also considered the possibility that there could be synergy between NO and H$_2$S thus increasing potency and perhaps efficacy.

**NOSH-NSAIDs have enhanced GI safety profiles**

We used an acute model of ulcerogenesis to compare the effects of three traditional NSAIDs, aspirin, naproxen and sulindac, to their NOSH counterparts. We chose these three NSAIDs because aspirin is used extensively worldwide not just as a ‘painkiller’ but for its fever and blood-clot-reducing properties and is used in patients who have had a heart attack, it has also been shown to have chemopreventive properties. Naproxen is mainly used as a potent anti-inflammatory and is employed in the management of osteoarthritis, but is known to have significant GI toxicity. Sulindac is used in the management of patients with familial adenomatous polyposis (FAP), a disease which is characterized by hundreds of colorectal adenomatous polyps that eventually progress to colorectal cancer; sulindac use is limited by its toxicity, which can affect up to 20% of patients.

Rats treated with just the vehicle had a normal glandular region on the surface of their stomach, i.e. no ulcerative damage. However, administration of aspirin, naproxen or sulindac resulted in extensive mucosal injury, depicted as bleeding ulcers. Unlike these NSAIDs, treatment with NOSH-aspirin, NOSH-naproxen or NOSH-sulindac at equimolar doses did not produce any significant ulcerative damage, representing a remarkable enhancement in GI safety (Figure 2). This observation is in line with the protective roles of NO and H$_2$S within the gastric mucosa.

**NOSH-NSAIDs – basic pharmacological properties**

In the United States, over 30 million people use NSAIDs on a daily basis, therefore a large number of people can potentially be subjected to untoward effects. Having established that NOSH-NSAIDs were GI safe/friendly we then evaluated these novel chimaeras against their respective native NSAIDs in various in vivo settings addressing the four ‘As’.

We compared the anti-inflammatory properties of aspirin, naproxen and sulindac, to that of NOSH-aspirin, NOSH-naproxen and NOSH-sulindac in rats. In general, all three NSAIDs and their respective NOSH counterparts caused a significant reduction in inflammation. Results for aspirin versus NOSH-aspirin are shown in Figure 3, we have reported similar results for NOSH-naproxen and NOSH-sulindac.
It is well known that NSAIDs exert a moderate anti-pyretic effect when administered orally. We therefore wanted to determine the decrease in body temperature induced by NOSH-NSAIDs compared with that obtained with NSAIDs (also in rats). Qualitatively similar results were obtained for all NSAIDs/NOSH-NSAIDs, with the results for aspirin and NOSH-aspirin shown in Figure 4A.

NSAIDs in general are the mainstay in the treatment of pain. In that respect, we compared the analgesic effects of NOSH-NSAIDs with that obtained with NSAIDs (also in rats). Qualitatively similar results were obtained for all NSAIDs/NOSH-NSAIDs, with the results for aspirin and NOSH-aspirin shown in Figure 4A.

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Using NOSH–NSAIDs to target cancer

Studies evaluating the effect of NOSH-NSAIDs on the growth of a variety of cancer cells showed that they are much more potent than the corresponding traditional NSAIDs at inhibiting the growth of these cultured cancer cells. Chemopreventive properties of the two types of NSAIDs were evaluated using colon, breast, pancreas, prostate and lung cell lines, as well as those from T-cell leukaemia. For NOSH-sulindac the potency enhancement over sulindac ranged from 1,000–10,000-fold; for NOSH-naproxen the increase over naproxen was 16,000–34,000-fold; and for NOSH-aspirin the potency enhancement over aspirin was approximately 16,000–100,000-fold. After 72 hours in colon cancer cells, this enhanced potency increased to about 250,000-fold. Although these NOSH-NSAIDs exhibited potent anti-cancer activity profiles, they minimally affected normal cells. It is important to note that these studies strongly suggest that the actions of NOSH-NSAIDs appear to be a generalized property (i.e. their effect is tissue-type independent) and that NOSH-aspirin is the most potent hybrid.
produce HSNO, which is a highly reactive intermediate. In a mouse model of colon cancer, NOSH-aspirin was shown to be significantly more effective (around 5-times more potent) at reducing tumour growth and mass than aspirin alone, without the adverse GI effects of aspirin. Clearly, as a chemopreventive agent, NOSH-aspirin is superior to aspirin both in terms of efficacy and safety. These results further strengthen our original hypothesis that incorporating NO and H₂S-releasing moieties within the aspirin molecule would enhance both its activity/potency and safety profiles.

Similarly, studies with NOSH-naproxen versus naproxen using an in vivo xenograft mouse model of colon cancer showed that NOSH-naproxen treatment resulted in large reductions in tumour volume and mass. The decrease in tumour size and mass was associated with inhibition of cell proliferation and induction of apoptosis. The dose of NOSH-naproxen used in this mouse model was well tolerated with no apparent harmful side effects or overall gross toxicity; but all the

Mechanistically, using HT-29 colon cancer cells as a model, the cell growth inhibition exhibited by NOSH-NSAIDs was a result of a reduction in cell proliferation and cell cycle arrest (G₀/G₁ or G₂/M), leading to increased apoptosis.

However, the underlying mechanism(s) for the enhanced potency of NOSH-NSAIDs is not apparent and there is no information available regarding the kinetics of NO and H₂S release and their potential interactions. What is certain is that both NO and H₂S contribute towards the potency of the intact molecules. This is based on our earlier observations where we showed that the biological activity of aspirin plus SNAP (S-Nitroso-N-acetyl-penicillamine, which releases NO) plus ADT-OH (5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione, which releases H₂S) was not the same as the biological activity of the intact NOSH-aspirin molecule. Thus, the sum of parts did not equal the whole. These molecular aspects need further investigation as it has recently been reported that NO can react with H₂S to produce HSNO, which is a highly reactive intermediate.

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mice given naproxen alone died by the end of the second week of treatment from what appeared to be GI bleeding and damage to other organs such as the spleen and liver.

**Future directions**

Our studies have established three key features of NOSH-NSAIDs: (i) NO and H$_2$S are both required for the enhanced potency of NSAIDs for inhibiting cancer cell growth, (ii) this enhanced potency is manifested in cancers of varied tissue origin and (iii) NOSH-aspirin is consistently the most potent NOSH-NSAID regarding these properties. In our proof-of-concept animal studies, we have demonstrated that NOSH-NSAIDs are essentially devoid of any GI side effects even though they reduce gastric tissue prostaglandin E$_2$ levels. The hybrid molecules retain all the positive pharmacological attributes of their respective parent NSAID. That is, they have potent anti-inflammatory, analgesic, anti-platelet and anti-platelet activities. In addition to their GI safety, NOSH-NSAIDs might also prove to have enhanced cardiovascular and renal safety profiles due to the released NO and H$_2$S. NOSH-NSAIDs are potentially useful as chemopreventive and/or chemotherapeutic agents against many types of cancer. A cartoon summarizing the classic pharmacological effects of NOSH-aspirin is depicted in Figure 5.

Current work in my laboratory is directed towards understanding the mechanisms of action of these novel compounds, focusing on molecular targets that are relevant to inflammation and cancer, and to possible interactions between NO and H$_2$S in producing a new signalling entity. These unique agents are a new class of anti-inflammatory pharmaceuticals and we are focusing on developing these for various patient applications. For example, our lead compound, NOSH-aspirin (NBS-1120) is being developed for use against a number of human malignancies. Other agents, such as NOSH-naproxen (AVT-219), are under active investigation for various patient applications.

Khosrow Kashfi is an Associate Medical Professor of Pharmacology at the City University of New York School of Medicine and is a Fellow of the Royal Society of Chemistry. His research is currently focused on the molecular targets of NOSH-NSAIDs in cancer. He is the co-inventor of this class of compounds and holds a number of patents in this general area. He has set-up a company around this technology, Avicenna Pharmaceuticals Inc., and is working towards taking some of these compounds to the clinic. Email: kashfi@med.cuny.edu.

**Further reading**

Reactive oxygen species: rapid fire in inflammation

Sonia Ingram and Marina Diotallevi (Brighton and Sussex Medical School, UK)

Everyone has encountered it at some point: inflammation. That horrible feeling when you’ve hurt yourself and the skin and tissue around the injury swells, goes red, feels hot and painful. It is even worse if it gets infected, then you really know about it! You can feel sick, weak and feverish as your body tries to fight off the infection and heal itself.

Inflammation is really important for keeping us healthy. Sometimes, however, the body’s inflammatory response can be a bit overzealous, not shutting down when it’s supposed to, which can lead to various problems and even a state of disease. To fully understand and be able to effectively treat these diseases, we need a better understanding of how and why this chronic inflammation occurs. Could a crucial element in our lives, oxygen, be key to furthering our understanding?

Inflammation and oxidative stress

Acute inflammatory responses are essential for eliminating infections and during wound healing, but become detrimental if they are not resolved and become self-perpetuating. This chronic response is sometimes observed under sterile conditions, when there are no pathogens present to attack but the inflammatory response persists, resulting in damage to the body’s own cells and tissues. The mechanisms underlying the chronic inflammatory response are not clear; however, ROS are thought to be involved.

The term ROS encompasses the superoxide anion ($\text{O}_2^-$), hydroxyl radical ($\text{OH}$) and hydrogen peroxide ($\text{H}_2\text{O}_2$) amongst others. ROS are the intermediate breakdown products of molecular oxygen, and as aerobic organisms we constantly break down oxygen and produce ROS as we respire. As well as being formed routinely during cell metabolism, ROS are important in pathogen defence during respiratory burst. When certain leukocytes come into contact with bacteria or fungi, NADPH oxidase, an enzyme on their outer membrane, is activated to rapidly produce large amounts of superoxide which effectively kills the pathogen.

