"For quite a long time, it was very expensive to do image creation and processing, but PCs and graphic cards are today so cheap that even small institutes can afford them," Hegerl said. In addition, improvements in the software make the technology more accessible for non-specialists. "In the future, [scientists] may become more able to do this themselves, so they no longer need a computer expert… which removes the need for people like myself," Ritchie said. However, Dewey warned that scientists should not leave the field to commercial developers but should stake their own claim. "It really is very important that all of these processes use open standards and that all software is open," he commented, to prevent monopolization by companies or the problem of competing software based on proprietary data.

Buying the software and hardware does not automatically guarantee exciting insights or fascinating animations; it still requires considerable expertise. "First you need a good idea," and experience in knowing which applications and processes are optimal for analysing and visualizing the object in question, Hegerl said. "I generally spend more time thinking about the problems than doing the calculations," Ritchie commented. Similarly, animations such as Sannuga's ribosome or the ATPase machinery are not completed within a few days. "To master an advanced software such as Maya, one would require considerable training, maybe a year or more," Sannuga commented. "Scientists with limited resources and animation skills would be able to produce less complex animations, but they would still require experts in molecular animation when producing more challenging animations."

Beyond their scientific use, these images and movies can fascinate laypeople too. "It is really impressive to be able to see into a cell," said Hegerl, describing the attraction of such images. Furthermore, the fascination and sometimes beauty of computer-generated scientific images and animations would make powerful tools for presentations and in education. "This is coming and it makes sense. I think we should use it in schools in the long term," Hegerl said, adding that the institute already regularly receives requests from universities to use their images for teaching.

It is not only educators who are interested, but also people specialized in the arts. The latest attraction at the Denver Museum of Nature and Science (CO, USA) is a 23-minute scientific animation of a flight through a black hole—to "the other side of infinity", as their website explains. The simulation, using animations created by Andrew Hamilton, Professor of Astrophysics at the University of Colorado, Boulder, is based on hundreds of hours of computing time at the University of Illinois National Center for Supercomputing Applications, to calculate how light behaves at the event horizon and inside the black hole (Johnson, 2006). If children can be thrilled by flying through a black hole, they might be equally exhilarated by flying through a cell. To this end, computer graphics are not only a great new tool for the researcher but also a way to fascinate the general public with science.

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The infection connection

Helicobacter pylori is more than just the cause of gastric ulcers—it offers an unprecedented opportunity to study changes in human microecology and the nature of chronic disease

When Barry Marshall drank a broth of *Helicobacter pylori* in the mid-1980s, he hoped to prove that the bacteria cause gastric ulcers, a common complaint worldwide. Sure enough, Marshall, then at the Royal Perth Hospital in Western Australia, soon developed gastritis, the precursor to stomach ulcers and gastric cancer. In addition to earning him the 2005 Nobel Prize in Physiology or Medicine with his colleague Robin Warren, this unorthodox—and now infamous—experiment proved that a bacteria caused a chronic disease that had previously been attributed to genetic and lifestyle factors. During the same period, researchers have proposed bacterial and viral triggers for many other chronic diseases, such as cancers and coronary heart disease. But Marshall and Warren's discovery has far wider implications than curing a single disease. "Human microecology is changing, and these changes have manifestations in terms of disease," said Martin Blaser, Chair of the Department of Medicine at New York University (NY, USA). To understand the impact of these changes, researchers need to take a closer look at host–pathogen interactions—both good and bad.

"What's interesting about *Helicobacter* is that everybody is focusing on its pathogenic role and not enough on its role as an indigenous organism," Blaser said. He thinks that the microbes that live inside humans are not there accidentally—over the course of human history, they have adapted to humans and have made trade-offs to become obligate parasites. As a result, their evolution is inextricably linked to human evolution, as well as to human disease. "Our microbes are a part of human physiology … as much a part … as our liver, or our kidneys, or our heart," Blaser commented. "They are a metabolic compartment or a series of metabolic compartments in the human body and how they behave and … their characteristics are relevant to health and disease."

KOCH'S POSTULATES

In 1883, German bacteriologist Robert Koch presented three rules to prove the pathogenicity of an organism. A fourth was added in 1905 by E.F. Smith. These rules are commonly referred to as Koch's postulates.

