be separated from the rest of the ocean in the way that laboratory experiments can be constrained by beakers. To overcome this problem, Smetacek *et al.* used an ocean eddy near Antarctica as a 'beaker' (Fig. 1). This solution seems to work well — the authors provide considerable evidence that the upper and lower layers of the eddy moved together coherently, and that the eddy had exchanged less than 10% of its content with the surrounding ocean by the end of the experiment.

The authors introduced dissolved iron(II) sulphate ($FeSO_4$) over a 167-km^2 patch in the eddy's core, so that the concentration of iron at the ocean's surface reached a level known to stimulate phytoplankton growth. The consequences were substantial: phytoplankton biomass more than doubled in 24 days, with 97% of the observed increase in chlorophyll associated with large diatoms, a class of phytoplankton that has high iron requirements. Along with this growth, the authors observed a reduction in levels of dissolved inorganic carbon (DIC) and of several nutrients (nitrogen, phosphorus and silicon). Data collected from stations outside the eddy, used as controls to monitor non-fertilized conditions, showed no such effects.

The scientists kept up their study for a full 37 days — longer than any other OIF experiment — and so were able to document the collapse of the diatom bloom through the formation of rapidly sinking aggregates of dead phytoplankton and zooplankton faecal pellets that carried carbon to the deep ocean. The last 13 days of observations were crucial to their success, because they enabled the authors to calculate the depletion of dissolved and particulate carbon at the surface and subsequent increases in particulate organic carbon at depth. Such 'budgets' are notoriously tricky to close in OIF studies, because of the difficulty in quantifying carbon losses that occur through air–sea gas exchange and physical mixing at the fertilized patch's boundaries, and because it is hard to account for variability in carbon levels within and outside the patch. In this case, however, the combination of evidence was clear: the iron-induced diatom bloom led to the export and sequestration of about one mole of carbon per square metre of ocean surface, from the uppermost 100 metres of ocean. In fact, one of the methods used by the authors suggested that, at its peak, carbon flux was the largest ever recorded in the Southern Ocean.

The implications of these findings are several-fold. First, a measure of the efficiency of carbon export in the experiments can be obtained by dividing the amount of DIC removed from the upper 100 metres of ocean by the amount of iron added. This measure — the carbon/iron molar ratio — is crucial for geoengineering proposals, which must specify how much iron will be needed to affect climate. In the laboratory, the ratio can be 100,000 or more[4]. By contrast, the ratios reported in previous OIF experiments[3] have been much lower, in part because iron uptake by plankton in the ocean is inefficient compared with that under laboratory conditions, but also because of differences in the amounts of iron and carbon that are recycled at the surface, or which sink to depth. Smetacek *et al.* report that the carbon/iron molar ratio in their long experiment was 13,000 — higher than in the previous OIF studies — and argue that this number would have increased further had they followed the bloom for longer.

Furthermore, the authors' results defied expectations[5] that the availability of light would limit phytoplankton growth in their experiment. Phytoplankton grow in the 'mixed layer' of the ocean, the region in which the uppermost layers of the ocean are homogenized by wind and other physical effects; the mixed layer in Smetacek and colleagues' experiment was deep, extending down to 100 metres, where little light would penetrate. Comparison of Smetacek and colleagues' study with naturally occurring blooms[6,7] in iron-rich waters near islands in the Southern Ocean also suggests that their experiment was similar to natural OIF events, and that higher sequestration was potentially possible.

Although the authors conclude that OIF does indeed sequester carbon in the deep ocean, questions remain about the possible unintended consequences of geoengineering. For example, OIF might cause undesirable effects, such as the production of nitrous oxide (a more potent greenhouse gas than carbon dioxide); oxygen depletion in mid-waters as algae decompose; or stimulation of a toxic algal bloom. And, as with all carbon-removal methods, OIF is no silver bullet for mitigating climate change. The ocean's capacity for carbon sequestration in low-iron regions is just a fraction of anthropogenic CO_2 emissions, and such sequestration is not permanent — it lasts only for decades to centuries. However, humans have already embarked on an ocean geoengineering experiment through our energy practices (which are affecting climate and acidifying the seas), by fishing, and through our other uses of ocean resources.