As the name suggests, ROS are highly reactive – able to oxidize a multitude of molecules, proteins, lipids and even DNA. This reactivity can be harmful if the wrong entity is oxidized, but the body is efficient at breaking down ROS into safer molecules, or utilizing their reactivity in cell signalling. Interestingly NADPH oxidase, the enzyme responsible for respiratory burst, is also present in a variety of cells not involved with inflammation, where it produces...
lower ROS levels than in leukocytes. This intentional production strengthens the idea of ROS mediating cellular functions.

Antioxidants are responsible for maintaining the correct levels of ROS; there need to be enough ROS for signalling and physiological functions to be carried out, but not so many as to cause host damage. When this fine balance is dysregulated and there are too many ROS or not enough antioxidants, a state of ‘oxidative stress’ occurs.

Oxidative stress has been widely implicated in many sterile inflammatory diseases, varying from ischaemia-reperfusion injuries through to neurological disorders and ageing (Figure 1). Increased ROS can trigger immune responses via two main mechanisms: (i) by incorrectly oxidizing proteins, lipids or DNA, which immune cells then do not recognize as safe and launch an immune response against, or (ii) by activating redox-sensitive proteins inside cells which can then partake in inflammatory signalling. Examples of this include the pro-inflammatory signalling protein MAPK and the transcription factor NfκB, which transcribes and increases the expression of many pro-inflammatory genes.

Oxidative stress and ROS are elevated and have many pro-inflammatory roles in sterile inflammation. Could antioxidants be used to decrease ROS and therefore reduce inflammation?

**Antioxidants in sterile inflammation**

Antioxidants can be broadly defined as ‘any substance which significantly delays or prevents oxidation, or removes oxidative damage to a target molecule’. A wide range of antioxidants, with different mechanisms of action, target molecules and efficiency rates all work together to regulate the oxidation state in the body. Some antioxidants, such as glutathione (GSH), the main cellular antioxidant, are synthesized in our bodies, but others, including vitamins C and E and molecules such as selenium, are obtained through our diet, particularly a ‘green diet’ consisting of fruit and vegetables.

Over the last 20 years, the number of clinical trials investigating antioxidants to reduce ROS-induced inflammation has increased exponentially. Antioxidants are not generally considered to be harmful compounds, making them an attractive treatment option. In particular, N-acetyl cysteine (NAC) (a precursor of GSH), vitamin E and coenzyme Q have been popular subjects of clinical trials. However, the results of these trials have been overwhelmingly disappointing, with little benefit of antioxidant therapy, and in some cases treatment was even found to be detrimental.

One clinical trial in 2011 looked at the effects of selenium (a component of some antioxidant enzymes) and vitamin E (an antioxidant in its own right), in preventing prostate cancer. These compounds were given to healthy volunteers and were found to actually increase the risk of prostate cancer. This contradictory effect of selenium was also found on a larger scale: in China, some areas with unusually low soil selenium are associated with an endemic osteoarthropathy, an inflammatory disease of joints and bones, whilst in Iran some areas with high soil selenium levels correlate with increased risk of oesophageal cancer within the population. Although caution should be exercised when interpreting these results, they indicate that different mechanisms are involved. Sayin et al. recently showed that antioxidants could actually provoke cancer by blocking the apoptosis signal triggered by ROS. All of these results have shown that down-regulating inflammation using antioxidant therapy is not as straightforward as once thought, and the right antioxidant balance is crucial.

The failure of antioxidant trials have been blamed on many things. First, the therapies used have been non-specific ROS scavengers and therefore the development of direct inhibitors of the enzymes that produce ROS, or targeted to molecules which are oxidized (modified by ROS), may be more effective. Secondly, antioxidant treatment in established disease may be too late to avoid, and certainly reverse, symptoms. Thirdly, the oxidation state may differ between diseases and...
even between individual patients so tools to detect the oxidation level should be developed to establish this before treatment. In addition, there are many anti-inflammatory mechanisms inside cells which are redox sensitive. As mentioned above, NFκB is one example of a redox-sensitive transcription factor which is pro-inflammatory. Nrf2, on the other hand, is a redox-sensitive transcription factor which is anti-inflammatory and transcribes many antioxidant proteins. ROS-dependent signalling, or ‘redox signalling’, can therefore be pro- or anti-inflammatory and a better understanding of this process is required to develop effective antioxidant therapies.

**Thiol redox signalling**

Intracellular proteins can signal to each other through a number of mechanisms. The most well-known include phosphorylation and ubiquitination, where enzymes add tags, phosphate groups or ubiquitin, respectively, onto other proteins to change their structure, function or how they are recognized by other proteins. To be classed as signals rather than protein damage, these mechanisms must be reversible so that proteins can return back to their original state. Thiol-disulphide exchange is a form of fast and fully reversible redox signalling.

Redox signalling uses ROS in place of phosphate or ubiquitin during signalling. ROS are highly reactive with protein thiols, which are small molecular groups comprised of one sulphur and one hydrogen atom (-SH). Of the 21 amino acids found in eukaryotic proteins, only cysteine contains thiols and the position of cysteines within proteins dictates whether or not their thiol can react with ROS. When thiols are able to react, and become oxidized by ROS, two thiols can bind together to form a disulphide (-S-S-). This disulphide bond can be broken and returned back to two separate thiols by enzymes or a reducing (rather than an oxidizing) environment (Figure 2).

The latest findings show the importance of redox enzymes in inflammation. These proteins play the role of ROS recipients inside the cell, but they also have proper inflammatory functions when outside cells. For instance, peroxiredoxin (Prx)1, a redox enzyme, when outside the cell can activate surface receptors of inflammatory cells, whilst Prx2, a similar enzyme, can activate the release of inflammatory cytokine TNF. Thioredoxin (Trx), another redox enzyme, acts like a chemokine by attracting immune cells towards it.

There are many receptors on the surface of immune cells that contain redox-sensitive thiols, and therefore can be modified by oxidation and redox enzymes. However, the mechanism of redox enzyme release appears to affect their subsequent function. For example, these enzymes can be released into the extracellular space via two main routes: (i) when cells are necrotic there is uncontrolled cell death and everything inside is released, or (ii) through exosomes, which are tiny sealed packages released from activated cells and phagocytosed by recipient cells. Redox enzymes released by necrosis are immediately in the extracellular space and able to modify any redox-sensitive thiols they encounter, whereas when contained in exosomes they do not encounter any thiols until they are safely delivered to the recipient cell. The theory is that these different mechanisms of release enable redox enzymes to communicate different messages depending on what is required for the right immune response.

**Future directions**

Fast and efficient communication between cells is imperative for the body to respond to infection or injury in the right way. There are many different types of cellular communication. Redox signalling is a super-fast communication method which utilizes ROS produced from NADPH oxidase and during
aerobic respiration. The exact mechanisms of redox signalling, such as thiol-disulphide exchange and the role of redox enzymes, are now beginning to emerge. This knowledge may help classify different types of patients and diseases, or help create more highly targeted antioxidant therapy for use in sterile inflammatory diseases.

**Abbreviations**

MAPK – mitogen-activated protein kinase

NFκB – nuclear factor light-chain-enhancer of activated B cells

Nrf2 – nuclear factor (erythroid-derived 2)-like 2

NADPH oxidase – nicotinamide adenine dinucleotide phosphate-oxidase

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**Further reading**

- **Pick, E., Kroizman, T. and Abo. A. (1989)** Activation of the superoxide-forming NADPH oxidase of macrophages requires two cytosolic components—one of them is also present in certain nonphagocytic cells. J. Immunol. 143(12), 4180–4187

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Sonia Ingram (Leach) graduated with a BSc in Pharmacology from the University of Bath in 2010. She then undertook conservation work on coral reefs in Indonesia and lived in the Australian outback for a year. On returning to the UK, she worked in medical communications until 2013 when she realized her heart was in medical research. After completing an MSc in Transfusion and Transplantation Science from the University of Bristol, Sonia embarked on her PhD project at Brighton and Sussex Medical School, under the supervision of Professor Pietro Ghezzi. Her project focuses on the regulation of redox enzymes during inflammation and their role in autoimmune disease. Email: s.leach@bsms.ac.uk.

Marina Diotallevi completed her undergraduate degree in Life Science at the University Pierre and Marie Curie, Paris, in 2011. She stayed on to complete a master's degree which specialized in the Biochemistry of Ageing and Immunology (2011–2013). Since the beginning of 2014, she has been doing her PhD at Brighton and Sussex Medical School under Professor Pietro Ghezzi's supervision looking at the redox mechanism underlying inflammation, in particular, the analysis of thiol oxidation modification in macrophages. Her scientific interest focuses on the biochemistry and structure of macromolecules and their associated function. Email: m.diotallevi@bsms.ac.uk.
Hypoxia and inflammation

Uncontrolled or non-resolving inflammation is central to the pathophysiology of clinically important conditions including inflammatory bowel disease (IBD), psoriasis, atherosclerosis and arthritis. A combination of increased oxygen demand and decreased supply renders the local microenvironment of chronically inflamed tissues oxygen deprived (hypoxic), leading to the expression of a programme of genes that promote adaptation to the hypoxic challenge. This ancient and ubiquitous adaptive transcriptional pathway is governed by a transcription factor termed the hypoxia-inducible factor (HIF). Originally identified in the search for regulators of hypoxia-induced erythropoietin expression and adaptation to high altitude, HIF has been more recently recognized as a major regulator of immune cell function, which is central to the control of immunity and inflammation. Indeed, recent studies have demonstrated that the use of drugs targeting the HIF pathway may be of benefit in the treatment of chronic inflammatory disease.