1. The suspected causal organism must be constantly associated with the disease.
2. The suspected causal organism must be isolated from an infected host and grown in pure culture.
3. When a healthy susceptible host is inoculated with the pathogen from pure culture, symptoms of the original disease must develop.
4. The same pathogen must be re-isolated from hosts infected under experimental conditions.

For chronic diseases, it is often not possible to use Koch's postulates to make a microbial prognosis. This may be due to difficulties isolating the suspected causal organism: if it is in an inaccessible (such as brain) or non-sterile part of the body; if it is ubiquitous in patients with and without the disease; or if isolation methods are insufficient. As it is difficult to make accurate diagnoses for complex disorders such as chronic fatigue syndrome or schizophrenia, linking these conditions to infectious triggers becomes even more complicated. And as chronic diseases often arise long after the initial infection (at which point the trigger may have been cleared from the body), and depend on host factors, Koch's postulates may be insufficient to prove a pathogenic link.

> …the biology of a short-term infection differs markedly from that of a persistent colonization, even for the same infectious agent

The link between chronic diseases and infectious agents dates as far back as the early 1900s, when F. Peyton Rous showed that viruses could cause cancer. Since then, many more researchers have tried to confirm similar links, despite difficulties using Koch's postulates to prove causation (see sidebar) and opposition from some members of the scientific community. Now, "the scientific arguments tend to be around 'which' infectious agent and 'how', rather than 'whether'," said Ian Frazer, Director of the Centre for Immunology and Cancer Research at the University of Queensland, Australia. Efforts to determine 'how' are now focusing on the distinction between acute infection and persistent colonization, and the long-term balance between man and microbe.

Evidence points to *H. pylori* being an ancient and persistent guest in the human gastrointestinal system, rather than an acute infection. But in the past few decades, the prevalence of *H. pylori* has declined, offering an unprecedented opportunity to track the demise of a member of the normal human flora. In developed countries, the bacteria now affects 20–50% of adults, but studies show that there has been a 50% decline in the prevalence of *H. pylori* in the USA since 1968 (www.helico.com). At least three factors have a role in its disappearance: cleaner water, less crowding and better hygiene, and the widespread use of antibiotics over the past 60 years.

The true nature of the bacteria's relationship with humans is only beginning to emerge, and reveals a complex mixture of pathogenicity and protection. "I don't use the term infection for *Helicobacter* anymore, I only use the term colonization," Blaser said. "I think it's normal flora just like *Escherichia coli* or Bacteroides." In addition to *H. pylori*, the human body houses hundreds of strains of commensal bacteria and viruses, which may persist for months, years or even a lifetime. For the most part, these microbes cohabit happily with their host, and rarely cause problems—indeed, their presence is often seen as beneficial rather than detrimental.

If it is not the mere presence of a microorganism that triggers disease, then other factors must be at play. Indeed, the biology of a short-term infection differs markedly from that of a persistent colonization, even for the same infectious agent. "Clearly, persistent infection is a dynamic state and not one of quiescent bacteria held in check by the force of the host immune system," wrote Stanley Falkow, Professor of Microbiology and Immunology at Stanford University School of Medicine (CA, USA; Falkow, 2006). Thus, "[t]he outcome of confrontation between a human body and a pathogenic microorganism is determined by what each of the players brings to the fight," according to a recent report on 'Microbial Triggers of Chronic Human Illness' from the American Association of Microbiology (AAM; Carbone *et al*, 2005).

> …"[t]he outcome of confrontation between a human body and a pathogenic microorganism is determined by what each of the players brings to the fight"

Although *H. pylori* may be the most well known, many other bacteria and viruses have been linked—either definitively or loosely—to chronic conditions (Table 1). Human papillomavirus (HPV) has an important role in cervical cancer, as do hepatitis B and C in hepatic cancer, Epstein–Barr virus in lymphoma and schistosomes in bladder cancer. Infectious agents have also been suggested to contribute to leukaemia, Crohn's disease, arthritis and rheumatic fever. And for decades, scientists have attempted to link chronic infection with cardiovascular disease, with mixed success.