Most scientists would agree that we are nowhere near the point of recommending OIF as a geoengineering tool. But many think[8,9] that larger and longer OIF experiments should be performed to help us to decide which, if any, of the many geoengineering options at hand should be deployed. EIFEX certainly does not answer all of the questions about geoengineering, but by showing how the addition of iron to the ocean not only enhances ocean productivity, but also sequesters carbon, it is one of the best OIF studies so far. ■

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CARDIOLOGY

Bad matters made worse

Heart attacks occur when lipoprotein-driven inflammation called atherosclerosis triggers blood clotting in the arteries. It seems that the attacks can, in turn, accelerate atherosclerosis by fanning the inflammation. SEE LETTER P.325

IRA TABAS

Heart attack, or myocardial infarction, is a leading cause of morbidity and mortality worldwide, and people who have had one infarction are at increased risk of another in the first year or so after the attack[1]. Myocardial infarction results from acute, occlusive thrombosis (blood clots) within the coronary arteries. These clots form at sites of atherosclerosis, a chronic disease process in which fat and cholesterol build up along the artery walls[2]. Atherosclerosis starts when circulating fat-carrying particles called lipoproteins, most notably low-density lipoprotein (LDL), are retained in the subendothelium, a tissue layer in the artery wall[3]. This induces an inflammatory response that involves the influx of immune cells called monocytes, which differentiate into other inflammatory-cell types, including phagocytic cells called macrophages and dendritic cells[3,4]. On page

325 of this issue, Dutta *et al.*[5] show that in mice with atherosclerosis, myocardial infarction leads to increased monocyte recruitment and enhanced atherosclerosis. If these processes are similarly linked in humans, the findings may have implications for therapeutic strategies in human heart disease.

The idea for this study came from the observation that a high monocyte count in the blood after myocardial infarction is a risk factor for repeat infarction, and that, in mice, infiltrating spleen-derived monocytes have a role in atherosclerosis[6]. If there is a cause–effect relationship between monocyte count and atherosclerosis, this would suggest that the number of circulating monocytes, particularly monocyte subclasses that are especially inflammatory, could be a rate-limiting factor.

To explore these issues, Dutta and colleagues used a mouse model of atherosclerosis in which the mice lack apolipoprotein E (APOE), a protein that facilitates the removal of certain types of atherosclerosis-promoting lipoproteins from the blood. The mice were also fed a high-cholesterol diet. Mice do not normally develop atherosclerosis because they have low levels of atherogenic lipoproteins, but the combination of a high-cholesterol diet and the absence of APOE in this model induces atherosclerosis. However, even this robust model does not cause acute thrombosis and myocardial infarction, probably owing to several physical and biochemical factors. So the authors modelled heart attack in the atherosclerotic mice by clamping shut their left coronary artery.

Within 1–3 weeks after myocardial infarction, the atherosclerotic lesions in the aortas of the 'heart attack' mice were approximately 40% larger than those in sham-operated animals, which had atherosclerosis but had not undergone infarction. The mice with infarction also had elevated blood monocyte counts, and the lesions themselves contained greater numbers of inflammatory cells and showed signs of disease progression, including larger regions of dead cells (necrosis). Furthermore, the authors provide evidence that the sympathetic nervous system (SNS) was activated in the mice following myocardial infarction, and that this led to an expansion of monocytes in their spleens. This was followed by monocyte delivery to the blood (a process called monocytosis) and then to the atherosclerotic lesions (Fig. 1). The SNS is associated with the 'fight or flight response', so it may be that in humans, and perhaps in this mouse model, SNS activation following myocardial infarction is a response to pain, anxiety and an acute decrease in heart function. The authors also showed that splenectomy or drug-induced blockade of the SNS lowered post-infarction monocytosis in the mice, although they did not report whether these interventions affected progression of atherosclerosis per se.