Adaptation to hypoxia

Molecular oxygen (O\textsubscript{2}) is often viewed as a paragon of purity and the key ingredient in the air that we breathe, essential to the sustenance of life. However, when seen over geologic time, the impact of O\textsubscript{2} on life on Earth has a more chequered history. The levels of O\textsubscript{2} in the Earth’s atmosphere remained extremely low for the majority of geologic time when the planet was largely populated by anaerobic prokaryotes (bacteria). It is only in the relatively recent past (the last billion years or so!) that atmospheric O\textsubscript{2} levels started to rise towards current levels. The accumulation of atmospheric O\textsubscript{2} was primarily a result of the expansion of the photosynthesizing cyanobacteria (blue-green algae) mainly present in the oceans of the planet. Initially, oxygenation of the atmosphere represented a major threat to the existence of life as O\textsubscript{2} proved toxic for the majority of early life, which up until that time had relied on anaerobic metabolism for survival. In fact, these organisms were unable to survive in the reactive chemistry of the oxygenated atmosphere, having not yet evolved sufficiently protective antioxidant capabilities. Thus, the accumulation of atmospheric O\textsubscript{2} provoked a mass extinction, termed the Great Oxidation Event (GOE). However, of the organisms that were able to survive the GOE, many would go on to thrive. These select groups of organisms developed not only the capacity to withstand the chemical challenges posed by atmospheric O\textsubscript{2} but also evolved to harness its inherent chemical energy to improve bio-energetic efficiency. The evolution of oxidative metabolism resulted in a significant step-up in the bio-energetic capacity of these nascent eukaryotic cells, leading ultimately to their ability to develop into multicellular animals (metazoans). Therefore, molecular O\textsubscript{2} provided the fuel, which in combination with the incorporation of mitochondria, improved the cellular metabolic efficiency necessary to allow the evolution of metazoan life on Earth to occur. A consequence of these events is that present-day respiring organisms (such as humans) have evolved to be extremely reliant on an uninterrupted supply of O\textsubscript{2} for their existence, due to the requirement for O\textsubscript{2} in order to satisfy biochemical demand. Therefore, O\textsubscript{2} deprivation (hypoxia) at the organism, tissue or cellular level represents a severe threat to survival.

Despite the fact that sufficient O\textsubscript{2} is present in the Earth’s atmosphere to sustain aerobic life, hypoxia is a commonly encountered challenge. For example, tissues such as the kidney, retina and intestinal mucosa normally experience very low levels of O\textsubscript{2}, even in a healthy state. Whole body hypoxia may occur due to events as diverse as ascent to high altitude, extreme exercise, suffocation or carbon monoxide poisoning. Furthermore, at the tissue/cell level, hypoxia may occur as a result of vascular occlusion such as in the case of heart attack or stroke. Because of the importance of a constant supply of O\textsubscript{2} to the maintenance of tissue survival, it is perhaps not surprising that metazoans rapidly evolved protective mechanisms to promote adaptive responses aimed at increasing the chances of surviving exposure to hypoxia.

Managing hypoxia on a molecular level

In humans and many other mammalian species, the capacity to adapt to hypoxia occurs at two main levels. Firstly, the physiological response to hypoxia is governed by the rapid activation of the carotid bodies to decreased partial pressure of O\textsubscript{2} in the blood leading to a neuronal response, which results in an increased rate and depth of breathing leading to an increase in blood O\textsubscript{2} levels. The second line of defence against hypoxia occurs at the cellular level and involves the transcriptional up-regulation of genes that promote adaptation of an organism to hypoxia.
Erythropoietin (EPO) is a hypoxia-responsive gene, which is expressed primarily by a subset of fibroblast-like cells located in the tubular interstitium of the kidney in adults. Initial studies into the mechanisms that link hypoxia to EPO expression identified the hypoxia-inducible factor (HIF) as the primary transcription factor involved. Under conditions of hypoxia, it was found that HIF accumulated in cells, bound to the EPO enhancer and induced expression of the gene. However, it rapidly became clear that HIF was neither restricted to expression in the interstitial cells of the kidney nor restricted to the regulation of EPO. Indeed, HIF is now recognized as a ‘master regulator’ of the cellular response to hypoxia, ubiquitously expressed and governing the expression of hundreds of genes that promote multiple aspects of hypoxic adaptation, including blood vessel growth (angiogenesis) and metabolic adaptation. The appreciation of the global role of HIF led to an intense search for the O₂ sensor(s) responsible for its sensitivity to hypoxia.

In 2001, simultaneous publications identified these sensors as a family of O₂-dependent enzymes termed HIF hydroxylases that included PHD1-3 and FIH. These HIF hydroxylases repress the HIF pathway in normoxia and therefore their inhibition in hypoxia allows a rapid activation of an adaptive HIF-dependent transcriptional response. Importantly, these are druggable enzymes and hydroxylase inhibitors have now been identified as a new class of drug with the potential to treat anaemia due to their ability to increase haemoglobin and thus the O₂-carrying capacity of blood. In 2016, the importance of the discovery and elucidation of the HIF pathway was recognized with the presentation of the Lasker Basic Research Award to Gregg Semenza, Peter Ratcliffe and William Kaelin.

**Hypoxia and inflammation**

It is now appreciated that chronically inflamed tissues such as the intestinal mucosa in inflammatory bowel disease and the arthritic joint are profoundly hypoxic. This ‘inflammatory hypoxia’ is the result of a combination of increased O₂ demand by infiltrating inflammatory cells such as neutrophils in combination with decreased O₂ supply resulting from inflammation-related vascular dysfunction. Neutrophils in particular are rampant consumers of O₂ at sites of inflammation as they use O₂ as they use it as part of the oxidative burst to generate reactive oxygen species (ROS) to kill invading pathogens. Therefore, immune cells in an inflammatory microenvironment are frequently exposed to significant levels of hypoxia. Notably, this low O₂ environment is markedly different from the more abundant O₂ levels these cells experience in the circulation prior to being recruited to an inflammatory locus (figure 1). Furthermore, in mice that specifically lack HIF in individual immune cell types, it has been clearly shown that HIF is a major regulator of immune cell function. This link between hypoxia and immunity/inflammation is largely mediated through HIF-dependent changes in immune cell metabolism and a consequential change in immune cell effector function. Therefore, HIF plays an important role as a lynchpin between altered immune cell metabolism and effector function and is a key regulator of immunity and inflammation.
The impact of HIF on immune cell effector function varies depending on the degree of hypoxia experienced and the immune cell type involved. For example, in neutrophils, the HIF pathway promotes cell survival, whereas in subtypes, it regulates the balance between pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. In B-lymphocytes, HIF regulates antibody production whereas in T-lymphocytes, it regulates differentiation, survival and tumour-killing capacity. Therefore, HIF regulates immune cell effector function in a cell-type-dependent manner. The role(s) of HIF in individual cell types is complex and may be either pro- or anti-inflammatory depending on the context of activation. However, a number of studies have demonstrated that pharmacological activation of this pathway drives a largely anti-inflammatory outcome, which has been demonstrated in models of intestinal inflammation, radiation injury, sepsis, renal transplant and bacterial infection and raises the possibility of repurposing hydroxylase inhibitors as a new class of anti-inflammatory therapeutics.

In summary, it is now clear that in many inflammatory conditions, microenvironmental hypoxia is a prominent feature and, through the HIF pathway, is a major determinant of immune cell activity. Future studies will determine whether the preclinical anti-inflammatory effects of pharmacologic hydroxylase inhibition can be translated into clinical benefit for patients with inflammatory conditions.

Cormac Taylor, PhD, holds an appointment as Professor of Cellular Physiology at the School of Medicine and Medical Science and the School of Medicine and the Conway Institute, University College Dublin, Ireland. Current research in the Taylor lab is directed towards developing our understanding of the physiological and pathophysiological mechanisms by which changes in microenvironmental oxygen levels regulate gene transcription in eukaryotic cells. A key focus of this work is the identification of new therapeutic targets in inflammatory disease. Email: cormac.taylor@ucd.ie.

Dr Eoin Cummins, PhD, holds an appointment as Assistant Professor of Physiology at the School of Medicine and Medical Science and the School of Medicine and the Conway Institute, University College Dublin, Ireland. Current research in the Cummins lab is directed towards understanding the role of carbon dioxide in the regulation of inflammatory gene transcription in eukaryotic cells. Email: eoin.cummins@ucd.ie.

Further Reading


Additional reading

Although inflammation is a necessary biological process in response to injury and disease, at abnormal levels it is also responsible for a significant annual health burden. Immune-mediated inflammatory disease is present at a prevalence of about 7% in the Western world and with an ever aging population this is set to rise. While treatments for some of the more common inflammatory disorders such as rheumatoid arthritis (RA) do exist, there is still a large unmet patient need as many sufferers do not achieve remission of symptoms even when using currently available therapies. Immuno-inflammation is one of GlaxoSmithKline (GSK)'s key therapeutic areas of interest. Helen Albert speaks to Paul-Peter Tak, Senior Vice President and Chief Immunology Officer at GSK, about his career and the work he is doing at GSK to encourage scientific innovation and target unhealthy inflammation in all its forms.

**Targeting immuno-inflammation: where industry and academia interface**

Paul-Peter Tak began his career in academia with a PhD in Immunology at the University of Leiden in the Netherlands. He moved to the Academic Medical Center (AMC) of the University of Amsterdam in 1999, where he became Professor of Medicine. During that time, he worked very closely with the biotech and pharmaceutical industry as a consultant and started several companies including a biotech company developing a gene therapy for RA called Arthrogen. In 2011, he joined GSK as Senior Vice President and Global Head of Research and Development (R&D) in Immuno-inflammation, while still maintaining research links with the AMC. In Jan 2016, he started as Senior Vice President for a group of therapy areas covering immuno-inflammation, oncology, dermatology and infectious diseases. He also currently leads the Development Steering Team which oversees late-stage development in R&D at GSK.