However, despite the growing evidence, the role of infectious agents in a wide range of chronic diseases is still contested. Ruth Itzhaki, a professor in the Molecular Neurobiology Laboratory at the University of Manchester, UK, faced an uphill battle against some members of the Alzheimer's disease research community with her work on the link between herpes simplex virus type 1 (HSV1) and

Table 1 | Chronic diseases for which there is evidence or suspicion of an infectious aetiology

Infectious agent	Chronic disease(s)
Borellia burgdorferi	Lyme disease
Campylobacter jejuni	Guillain–Barré syndrome
Chlamydia pneumoniae	Atherosclerosis Alzheimer's disease
Chlamydia trachomatis	Reiter's syndrome and reactive arthritis Pelvic inflammatory disease
Cytomegalovirus	Post-transplant accelerated atherosclerosis
Enteroviruses	Diabetes
Epstein–Barr virus (EBV)	Burkitt's lymphoma Nasopharyngeal cancer Chronic fatigue syndrome Multiple sclerosis
Escherichia coli O157:H7	Haemolytic–uraemic syndrome
Group A streptococcus	Post-streptococcal glomerulonephritis
Group A *Streptococcus agalactiae*	Obsessive–compulsive disorder
Hepatitis B virus (HBV) Hepatitis C virus (HCV) HBV and delta virus	Hepatocellular carcinoma, chronic hepatitis
HBV	Polyarteritis nodosa
HCV	Mixed cryoglobulinemia
Helicobacter pylori	Gastric lymphoma MALT lymphoma Peptic ulcer disease Sjogren's disease Primary biliary cirrhosis
Herpes simplex virus	Bell's palsy
Histoplasmosis	Chronic pericarditis
Human papillomavirus	Cervical carcinoma Larynginal papilloma Penile cancer Anal cancer Vulvar and vaginal intraepithelial neoplasia Venereal warts Common warts Head and neck cancer
Human T-cell lymphotropic virus type 1	Adult T-cell leukaemia Tropical spastic paraparesis
Influenza (intrauterine exposure)	Schizophrenia
Kaposi's sarcoma-associated herpes virus	Multicentric Castleman's disease Lymphoma Kaposi's sarcoma
Measles	Sub-acute sclerosing panencephalitis
Mycobacterium leprae	Leprosy
Mycobacterium paratuberculosis	Crohn's disease
Mycobacterium tuberculosis	Tuberculosis
Parvovirus B19	Anaemia; arthritis
Prions	Creutzfeldt–Jakob disease Kuru Familial insomnia Amyotrophic lateral sclerosis
Rubella	Post-rubella arthritis syndrome Congenital rubella syndrome
Simian virus 40	Mesothelioma
Syphilis	Tertiary and neurosyphilis
Tropheryma whippleii	Whipple's disease

Adapted from tables 1 and 2 in Carbone *et al* (2005) with kind permission from the American Association of Microbiology.

the disease. "All they are interested in is amyloid," she said. "When we first showed the presence of viral DNA in elderly brains, we met huge incredulity. Since then about seven other groups have broadly substantiated this, and the results have generally been quietly accepted."

More tenuous links have been suggested between microbial agents and conditions, such as obesity and diabetes. Human adenoviruses increase adiposity in humans (Atkinson *et al*, 2005) and other animal models (Whigham *et al*, 2006; So *et al*, 2005). Although Blaser noted that a link between *H. pylori* and obesity and diabetes in humans is "preliminary and not supported", he pointed out that the regulation of hormones involved in energy homeostasis changes in the presence or absence of the bacteria. "*Helicobacter* status affects the status of hormone production in the stomach."

But to conclude that all disease-causing agents are bad is certainly too simple. For *H. pylori* colonization, for example, "there's a biological cost to carrying it—some people will get ulcers, and some people will get cancer," Blaser said. "But there may be some biological benefits." In parts of the world where *Helicobacter* incidence is decreasing, ulcer disease and gastric cancer are also declining in incidence, as is expected. However, the number of cases of esophageal cancer, specifically adenocarcinoma of the esophagus, is rising. "The data indicate that it's the most rapidly rising cancer in developed countries like US, UK, Norway, and Australia, where this has been studied," Blaser said.

The question then is whether a link exists between these cancers and the decrease in *H. pylori* incidence. Paradoxically, over the course of a lifetime, *H. pylori* decreases gastric acidity. "You'd think there should be a lot of acid because of ulcer disease but ulcer disease is not well understood," Blaser explained. Essentially, the inflammatory process caused by long-term *H. pylori* colonization reduces gastric function in terms of acid production. As people age, and as their stomachs age, they produce less acid—part of the pathogenesis of gastric cancer. For an individual without *H. pylori*, the stomach pumps out full levels of acid for decades, thus affecting their unprotected esophagus, "which, I would

argue, has never in evolutionary history been in this situation before."