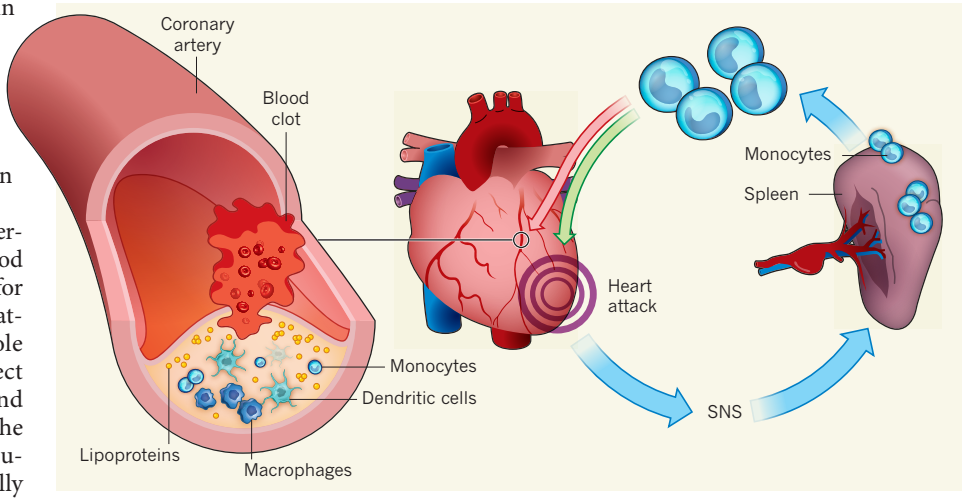

Figure 1 | A cycle of damage and repair. During atherosclerosis, fat-carrying lipoproteins are retained in the artery wall. This induces an inflammatory response that is characterized by an influx of immune cells called monocytes, which differentiate into other inflammatory cells, such as macrophages and dendritic cells. Blood clots at sites of atherosclerosis can block the arteries and cause heart attacks. In a mouse model of atherosclerosis, Dutta *et al.*[5] show that heart attack activates the sympathetic nervous system (SNS), and that this promotes monocyte production in the spleen. The authors demonstrate that the monocytes move from the spleen to the blood and then to atherosclerotic lesions in the arteries, where they accelerate the progression of these lesions (red arrow). However, monocytes are also likely to be important in the repair of heart muscle tissue (green arrow) following heart attack[9], and this dual role could complicate attempts to treat post-heart-attack atherosclerosis by targeting monocytes.

Might this process of heightened susceptibility following myocardial infarction also be important in humans? The mouse model used by Dutta *et al.*[5] has many obvious differences to human disease, most notably the absence of atherosclerosis-driven acute thrombosis or infarction, and the possible confounding effects of the absence of APOE, which has several other biological actions besides lipoprotein clearance. However, the atherogenic process itself is similar to that which occurs in humans, and the links to post-infarction monocytosis and SNS activation in humans add relevance.

Even so, the mechanism underlying post-infarction susceptibility in humans is likely to be multifactorial. For example, heightened systemic and cardiac inflammation could fuel the inflammatory response by mechanisms over and above raising monocyte numbers, and biological changes induced by the occluded artery or by tissue damage sustained in the attack might alter the subendothelium of nearby arteries in ways that promote lipoprotein retention. It should also be remembered that heart attacks often indicate the presence of environmental and/or genetic risk factors, and so are probably a marker of individuals predisposed to accelerated atherosclerosis due to a variety of factors beyond the actual infarction event.

The possibility that an SNS–spleen–monocyte pathway of accelerated atherosclerosis contributes to the increased risk of repeat events after heart attack could add perspective to current therapeutic strategies and suggest new ones. Several clinical trials have shown that post-infarction administration of high doses of statins — drugs that lower blood levels of LDL — have a substantial protective effect against repeat infarction. Reduction of LDL would be expected to interrupt the atherosclerotic process in the post-infarction period, as it does in other settings. However, some researchers interpret this rapid effect of statins as indicative of other mechanisms, such as anti-inflammatory activity, which is known to be a property of statins[7]. Low levels of high-density lipoprotein (HDL), a risk factor for myocardial infarction, have also been linked to increased monocytosis and atherosclerosis[8], so the current study could inform current human therapeutic trials designed to raise plasma HDL. It would also be interesting to assess whether beta-blocker drugs, which are often given to patients following heart attack, have any effect on monocytosis and atherosclerosis progression.

Finally, although therapeutic strategies aimed at lowering monocyte numbers may have broad benefit in blocking the progression of atherosclerosis, their applicability could be tempered by the fact that post-infarction monocytosis could also have a role in healing the damaged heart muscle[9] (Fig. 1). Indeed, this previous finding, in the context of Dutta and colleagues' study, raises the fascinating question of why these monocytes help tissue healing in infarcted heart muscle but worsen atherosclerosis. A crucial healing function of monocyte-derived phagocytes is in the clearance of dead cells[10]. It is possible that dead cells in atherosclerotic lesions are less easily recognized by the newly arriving phagocytes than are dead cardiac muscle cells. Alternatively, when the post-infarction phagocytic cells enter the lesions, they may be exposed

to factors that impair their ability to clear dead cells — factors that would not be present in the infarcted heart muscle. Thus, although it is likely that inflammation and monocytosis have several roles in the response to heart attack, further understanding of these processes is needed to intelligently translate these concepts into new therapies. ■

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CHEMICAL BIOLOGY

Greasy tags for protein removal

Most proteins in the human body are difficult targets for small-molecule drugs. This problem may have been overcome with the discovery of molecules that induce protein degradation, suggesting fresh, modular approaches to drug discovery.

TAAVI K. NEKLESA & CRAIG M. CREWS

It was recently discovered[1,2] that proteins covalently 'tagged' with small, synthetic, hydrophobic molecules are degraded by the cell's quality-control machinery. Writing in *Chemistry & Biology*, Long *et al.*[3] now report that non-covalent binding of such molecules also marks proteins for degradation. This finding could open up a wide range of proteins as targets for drug-discovery programmes.