What made you decide to move from academia to industry and how have you found the transition?

I'm a physician, a physician scientist, and I studied medicine to influence the life of patients in a positive way. I've always focused on three things in academia - trying to be a really good physician for my patients and to listen to them and give them the best treatments of the day, that's one. Second, to start to discover and develop new medicines for the future, and the third is of course about training and education of physicians who became medical specialists. I was the head of department in Amsterdam for 12 years, but I thought perhaps I could have a bigger impact on patients' lives if I joined a company like GSK. If you develop medicines here that really make a difference, you have the potential to touch the lives of millions of patients. I decided that with the resources, the technology, the high level of the science and the academic collaborations that GSK has that I could make a big difference here. So that was the reason that I joined. Of course it was a big step, because I was in a very senior role, also in an international organisation, and I didn't really know what I was signing up for. I had worked very closely with industry, but I'd never been an employee of industry. But I think from the day that I joined I really enjoyed it, it was like stepping into a warm bath in many ways. What I really liked was the rigour of the science here, the very high quality, the collaborative atmosphere and that people have a common goal to discover and develop medicines for patients who need them. At the same time, I have continued to be very strongly linked to the academic world, so I guess I have had the best of both worlds.

What is the GSK immunology network?

We have a lot of collaborations with universities and academic institutions and also with biotech and other pharmaceutical companies. I think we have more than 500 research partnerships, some of them are very strong. The Immunology Network, as I've called it, is I think, a very innovative model of working with academia and has different components. The first pillar of the network is the External Immunology Board; we work with absolutely top immunologists from around the world who all have a slightly different profile. For example, some people are focused on neuroimmunology, others on immunometabolics and so on. Then the second pillar is the immunology catalyst and this is for senior academics who want to come into GSK for an extended sabbatical. If selected, they can come and work in our facilities in Stevenage in the UK, which is one of the two major Research and Development hubs in the world for GSK, where we give them support in terms of postdocs, personnel and the lab, but they continue to do their own independent research. They have a badge to get into our facilities, but they are not GSK employees. They continue to be employees from their university and we reimburse the university so they keep their academic independence, which is deliberately the model. The third
component is the Immunology Innovations Fund that I started. If there is a great idea in the Immunology Catalyst, which does not fit into one of the current funding schemes, then I can use money from the Immunology Innovation Fund to bring it to the next inflection point and then we may decide to start a biotech company around it if the academics are interested, and then they would become the founders, or we might internalise it and it could become a GSK program. The 4th and last pillar is the organization of the Immunology Network Summit Meetings where the external immunology board members come together with the immunology catalyst members and the immunologists in GSK. They are a bit like Keystone meetings, the level is similar, and it creates something completely new I think. So in part it is about internalising the external world and bringing in the independent academic voice into GSK.

What do you think are the hot topics in inflammation research right now?

Well there is a lot going on in inflammation. We call it immuno-inflammation, because it’s quite difficult to distinguish between the immunology and inflammation, as it’s so strongly linked. It is a very important field in terms of the prevalence of disease. Immuno-inflammatory disorders are common and there is still a very big unmet need. There are many conditions where we don’t have any treatments. For some of the conditions where we do have quite a lot of treatments, like RA, there is still a very big unmet need, because at least 50% of the patients do not achieve remission and that is the goal of treatment. In the last 5 or 6 years, immunoinflammation at GSK has been quite successful. We’ve built a very strong and holistic portfolio. I will give you a few examples, as these are what we believe are hot topics. One example is the world of epigenetics, where of course we enter a completely new field where you ask the question, what are the factors that determine whether a gene is activated or switched off? How can we interfere with that? Another very hot area for us is the world of pattern recognition receptors. A very specific program, a key programme in our immune-inflammation therapy area unit, is around receptor-interacting protein 1 (RIP1) kinase, which plays a pivotal role in necrosis, apoptosis and necroptosis, all different forms of cell death, but also in cytokine signalling. So it plays a very important role in different diseases. Because this is such new biology, where we have developed a kinase inhibitor that only touches RIP1 kinase, you can see we have something that could be very interesting in terms of benefit-risk ratio. We have very strong preclinical package in a whole variety of disease models and we’ve published extensively on this. But then of course with such new biology the question is where is it going to work? Therefore, we’re using a systematic experimental medicine approach, where in parallel we are testing the effects of a RIP1 kinase inhibitor in RA, psoriasis and ulcerative colitis, but there are also other programs outside the immune-inflammation area where we are exploring the role of RIP1 at this moment. So that is something that is very exciting for us. In addition, we have a focus on T-cell biology, especially Th17 biology, which is for us a very important field. We are also working on cytokines, chemokines and complement, and these are all key areas that I find very exciting at this moment in immuno-inflammation.

What have you discovered in your studies of vagus nerve stimulation in rheumatoid arthritis and why does the bioelectronic treatment approach hold promise for individuals with immune-mediated inflammatory disease?

This is work I completed outside of GSK. I am still affiliated at the University of Amsterdam and am still a non-salaried Professor there. I did this work during the last 10 years in Amsterdam and I’ve tried to
complete that. I discovered that the so-called alpha-7 nicotinic acetylcholine receptor (alpha-7) plays a key role in the joints in controlling inflammation. This is how we got interested in it. I used alpha 7 knockout mice and found that in models of chronic inflammation in RA these mice have increased arthritis and increased distortion of the joints. If you do the reverse and you give these mice nicotine, which triggers the alpha-7 receptor or specific alpha-7 agonists that activate this pathway, you can inhibit arthritis. In a collaboration with a company called SetPoint Medical, based in the US, we stimulated the vagus nerve, which also leads to activation of the same pathway, for 60 seconds per day and we found that you can reduce inflammation and protect joint destruction. We then did a clinical trial in humans and when we implanted the device in humans with RA, we could show there was a beneficial effect even in patents who are therapy resistant.

What research is GSK carrying out to help develop better treatments for RA?

We have several programs in RA, which is the most common chronic autoimmune disease. However, we are definitely not limited to RA in immuno-inflammation. We have a focus on rheumatology, so also the other rheumatological syndromes like osteoarthritis, Sjögren’s syndrome, systemic sclerosis etc., but also gastroenterology and dermatology, so that all sits in immuno-inflammation. The programs that we have that are developing treatments for RA at this moment at GSK include the interleukin (IL)6 monoclonal antibody sirukumab, which is currently under review by the regulators. The difference between sirukumab and let’s say tocilizumab, the anti-IL6 receptor antibody from Roche which is on the market, or sarilumab, which was recently approved, is that these other two medicines target a receptor, whereas sirukumab targets the ligand. We partnered with Johnson & Johnson on that program. In addition, an anti-granulocyte macrophage colony-stimulating factor (GM-CSF) monoclonal antibody is being tested for treatment of RA and currently in phase IIb trials. This may have different advantages compared to other medicines. First it’s a very different pathway targeting really the key effector cells in RA, namely the macrophages in the synovial tissue and the neutrophils in the synovial fluid. It works in a slightly different way to TNF blockers and it actually targets the monocytes and the macrophages that are the major sources of proinflammatory cytokines in the joint. But it has also been shown in preclinical models that GM-CSFs play a particularly important role in pain, so we are quite interested in the specific effect on pain in RA and osteoarthritis. And then I think there is still an outstanding requirement for a safe and effective small molecule and we hope that RIP1 kinase inhibitor might play that role. Then we have a few other programs that we have not disclosed as well.

What promising developments in the inflammation arena in general have made over the last couple of years, both by GSK and others?

Well I would pick probably the IL17 inhibitors and the IL23 inhibitors, as I think they are very important. I think anti-GM-CSF also has enormous potential. We are very excited by this medicine for a variety of immune mediated inflammatory disorders, some of which I have spoken about. I think we have a systematic approach to extend this to different indications, where a specific mechanism may play an important role. Again with RIP1 there is huge interest from the
scientific community, so I think that’s another very important development as well. And then of course there are the Janus kinase (JAK) inhibitors, the highly selective JAK1 inhibitors, which are of interest. Of course we need to see over time what the benefit-risk ratio is, if we get more experience, but definitely an interesting development.

**In addition to developing new treatments for inflammatory disease, have you had much success repurposing old drugs for new indications?**

I would not even call it repurposing of medicines, because ideally we need to do this at an early stage of development and go into different indications where we believe a specific mechanism plays a role. I think RIP1 is probably a very good example, but for all medicines developed, we take the approach to investigate multiple indications. Especially in medical specialities like rheumatology and gastroenterology, what we call a disease is not really a disease, it’s a syndrome defined by clinical signs and symptoms. These conditions are heterogeneous, and may be driven by completely different mechanisms. Interestingly, you can see on average the same efficacy if you treat a patient with RA with different therapies that target different pathways. For example, TNF blockers compared to rituximab, which targets the B cells, compared to tocilizumab, which targets the IL6 receptor. The mechanisms are completely different, but also the patients who respond to these treatments not the same necessarily, highlighting the importance of individualized health care approaches to improve treatment effects. The other way around, all of these medicines may work in diseases other than RA. Another example would be belimumab, which has been approved for treatment of lupus now in four phase III clinical trials. They were all positive which is quite amazing, because it’s such a difficult disease to treat and many competing molecules have failed. Then the question is could it work in other autoantibody dependent immune mediated inflammatory disorders? And we’ve tested it in different conditions. There is a clinical trial going on in Sjögren’s syndrome, another autoimmune disease characterised by autoantibodies. We have also tested it in very rare diseases like idiopathic membranous glomerular nephritis, which is a truly autoantibody dependent disease. In a small experimental medicine study, we could show that there was a very significant decrease in the levels of autoantibodies, followed by a very significant decrease in proteinuria, which is a key hallmark of the disease. So that’s an example of what I call expansion of indications, where you really get more confidence in the mechanism and where you can see based on the molecular events rather than just on signs and symptoms. Maybe a good example from respiratory would be mepolizumab, an anti-interleukin 5 antibody that we have developed for asthma that we are now testing in a variety of different diseases that are all characterised by increased eosinophils, because IL5 drives eosinophilia. We have just announced that we are going to start a phase III clinical trial in nasal polyps. We are testing it in COPD, there is a condition called hypereosinophilic syndrome (HES) where we are testing it. There is a condition called eosinophilic granulomatosis with polyangiitis (EGPA), where we have published positive results. We are also testing it in atopic dermatitis, so I think that creates a very mature example of how based on the mechanism in common diseases like eczema, it is also possible to treat rare diseases like EGPA.