Other factors—not yet fully understood—undoubtedly play a part. Not everyone who has *Helicobacter* will develop gastric cancer, and not everyone without the bacteria will develop esophageal cancer. "These are not all or none relationships," Blaser explained. "It's about trends." When the incidence of a disease rises so rapidly in such a short space of time, evolutionary factors cannot be the cause—environmental factors must be to blame. And "the place to begin looking is in our human environment, our indigenous microbes. That's the smoking gun," Blaser said.

Part of the reason why *H. pylori* receives such attention is because Marshall and Warren's research led to a cure: antibiotics are now routinely used to cure gastric ulcers. Similar excitement surrounded the development of a prophylactic vaccine against HPV infection, based on research by Frazer and colleagues at the University of Queensland. Gardasil™ (Merck & Co; Whitehouse Station, NJ, USA) and Cervarix™ (GlaxoSmithKline; Uxbridge, UK) are 100% effective in preventing HPV16 and HPV18 infections. Frazer and his colleagues are now working on therapeutic vaccines to eliminate existing HPV infections. "We hope that the answers obtained from that work might be more generically applicable to the field of therapeutic vaccines," he said.

> …despite the growing evidence, the role of infectious agents in a wide range of chronic diseases is still contested

But if other infectious agents share *H. pylori*'s two-sided nature, will such eradication attempts cause more trouble than they are worth? The difficulties in balancing the risks and benefits "should not be a hindrance to developing vaccines and antimicrobials", according to G. Singh Chhatwal, who studies the role of streptococci in rheumatic heart disease and other diseases, at the Department of Microbial Pathogenesis and Vaccine Research at the German Research Centre for Biotechnology in Braunschweig. Frazer, conversely, sees no danger in eradicating HPV to decrease the risk of cervical cancer. "High risk HPVs, such as HPV16, are all bad news, wherever they are in the body," he said. "No epidemiological evidence suggests that their elimination would lead to any new problems." In fact, most of the HPV types that humans carry are benign and successful ectoparasites—they cause no disease. HPV16 is what Frazer called a "successful failure"—successful because it is so widespread, but a failure "because it causes enough problems for the 1% of people who get cancer attributable to it that we will go out of our way to eliminate it by developing a vaccine!" There are other, less successful failures in the HPV family that are not as widespread but cause cancer, and they too will be targeted. "We'll then be left with the truly successful HPVs that cause no disease," Frazer said.

> As eradication programmes are based on cost–benefit analyses…their implementation will probably differ depending on the disease

The high-profile cases of gastric ulcers and cervical cancer may offer hope that more chronic diseases could be cured, but this assumption still largely ignores the human role in the interaction between host and microbe. "Unless we know that, then the story's not complete," Chhatwal said. "It's important to understand these problems in terms of risk relationships," Blaser said. "In my mind, *Helicobacter* doesn't cause ulcers [and] gastric cancer, it increases the risk for ulcers [and] gastric cancer—just as smoking doesn't cause lung cancer, it increases risk." Frazer agreed: "Behavioral risks for cancer are clearly additive to the infectious risks." Itzhaki's research highlights the importance of genetic factors: she and her colleagues found that HSV1 is a strong risk factor for Alzheimers' in carriers of the type 4 allele of the apolipoprotein E gene (Itzhaki *et al*, 1997; Itzhaki & Wozniak, 2006).

As eradication programmes are based on cost–benefit analyses—both financial and biological costs—their implementation will probably differ depending on the disease. Speaking in a personal capacity, Kathryn Carbone, one of the authors of the AAM report, said that, although each medical decision balances advantages and disadvantages, "as facts come to light, they contribute to changing the risk–benefit of any medical decision." What makes these decisions even more difficult is that the disadvantages—such as the appearance of an unexpected disease—can take years or even decades to reveal themselves.

H. pylori has proven to be important as "as a disease-causing agent…as an indicator of changing human microbe ecology and…as a disease protecting agent," according to Blaser. However, understanding the protective role of microorganisms in relation to disease might be a bigger leap than accepting causation. "Scientists want to see black and white, not the grey," Chhatwal said. And as Blaser pointed out, "Most of the microbes in the human body are either uncharacterized or under-categorized." The changing nature of human microecology has consequences, good and bad. In Blaser's opinion, microbes are an extended part of the human genome. Now that the Human Genome Project is complete, "I think we need a second human genome project that explores our microbial genomes."

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