The dearth of newly approved drugs in the past decade reflects the challenges faced by the pharmaceutical industry. Although advances in genomics have identified many proteins that are implicated in disease, many of these proteins — especially those that are not enzymes — are not currently viable drug targets. In fact, it has been estimated that only about 15% of the human proteome is 'druggable' with small molecules[4].

Many attractive drug targets have therefore been dubbed 'undruggable'. For instance, there are roughly 1,400 human transcription factors — proteins that regulate messenger RNA synthesis from DNA, but which lack enzymatic activity. These proteins remain largely undruggable, despite the fact that aberrant expression of some of them is known to cause cancer. One possible solution to this challenge has been the development of small interfering RNAs (siRNAs), which intervene in gene expression by binding to mRNA. However, delivering siRNAs to their targets *in vivo* has been a difficult hurdle to overcome, and so small molecules that can affect the function of undruggable proteins are needed.

Another emerging approach is to destroy, rather than inhibit, target proteins in cells. Normal protein turnover in cells is mainly mediated by the ubiquitin–proteasome system (UPS), which tags unwanted or misfolded proteins with chains of the ubiquitin protein. Once ubiquitinated, the marked proteins are recognized by the proteasome, a large, barrel-like molecular machine that cleaves proteins into small peptides. Efficient removal of unwanted proteins is key to cell survival, as evidenced by the development of proteasome inhibitors as effective antitumour agents[5].

Several strategies have been reported that co-opt the UPS for targeted protein degradation. One of these uses 'proteolysis-targeting chimaeric molecules' to bring the protein of interest close to a ubiquitin ligase (an enzyme that mediates the ubiquitination of a target protein), thus bringing about protein ubiquitination and subsequent degradation[6].

An alternative approach is to mimic a misfolded protein state using small molecules. Normally, the 'greasy' (hydrophobic) side chains of polypeptides are buried in the interior of a globular protein, with the hydrophilic amino-acid residues lying at the surface. Even a small increase in surface hydrophobicity can make a protein unstable. For instance, the deletion of a single amino acid from the CFTR protein is the main cause of cystic fibrosis. The deletion results in the exposure of hydrophobic patches on the surface of CFTR, leading to misfolding and subsequent degradation of the protein (Fig. 1).

We have recently shown[1,2] that the covalent attachment of a synthetic hydrophobic group (such as adamantane, a bulky hydrocarbon) to the surface of proteins attracts chaperone proteins whose job it is to help refold misfolded proteins, or, if they cannot be refolded, to target them for degradation by the proteasome. But most drugs bind to proteins through non-covalent interactions, and it was unclear whether non-covalently bound molecules could also trigger this sequence of events.

Long *et al.*[3] have settled this concern. They investigated the biological effect of attaching a hydrophobic group (Boc_3Arg, a modified arginine amino acid) to trimethoprim (TMP), a ligand molecule that binds non-covalently to the dihydrofolate reductase (DHFR) enzyme from the bacterium *Escherichia coli*. The authors observed that TMP–Boc_3Arg induces 30–80% DHFR degradation in mammalian cells, depending on the rate of DHFR synthesis. This effect could be blocked either by TMP, which competes with TMP–Boc_3Arg for binding to DHFR, or by inhibitors of proteasome activity.

The authors also demonstrated that the glutathione S-transferase (GST) enzyme is degraded when treated with a compound in which Boc_3Arg is attached to ethacrynic acid (EA), a GST inhibitor that becomes covalently bound to the enzyme's active site. This demonstrates that the degradation effect of Boc_3Arg occurs for at least two enzymes. Long *et al.* went on to make a fusion protein in which DHFR is attached to GST, and then treated cells producing the protein with either TMP–Boc_3Arg or EA–Boc_3Arg. They observed that DHFR–GST was degraded more efficiently by EA–Boc_3Arg, which binds covalently to the protein, than by TMP–Boc_3Arg, which binds non-covalently. This suggests that the covalent attachment of hydrophobic tags to enzymes is the more effective strategy for protein degradation.

As TMP is a high-affinity inhibitor of *E. coli* DHFR, further studies are needed to determine whether a small molecule that is both a protein inhibitor and a degradation signal is more effective in abrogating protein function than a simple inhibitor. As pointed out by the authors, the case of botulinum toxin illustrates the advantage of the degradation approach. The most potent form of this toxin, which causes muscle paralysis, has a half-life in the body of about 3 months. Although an inhibitor of the toxin would be able to suppress toxicity in the short term, elimination of the toxin is obviously a preferable therapeutic approach.

However, the Boc_3Arg moiety is large (almost 500 daltons in mass), and large molecules often have poor pharmacokinetic properties that limit their use as drugs. So, appending it to an existing inhibitor could potentially worsen that inhibitor's pharmacokinetic properties. Curiously, even though TMP has high affinity for *E. coli* DHFR and is thought to have excellent cell permeability,