**What do you think the future holds for inflammation research and the development of new therapies for inflammatory disease?**

The future is to induce remission in all patients. Something that we only achieve in a minority of patients at the moment and in many diseases we don't achieve it at all. To do this, we need to use different modalities where necessary. I spoke about small moleculesand biopharmaceuticals, but we will also use other approaches. Ultimately the goal should be to cure the patients or to even prevent the disease. You may have heard me speak about type 1 diabetes in the past, which is also an autoimmune disease. Based on autoantibody profiles you can identify people who are at risk of developing the disease and during that stage you could perhaps interfere and stop the process from developing towards full-blown clinically established disease. I have done a similar study in RA, wearing my academic hat again. So I think that is the future - remission, cure, prevention - using different modalities and with a deep molecular understanding of the subsets of the disease.

### Further reading

Parliamentary Links Day 2017

The theme for this year’s Links Day was UK Science and Global Opportunities, a somewhat timely topic in light of last year’s Referendum result. This annual event brings together scientists, politicians and Learned Societies to network and discuss topical issues in science policy.

Links Day 2017 was opened by the Speaker of the House of Commons, the Rt Hon John Bercow MP, who emphasised the importance of maintaining links with our European neighbours in light of Brexit. Chair Designate of the new research funding oversight body UK Research and Innovation (UKRI) Sir John Kingman also spoke. He emphasized the strengths of the UK in science and the contribution that the sector makes to the economy. He also outlined in more detail the role UKRI would play saying that they aim “to be a voice for everything that matters for science and innovation”. He explained that UKRI will have strategic oversight on capital spending and investment in research. “The major parties want to invest in science and research and it is our responsibility to make sure they continue to do so”, he added.

The third speaker, Minister for Universities, Science, Research and Innovation Jo Johnson MP, said that there is a need to develop more areas of “outstanding science” and to address “economic imbalances” in science and research that exist between different areas in Britain. However, he emphasised that investing in science and engineering continues to be a priority for the UK Government.

He acknowledged that Brexit presents a “whole new set of challenges”, adding that “we want the UK to remain the go to place for innovators, scientists and investors”.

Commenting on the formation of UKRI, he said: “It will play a central role in enhancing the UK’s productivity and competitiveness in years to come”.

Two panel sessions followed, both of which were chaired by Pallab Ghosh from the BBC. The first session tackled ‘Science and Europe’ and the second focused on ‘Science and the World’. Both sessions included five speakers with a scientific, educational or political...
background who spoke briefly before taking questions from the audience.

Concerns about the impact of Brexit dominated the first session, with Professor Roberto di Lauro from the Embassy of Italy and Dr Lorenzo Melchor from the Embassy of Spain both expressing apprehension about the impact of Brexit on European researchers working in the UK. Professor di Lauro said that in a questionnaire filled out by over 6000 Italian researchers working in the UK, 79% said they were thinking of leaving following last year’s Referendum result.

Chi Onwurah MP, Shadow Minister for Business, Energy and Industrial Strategy, stressed the importance of maintaining good relationships with Europe saying "innovation depends on the free flow of ideas and people".

In the second session the importance of remaining competitive in a global market and encouraging cross-country collaborations to maintain high quality research and innovation were highlighted.

Malcolm Brinded from the Royal Academy of Engineering said that there is "a major engineering skill shortage that seriously damages our competitiveness", adding that "we need to get more UK engineers excited about global opportunities".

Professor Sir John Holman from the Royal Society of Chemistry acknowledged that while the UK currently has a strong science sector, “we have got to run even faster if we are going to maintain that position in the face of current challenges.”

Holman and the other speakers stressed the importance of recruiting and retaining good science teachers to ensure that young people continue to enter science and engineering.

Professor Dame Jocelyn Bell Burnell, President of the Royal Society of Edinburgh, suggested that “international development opportunities are a great way of getting young people engaged in science”. She added that the importance of good careers advice cannot be underestimated.

Stephen Metcalfe MP, immediate past chair of the Science and Technology Select Committee for the House of Commons, concluded the event.

“We are achieving a great deal at the moment, but there is always more that can be done”, he commented. "We need to create a vision that attracts and not repels the best scientists in the UK.”
From womb to tomb: stress across a lifetime

Stress. What does it mean to you? We probably all refer to it after a long or strenuous day at work, during a particularly heated family argument, or when thinking about our finances. But what does the word really mean, and why is it so important in our everyday lives? Can it increase our risk of developing serious health conditions such as obesity, heart disease, and lung cancer? Is it something that can affect our children as they develop in the womb? Is it even something that can affect our children’s children? It may seem surprising, but the answer to all of these questions is yes.

"We all need a bit of stress in our lives. It’s a survival mechanism”

Don’t get me wrong, we all need a bit of stress in our lives. It is after all a survival mechanism. When we experience a stressful situation, our bodies are elegantly prepared to respond in an appropriate way. Imagine walking into work on a Monday morning, and you start to smell smoke. A bit concerning? You walk a little further and you start to see the smoke too. Getting a little stressful in here, right? You reach the end of the corridor, and your office is on fire. You’re pretty stressed right now.

As soon as you smell the smoke and see the fire, your nose and eyes send signals to a region of your brain called the amygdala, which is our body’s fear and emotion centre. Much like someone calling
999, the amygdala senses the stressful situation and sends a message to another brain region called the hypothalamus. The hypothalamus acts like a ‘stress call-centre’ for our body. Whenever the hypothalamus receives a stress signal, it tells our body to release stress hormones from the pituitary and adrenal glands.

“The fight-or-flight response helps us survive dangerous or harmful experiences”

This so-called HPA (hypothalamus, pituitary, adrenal) axis releases a surge of stress hormones such as cortisol and adrenaline, which allow us to either extinguish the fire like a firefighter, or run away. You may have heard of this as the fight-or-flight response, which helps us to survive dangerous or harmful experiences. So, if we need this response to survive, how can it be harmful to us? Imagine if the fire comes back on Tuesday, and every day next week, and the week after. Ok, a little bit unlikely - but for thousands, if not millions, of people, other types of chronic stress can change their health across their lifetime. When the HPA axis continually pumps out stress hormones, it changes the way we respond to stress, which can result in many different diseases such as depression, cancer, or heart problems.

The word stress comes from the Old French word ‘estrece’, which means narrowness or oppression. In a way, this definition still applies today – chronic or continued stress can oppress our bodies stress response system and change our biology permanently, in ways that we have never understood better than we do now.

“Stress comes in many forms and can affect fetal development”

Across our lifetime, our bodies go through several important stages of development. Probably the most obvious is before we are born, when we are just a fetus surviving courtesy of our mother in the safe confines of the womb. But, like a lot of things, the human body is not perfect, and the womb can’t always protect us to allow us to develop normally. We all know that women should avoid smoking and drinking during pregnancy, but stress can also come in many other forms. The surge of hormones and molecules released during maternal stress are thought to be sensed by the fetus and can affect development leading to diseases.

In 1944, the German-occupied western region of the Netherlands had a severe shortage of food rations. As a result, nearly every man, woman, and child was undernourished, having as few as 400 calories per day, and this was later called the Dutch Hunger Winter. Later, in the 1980s, a doctor named David Barker noticed something strange. Women who were a third of the way through pregnancy during the Hunger Winter were much more likely to have children who would grow up with serious health conditions such as obesity. And what about women who were later on in their pregnancy during the Hunger Winter? These women gave birth to children...
who would be much more likely to grow and develop mental health problems such as depression and schizophrenia. Babies who experience nutritional stress in the womb and who seem healthy at birth can often be afflicted for the rest of their lives with chronic health conditions.

But is this an isolated case? What do we know about other forms of stress during pregnancy? The answer is that we're learning more and more every day, with the help of pioneering scientific research. Pregnancy represents a critical period of brain development for the fetus and stressful situations experienced by the mother can interrupt these processes. Things like physical and verbal abuse, losing a loved one, experiencing a natural disaster, or even something as simple as catching a cold – these forms of stress are sensed by the developing fetus and can increase the chance of the child developing mental health problems such as schizophrenia, autism, and depression in later life.

Unfortunately, it doesn't stop there. Most of us know that our brain carries on developing until our mid-20’s, so it makes sense that we are still vulnerable to the effects of stress throughout our lives. Like the fire coming back every day, some children experience adverse childhood experiences (known as ACEs), such as verbal, physical, or sexual abuse on a regular basis. This is thought to permanently change the way their brain and body responds to the stress hormones cortisol and adrenaline. The number of ACEs that a child experiences can predict how likely they are to develop conditions like heart disease, illicit drug and alcohol addiction, and mental health problems like depression and schizophrenia.

"Epigenetics … a possible mechanism by which life experiences can alter how our bodies work"

So why is this happening? Are stressful experiences ingrained in our DNA? The answer to this is both yes and no. DNA is our body’s ‘blueprint’, and what makes us unique. This blueprint remains the same throughout our life, and acts like a script so that our bodies know what to produce to keep us alive. Scientists have recently discovered that there is another layer of information on top of our DNA, like a sheet of plastic on top of the blueprint, which can change how our DNA script is read. This discovery, known as epigenetics, is emerging as a possible mechanism by which life experiences can alter how our bodies work.

Neuroscientists have shown that when rats don’t care for their pups in the week after birth, the pups grow up to have different epigenetic marks on the
DNA in their brains. This changes their behaviour as adults, and can cause them to show symptoms of schizophrenia or depression. The lead scientist from this study, Dr Ian Weaver, Dalhousie University, USA, says that rats not caring for their pups causes similar diseases to those seen in abused children.

There’s plenty of scientific evidence to suggest that these life experiences can be passed onto our children through our epigenetic marks on our DNA, but what about our grandchildren? The recent migrant crisis in Europe has shown the world devastating pictures of families trying to escape war and poverty, leading to children floating in the water for days and people living on the streets and searching for food. A recent study used a scientific method known as a meta-analysis to show that the stress of being an immigrant increases the risk of developing schizophrenia not only for yourself, but more than doubled the risk for both your children and grandchildren. What can we do to stop this happening, for ourselves, and future generations?

“It’s not all doom and gloom – don’t stress about stress!”

Scientists are now trying to work out how life experiences affect the epigenetic status of our DNA. They hope that when they understand this, they may be able to prevent or reverse the changes that cause diseases. New technologies can measure different epigenetic marks, such as DNA methylation, which can affect how fast or slow our DNA script is read.

Since recognising the lasting effect on our wellbeing, health services have been committed to developing techniques to combat stress. It’s well-known that exercise is good for you and helps us lose weight, but it also helps to prevent schizophrenia and depression, and can even ward off Alzheimer’s and Parkinson’s disease.

Mindfulness is a relatively new technique that can be used to help people to relax, reduce stress, and ‘live in the present moment’. It’s a simple technique, similar to meditating, that encourages us to ignore the busy world around us, and be aware of our body and how we’re feeling. Sound a bit phony to you? Scientific studies have shown that structured mindfulness in children exposed to ACEs can significantly improve long-term health outcomes.

Mindfulness is also endorsed by the NHS, and recommended by the National Institute for Health and Care Excellence (NICE) to help fight depression.

It’s not all doom and gloom, so don’t stress about stress! Interest in this field of research has boomed in recent years, with many scientists looking to understand how maternal and lifelong stress impact disease development.

The annual Science Communication Competition is open to talented science communicators who can be undergraduate or postgraduate students; both members of the Society and non-members. Entries to in the written and video categories must be original works on a molecular bioscience topic and be targeted at the general public. Full details of the competition, including past winners and terms and conditions are available at www.biochemistry.org/GetInvolved/ScienceCommunicationCompetition.aspx

Further Reading

- How childhood trauma affects health across a lifetime, TED talk by Nadine Burke Harris (2014). Online at: www.youtube.com/watch?v=95ovJ3dsNk
Bioscience is one of the fastest developing disciplines and its impact on society economically, politically and ethically is not to be underestimated. For example, significant advances in genome sequencing, genome editing, systems and synthetic biology have all occurred within the last decade, if not within the past few years. The impact of this revolution in understanding of the biological world and the technology that it offers raises fundamental questions about what it is to be human and what is a meaningfully long life on an ever changing planet. How then, should the way that we teach the Biosciences ensure that people of all ages understand the subject in order to have informed opinions and be able to contribute to the advance of the subject in the future. To begin to address this weighty topic, a seminar was organised with German and Dutch colleagues at UCL Institute of Education in London, funded by the UCL Global Engagement Office. The topic of this seminar series was ‘Thoughtfulness in Biology Education’ and the participants ranged from Bioscientists, Science education researchers from across Europe, Education philosophers, university and school teachers, informal science educators and representatives from the Royal Society of Biology, the Wellcome Trust and the Biochemical Society. This one-day symposium included some very fruitful discussions of what thoughtfulness might mean in terms of Biosciences education, both in dealing with Biology as a discipline that spans the molecular, cellular, organismal and planetary realms, and in terms of considerations of justice which the term ‘thoughtfulness’ represents, for example in our relationship to non-human species. Some consensus was reached in the need to move away from a fact-based discourse of Biology competence towards a more holistic approach. This approach would encourage learners to place their understanding within the context of the whole of Biology, particularly as a discipline that impacts on such a wide spectrum of issues faced today. This would involve changes to how we teach the subject, to how we teach the teachers and to how we assess the students learning and teachers’ competencies. More research is needed on this topic and it was resolved that participants in the seminar would continue to work together to make more concrete proposals for the future.

If you are interested in this topic, you can follow the movement of Thoughtfulness in Biology Education via twitter @UCL BioThoughtfulness and #BioEd.

If you are interested in contributing to the movement, please get in touch with Dr Ralph Levinson (r.levinson@ucl.ac.uk) or Professor Stephen Price (stephen.price@ucl.ac.uk)
The last few months proved to be more eventful than many of us anticipated.

With the unexpected announcement of the general election, we worked hard with our partners to ensure that the priorities of the UK science sector were part of the conversation in the run-up to the opening of polling stations.

We contacted candidates in the election and the major party leaders directly, making the case to them that we need to ensure the UK science base remains world-leading, and to ensure all decision-making continues to be informed by evidence and after seeking advice from the scientific community where appropriate.

With the Brexit negotiations having begun we also highlighted the need for reassurance for EU national colleagues who at the moment live and work in the UK. This significant proportion of the UK biosciences community provides an invaluable contribution to the research and innovative work we do, but thousands still do not know if they will be able to remain here come March 2019.

Such uncertainty is leaving countless research groups in a state of limbo when considering their long term plans and goals, and risks slowing down the progress of UK science as a whole.

Through our Brexit analysis we’re looking to capture the impact that negotiations may have on the progression and growth of our sector, and we’re keen to hear from those who are affected by these issues, including members of the Biochemistry Society.

We want to hear from as many researchers, academics and others working in the biosciences as possible, in a bid to understand more about how the changing prospects of the community is impacting research and application.

The maintenance and indeed improving the strength of UK biosciences has always been a priority for us at the RSB; we celebrated the award of Accreditation for another 16 institutions’ bioscience degrees this year, bringing the total number of students enrolled in accredited courses to around 10,000, including many students specialising in biochemistry.

We have now accredited 215 degree programmes across more than 30 higher education institutions to date, and recently awarded the recognition to international programmes including for example the Xi’an Jiaotong-Liverpool University in China. A further 213 programmes have also been awarded Advanced Accreditation.

As part of our direct engagement with member organizations we came together with the Biochemical Society and others for another Twilight Meeting in May focused on Equality, Diversity and Inclusion (ED&I). This offered an important opportunity to share both the challenges we face as membership bodies and also stories and cases of improvement and good practice for ED&I initiatives.

The RSB continues its commitment to the ED&I framework championed by the Science Council, and we will be putting in place additional initiatives to increase diversity and inclusion in all areas of our activity.

In May we held our Annual General Meeting, which drew an unprecedented crowd and saw over one hundred members of the RSB attending; indeed we had to upscale arrangements to a larger venue than anticipated to ensure all could fit!

At the event we were delighted to announce that Professor Dame Julia Goodfellow will take up the presidential reins from May 2018.

We shared with those in attendance at the AGM how much we’ve grown as a membership organization; we’ve seen a significant growth in our membership over the past year, and more membership organizations have joined the RSB alongside the Biochemical Society.

All of our work is done on behalf of our members and we want members and organizations to feel ownership of the outputs, and hopefully to playing a part in ensuring that the UK biosciences continues to go from strength to strength.

We will continue to work hard to represent the UK biosciences community to those in the upcoming negotiations, and try to ensure the deal secured is the best deal for our sector and all of our collective members.
Upcoming Events

- **Glycobiology in Infectious Disease**
  4–5 September 2017, Keele, UK

- **Insulin and Exercise Signalling for Glucose Homeostasis and Metabolic Health**
  6–8 September 2017, Bath, UK

- **Hydrogen Bonds & DNA**
  10 November 2017, Nottingham, UK

- **Synthetic Biology UK 2017**
  27–28 November 2017, Manchester, UK

- **Understanding translational research**
  30 November – 1 December 2017, London, UK

- **Capture Hi-C: Practical approaches to mapping genome-wide regulatory interactions**
  December 2017, London, UK

- **New Approaches for Investigating Nascent Peptide Folding**
  11–13 December 2017, Cambridge, UK

- **Biochemical Basis of Respiratory Disease**
  8–10 January 2018, Nottingham, UK

- **Shaping your career in molecular biosciences: taking a wider view**
  15 January, 2018, London, UK

- **The Dynamic Cell III**
  19–21 March 2018, Manchester, UK

- **New Horizons in ESCRT Biology**
  17–20 April 2018, London, UK

- **30th Annual UK RNA Polymerase focused meeting**
  19–20 April 2018, London, UK

- **83rd Harden Conference Autophagy - from Molecules to Disease II**
  3–6 June 2018, Warwick, UK

Meeting Reports

### Ageing Cell Conference

**27–28 March 2017, Babraham Institute, Cambridge, UK**

The Babraham Institute’s ‘Ageing Cell’ conference, supported by the Biochemical Society, brought together over 200 delegates and showcased the very latest research on Ageing at a cellular level. In a programme designed to explore the intersections of research disciplines, the event did not disappoint.

Four major themes were highlighted during the two-day event. Day one focused on the Ageing Stem Cell and also explored the effects of Ageing on Immune System. Of particular note was Mark Lucanic’s talk on “Robust and reproducible chemical interventions in ageing” sponsored by the Biochemical Society, which highlighted a fascinating collaboration between the Buck Institute, University of Oregon, Rutgers University and the National Institutes on Aging. His talk described how the Caenorhabditis Intervention Testing Program works to identify robust chemical treatments that can delay ageing across diverse genetic backgrounds with highly reproducible results.

Day two themes of Signalling and Epigenetics of the Ageing Cell revealed pioneering research on topics including the epigenetic clock and senescence. These themed sessions, focused Q&A and networking all encouraged significant knowledge exchange and new collaborations have already been cited.

*Linden Fradet* (Babraham Institute)

![Ageing Cell conference group photo](image)

### Matrix proteoglycans – active participants in cell-ECM communication

**3–5 April 2017, St Catherine’s College Oxford, UK**

The Spring British Society for Matrix Biology (BSMB) meeting, supported by the Biochemical Society, focused on how extracellular matrix proteoglycans modulate cell physiology in health and disease. Talks from invited speakers highlighted the important roles that proteoglycans play in regulating cell behaviour in health and disease.

The 2017 Fell-Muir Award was presented to Cay Kielty (University of Manchester), in acknowledgement of her outstanding contributions to the field of matrix biology. Her keynote lecture described her work on fibrillin and its role in elastic tissue form and function.

Young scientists co-chaired the sessions and presented 8 of the talks selected from abstracts. Karen Onions (University of Bristol) won the oral presentation prize for her talk on use of VEGF-C to target endothelial glycocalyx dysfunction. There were 60 posters at the meeting, with Laura Ferreras (Newcastle University) and Andreas Romaine (University of Oslo) winning prizes for their posters on HS-3-O-sulfotransferase in renal fibrosis, and syndecan-4 in cardiac hypertrophic remodeling, respectively.

Feedback from the meeting has been overwhelmingly positive, with researchers enjoying the opportunity to hear about cutting-edge research and state-of-the-art analysis techniques in the glycobiology field.

*Linda Troeberg* (University of Oxford)
Prof. Anne Parle-McDermott joined the School of Biotechnology, Dublin City University (DCU) as a Lecturer in Genetics in 2006 from Trinity College Dublin (TCD) where she was a Lecturer at the Smurfit Institute of Genetics (2005-2006) and a postdoctoral research fellow in the School of Immunology and Biochemistry from 1999-2005. Anne completed her Ph.D thesis at the Royal College of Surgeons in Ireland in 1999 and was awarded her Ph.D by TCD in 2000. Anne is currently Associate Professor at DCU, Principal Investigator of the Nutritional Genomics Group and Deputy Head of the School of Biotechnology. Her research group focuses on understanding the role of folate metabolism in human health and the development of biosensors.

What motivated you to become a scientist?
Since my school days, I was always interested in Biology and so deciding to do a science degree at university seemed an obvious choice for me. What really sealed it for me to continue on at postgraduate level was my early laboratory experiences. I loved being in the lab doing experiments and I got a real taste for what research was really like as an undergraduate; a summer project in the US (US-Ireland Fund) and my final year project. I have had an amazing journey since then and it is such a privilege to work at something that you really enjoy and are passionate about.

What inspires you about molecular bioscience?
If we can get a grasp of some of the molecular detail, then we can get a glimpse of how cells function- molecular bioscience is the key to unlocking the mysteries of biology. I am amazed by the technical advances in the last 10-15 years….what we can do now is mind-blowing!

What are you reading at the moment?
Now that semester is over for another academic year, I’m catching up on a number of articles that are relevant for my research; at the moment my focus is on mitochondrial DNA and growing induced Pluripotent Stem cells.

What’s on your lab bench/desk right now?
Our school just got a new MinION DNA sequencer for teaching our undergraduates/postgraduates and for doing research. We’re having great fun getting it up and running and deciding what to sequence first.

What’s been the greatest challenge in your career so far?
It has to be securing an academic position and independent research funding. Making the transition from a Post-doctoral research fellow to independent investigator and ultimately a tenured academic position is challenging. There simply aren’t enough positions on offer and the timing has to be right also. You do find a range of different skills and talents are required; the most important probably being resilience!

What is your advice for someone who would like to pursue a career in molecular bioscience?
You must have a passionate interest in what you do- all careers have challenges and you need that interest and passion to make it all worth it.

What do you do in your spare time?
I love to read and I’m part of a bookclub that meets once a month. I enjoy movies particularly period dramas and sci-fi. I have recently taken up golf, I enjoy jogging (slowly) and I’ve just re-started playing basketball...not to mention, spending time with my family: my husband Paul and two children, Ciara and Fionn.

Ambassadors are a key group of members that help us to raise awareness of the Biochemical Society, promote its activities, recruit new members and act as the Society’s point of contact at their institution. If you would like to get involved as an Ambassador please contact: membership@biochemistry.org.

UK Dictyostelium meeting 2017

30–31 March 2017, University College London, UK

The UK Dictyostelium meeting returned to Central London for the first time in nearly 20 years with a very diverse programme, sampling the full range of biological interest that the organism, and its genetic and biochemical tractability, generates. The meeting began with a varied exposition of the rich genomics expertise available to the community, from functional genomics, through to the exciting discovery of novel mobile genetic elements.

The motility session did not disappoint, introducing mechanisms and models that may open the door to historically intractable problems, such as how neuronal growth cones can migrate metres in the correct direction during development.

A more recent major contribution of the organism has been in the world of single cell biology, where the organism was used to pioneer methods to image the transcription of individual genes in single living cells. Several talks spanned this rapidly expanding subject, including two introducing single cell transcriptomics as a means to globally interrogate the processes that make cells different during development.

Jonathan Chubb (University College London)
When I became Chair of the Society’s Policy Committee in 2015, I never expected the area of science and education policy to become so busy. After 3 years as a member of the Committee I was already used to replying to Government consultations and requests for information. However, two General Elections in as many years and a momentous decision taken by the UK electorate to leave the European Union has left most people working in science policy reeling from the almost endless requests for information from the Government. The Policy team at the Biochemical Society is relatively small, being comprised of a science policy officer and Director of Society Programmes.

However, the Society packs quite a punch when it comes to the impact of its policy work, which is largely due to our close working relationship with a number of external policy organizations. Science policy work can be complex, wide ranging, reactive, but very rewarding and represents an important member facing activity for the Society. It is also a vital part of our democracy and the importance of science policy in serving the public interest should never be underestimated.

The policy landscape is often complex and the best results are usually achieved when working in partnership, as issues in science are often broad and cover a number of communities. We work with several learned societies and other organizations, in particular, with the Royal Society of Biology (RSB) and the Campaign for Science and Engineering (CaSE). RSB being an umbrella organisation, with a number of Member Organizations of its own, acts as a leader in policy influencing in Parliament on our behalf, and CaSE leads on the lobbying side. RSB organizes two important annual events at the Parliament, which we take part in – Voice of the Future and Links Day. The former is a great forum for young researchers to meet with ministers and MPs and ask science policy questions. Every year six of our student and early career members get to participate in the event. Applications for the event open in January, so keep an eye out.

Last year following the Society’s Governance Review, we restructured the Policy Committee into the Policy Advisory Panel and founded a new membership body – the ‘Policy Network’ – to support it. We are very keen for our members to input into our policy activities and joining the Policy Network is a great way to find out more about science and education policy developments, as well as to feed your views into our work and consultation responses. It is important that the Policy Network represents the Society’s membership. We welcome interest from members of any grade and based in any country, but in particular I would like encourage our student members, both undergraduates and postgraduates, to get involved. Science policy is a fascinating, ever changing and influential area of work that is well worth being involved in!

Brexit has been a topic of extensive discussion within the science community and beyond for months. It is important that UK remains a world leader in science and attracts the best talent from all over the world. As the Brexit process goes ahead, we hear more and more stories of how it has already impacted scientists in the UK. It is important to capture those case studies so they can be used when lobbying the policy makers. If you have already been affected by Brexit and would like to share your story, please get in touch with the policy team.

Another area that the Society is particularly committed to is equality, diversity and inclusion in science. We run a number of activities in this area, for example, we currently sponsor a Daphne Jackson Fellow and help people returning to work after a career break. In addition, we also support the in2science scheme, providing summer placements for talented students from disadvantaged backgrounds. Finally, we run a Diversity in Science scheme, which provides small grants to individuals, groups, charities and not-for-profit organizations to support and address issues relating to diversity in science. The scheme is in its 4th year and has already helped to fund a wide range of projects, change to ‘including Native Biochemists, I’m a Scientist, Get me out of here! and LGBTSTEMinar.. This year’s round of grants will open on 1 September. Please visit the Society’s website for more information.

For more information on the Policy Network or any other policy activities, please visit the Society’s website or email policy@biochemistry.org.
Last month it was a pleasure to welcome guests of the Biochemical Society and Portland Press to our annual Summer Party. We were fortunate to enjoy a warm summer’s evening on the terrace of 30 Euston Square, and I would like to thank our guests, who made it such an enjoyable evening, for joining us to celebrate the Society’s achievements in support of our community.

The 106th Annual General Meeting of the Biochemical Society took place this year on 5 July. Members present received an update on another busy year of activities, but for those who were unable to attend I’m pleased to share a few key updates from the meeting.

The Society’s membership is diverse – with members spread across over 90 countries, of whom nearly half are female and two fifths are students. I am also delighted that our paid membership has reached over 6,400 – the highest level since the Society’s records began in 1999. This thriving community is a really positive sign for the future of the discipline and we continue to support our members through a range of grants, bursaries and studentships (460 awarded in 2016) and a varied programme of scientific events, which in 2016 featured 29 conferences and training events attracting over 1,900 delegates. This year, we are also developing an exciting new online training portal that will help further our reach, and support the Society’s face to face training programme.

Extending beyond our membership, the Society continues to engage the public with key issues in order to broaden understanding of topics such as antimicrobial resistance, cancer treatment, and genome editing, with our public engagement debates last year reaching over 1,000 people. The Society’s education programme also continues to flourish, with over 10,000 registrants to date for our Massive Open Online Course in partnership with the University of East Anglia and FutureLearn, entitled ‘Biochemistry: the Molecules of Life’. Our popular scientific outreach grants scheme also helps support scientific outreach in different communities – don’t forget this year’s deadline is Friday, 22 September.

The Society continues to work closely with the Royal Society of Biology to influence science policy, and earlier this year we launched a Policy Network which to date has been joined by over 100 members. This will help us to engage our members with our policy work and thereby enable us to ensure their views continue to be channelled into national consultations.

As you will no doubt be aware, Portland Press launched two new journals this year (Neuronal Signaling, and Emerging Topics in Life Sciences with the Royal Society of Biology). It is pleasing to see that the number of publications across our journal portfolio rose by 18% in 2016, including a 66% increase in the number of Open Access publications. Don’t forget to visit www.portlandpresspublishing.com for information on the Open Access fee discounts available to all our members.

This year’s Annual General Meeting marked one year that has elapsed since the recommendations of the Governance Review were approved and it is encouraging to report that we have managed to implement the new governance structure fully within this period. At the meeting, we ratified appointments to three new positions created by the governance review project to ensure better representation of the interests of our membership at Trustee level. Dr Martin Pool (University of Manchester) joins the Council of Trustees as Local Ambassador representative; Dr Dominika Gruszka (the Francis Crick Institute) as Early Career Researcher representative; and Dr Malcolm Weir (Heptares) represents the 4.3% of our membership who have identified themselves as working in industry, a figure which we hope to increase in the next year.

Looking forward to next year, we will be welcoming some more new faces to the Society, including Professor Frank Sargent (University of Dundee), who will take over from Dr Nick Watmough as Honorary Treasurer for 2018. At the same time, Professor Sir Pete Downes from the University of Dundee, will take over the reins from Professor Sir David Baulcombe as the Society’s President from January 2018. I very much look forward to working with Pete, Frank and our dedicated Council of Trustees to drive forward the Society’s strategy which we will be discussing at the Trustees’ strategy retreat in November this year.

For Members interested in helping shape the activities of the Society, your opportunity awaits as we have several vacancies open for nomination in the forthcoming Biochemical Society Elections, including positions for Undergraduate, Associate, Emeritus and Industry member representatives on our new Membership and Nominations Committee. Members can nominate either themselves or colleagues quickly and easily through the new MiVoice nominations system – so please look out for further information which will be provided by email.
**Book Reviews**

**What You Need for the First Job, Besides the Ph.D. in Chemistry, Edited by Mark A. Benvenuto**

For students nearing the end (or even the beginning) of their graduate studies, the next steps in a promising career can seem fraught with difficulty. As with all aspects of research it is well worth speaking to those who have been there and done it successfully, or less successfully, and learn from their experience. What You Need for the First Job, Besides the Ph.D. in Chemistry is an attempt to distil a huge amount of useful advice into a single volume. This book is based on the symposium of the same name held at the 246th National Meeting of the American Chemical Society. Although there is an overt emphasis on careers in chemistry, many of the points made within are applicable to those pursuing a career in biochemistry or the wider biosciences.

Many postgraduates may remain uncertain as to their final choice of career and this volume focusses on three of the main areas – corporate, government and academic jobs. As you would expect, there is a degree of commonality between these and it is worth reading all sections even if you aspire to a career in one in particular. The first two chapters focus on corporate jobs and the notorious transition between academia and industry. Whilst there is a degree of overlap between the chapters, the points made are highly relevant and it is good to see different perspectives on the same issues.

The second section of the volume concentrates on corporate jobs, but, beyond that, also on skills development. An important aspect of the PhD is the skillset researchers acquire along the way and these chapters do an excellent job of drawing these out and encouraging early reflection on transferrable skills.

The final and largest section concerns academic jobs. Although the focus centres on the US system, much of the advice is highly relevant for other countries with excellent advice for job applications and interviews. Additionally, there is useful advice for those starting out in their academic positions with respect to teaching and pedagogy.

Although this is a relatively slim volume, it is more likely to be used as a reference into which postgraduates and academics can delve for advice rather than read from cover to cover, although I would recommend all researchers, chemist or not, to read this volume at least once.

**Alan Goddard** (Aston University, UK)

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**Oxidative stress: Diagnostics, Prevention and Therapy Volume 2 by Maria Hepel and Silvana Andreescu**

Oxidative stress is a term widely used in a number of different settings in both the research field and in the general public. A search on PubMed for ‘oxidative stress’ lists 164,271 publications currently accessible, increases of publications over the past 20 years. New and innovative work is being published constantly concerning oxidative stress, and it is brave to publish a book on the matter.

This volume comprises of a collection of research and review papers organized broadly into three overlapping parts titled oxidative stress, pathways and mechanisms, allowing the reader to gain a wide ranging snapshot of oxidative stress research.

The opening chapter summarises the key points on oxidative stress and human health. It successfully introduces the major themes that are developed with more depth later on in specific chapters. These chapters are very wide ranging and despite having a background in oxidative stress, I enjoyed seeing the work in different disciplines that I otherwise wouldn’t have been exposed to including oxidising pollutants, an interesting focus on biosensors and molecular probes, as well as the classic oxidative stress subjects (ageing, nitric oxide and blood vessels).

The figures held within this collection are of great benefit to the reader and are helpful to breakdown complex ideas. What is confusing is having black and white versions located within the respective article and the reprint of these figures in colour in the middle of the book. I don’t feel this adds much and if the authors deemed that colour versions of the figures were necessary, they should have been contained within the article itself.

In summary, this collection of review and research articles related to oxidative stress falls between two groups. Researchers already in the field are likely to be up to date on more recent articles and the detail contained within this would be too much for undergraduates or casual readers.

However, for researchers entering the oxidative stress field for the first time, this book provides an excellent summary and starting point to further explore the more recent publications.

**Gareth Anthony Nye** (University of Manchester, UK)
The Epigenetics Revolution by Nessa Carey

Nessa Carey has chosen the fast-paced field of epigenetics as the subject matter for her debut book, which is a very enjoyable read. There have been a number of fascinating discoveries made in the epigenetics field that have truly expanded our understanding of vital parts of human biology. These are described elegantly and at length by Carey. In many cases, the earliest discoveries came from developmental scientists such as Professor Sir John Gurdon as far back as the 1950’s, who used live toad models to successfully reprogramme an adult toad nucleus by inserting it into an unfertilised egg resulting in the creation of a new toad. This was an early demonstration of how genes can be switched on and off in a non-permanent way and is the system we now know as epigenetics.

X chromosome inactivation in females is explained very well in this book. Early predictions by Mary Lyon in the 1960’s to present day knowledge of the XIST gene, responsible for X inactivation, are all included. Diseases with epigenetic mechanisms such as Angelman Syndrome, Prader-Willi Syndrome and cancer are also covered extensively by Carey.

It can be difficult with a complex topic like this to get the right balance for a general audience. A number of strategies have been implemented to help the non-expert reader along the way. Time is afforded early in the book to describing basic genetics. This is a necessary introduction that is essential before moving on to the fundamentals of epigenetics. Time is also spent on careful description of the mechanisms underpinning epigenetic modifications such DNA methylation and histone acetylation. The ‘heaviness’ of the studies under discussion are also counteracted by giving us some light, general information on the scientists behind these fascinating discoveries.

On the flip side of this, for readers wanting further details about the science, the book is heavily referenced with Carey occasionally suggesting reviews that offer more comprehensive coverage of a topic. Overall, a good balance is struck between keeping an informed reader entertained while at the same time not alienating the person with a more basic level of knowledge.

Despite the huge advances in our understanding of epigenetics in a short space of time, we are given the impression that the best is yet to come. The reader is left in no doubt at the close of this book that this is a hugely exciting field which has fundamentally improved our understanding of genetics.

Maeve Kiely (University of Limerick, Ireland)
People in white coats
By Benoît Leblanc
(http://peopleinwhitecoats.blogspot.co.uk)
Crossword Competition

Win

This month’s crossword prize is an ‘In the Lab’ T-shirt (men’s or women’s fit) from The Flying Bulb.
Simply email the missing word, made up from letters in the highlighted boxes to biochemist@biochemistry.org, by Monday 4 September.
Please include the words ‘August crossword competition’in the email subject line.

Congratulations to June’s winner:

Letong Yuan from Imperial College London
The missing word from last issue’s competition was PANDEMIC.
Letong received a Biohazards themed gift box from Giant Microbes.

Terms and conditions: only one entry per person, entrant must be a current Biochemical Society member; closing date Monday 4 September 2017. The winner will be drawn independently at random from the correct entries received. The winner will receive an ‘In the Lab’ T-shirt (men’s or women’s fit). No cash alternative available. No employee, agent, affiliate, officer or director of Portland Press Limited or the Biochemical Society is eligible to enter. The winner will be notified by email within 7 days of the draw. The name of the winner will be announced in the next issue of The Biochemist. The promoter accepts no responsibility for lost or delayed entries. Promoter: Biochemical Society, Charles Darwin House, 12 Roger Street, London WC1N 2JU; do not send entries to this address.