The cancer-virus cures

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About 90% of the people in the world carry a latent virus known to cause cancer. On the fiftieth anniversary of its discovery, researchers are considering joining up their experimental treatments against these malignancies. **David Holmes** reports.

At the end of March, virologists and immunologists thronged the manicured lawns of the University of Oxford's Keble College as they came together to celebrate the fiftieth anniversary of one of the most extraordinary virological discoveries of the twentieth century. In 1964, three unknown researchers published a paper in *The Lancet*¹ describing a newly discovered virus that would turn conventional virological and oncological wisdom on its head. They were Michael Anthony Epstein, Yvonne Barr and Bert Achong. And although they weren't certain at the time, they had discovered the first virus that would be shown to cause cancer in humans: Epstein-Barr virus (EBV).

It's estimated that EBV causes over 200,000 cancers worldwide each year, most commonly Burkitt's lymphoma and other B cell lymphomas, nasopharyngeal carcinoma and about 10% of gastric cancers. Altogether, EBV-associated cancers account for approximately 1.5% of all cancers worldwide. But despite EBV being the first human tumor virus identified, there is still no specific treatment or prophylactic vaccine available against

EBV infection and its associated diseases. Although other tumor-causing viruses such as human papillomavirus (HPV) and hepatitis B virus have been the subjects of successful vaccination development programs, EBV has always seemed to slip through the cracks.

Burkitt's lymphoma occurs primarily in Africa, whereas nasopharyngeal carcinoma is most common in Southeast Asia, neither of which is an established market for pharmaceutical companies. "I think the pressure for a vaccine against cancer would have been stronger if, for example, nasopharyngeal carcinoma had been a common disease in Caucasians rather than in Southeast Asian people," says Alan Rickinson, of the University of Birmingham in the UK. However, he notes, "as we go through the twenty-first century, the pressure for vaccination within Southeast Asia will be very strong," and there are signs that EBV is at last being taken seriously by funding agencies. At the same time, several teams developing new approaches to tackling EBV-associated disease appear close to a breakthrough and, tantalizingly, have mooted the prospect of

collaborating to combine their approaches.

Although Achong died in 1996 and Epstein and Barr have long ago retired from life at the bench, each passing year uncovers new clues about how EBV causes cancer and new links between EBV and other diseases. A few weeks after delivering the keynote talk at the fiftieth anniversary conference, Sir Anthony (he prefers to be called Tony) cut a spry and dapper figure around the leafy Oxford neighborhood that is home to the University of Oxford's Wolfson College, his sparkling eyes and razor-sharp wit belying his advanced years. Incredibly, Epstein celebrates his ninety-third birthday this year, but, tucked away at Wolfson, where he is now an honorary fellow, he shows palpably youthful excitement in his voice as he recounts the remarkable "chain of chance" that led to his career-defining discovery and the progress that has since been made in understanding the role of EBV in cancer.

Under the microscope

After being demobilized from the British Royal Army Medical Corps in 1947 after

World War II, Epstein had been toiling at London's Middlesex Hospital in the deeply unfashionable field of chicken tumor viruses. Then, in 1961, a surgeon in the British colonial medical service named Denis Burkitt arrived at hospital on leave from his posting in Uganda to give the first talk outside Africa about a newly recognized childhood lymphoma.

Epstein took a seat in the audience out of curiosity, but as the presentation moved on to the epidemiology of Burkitt's exotic and bizarrely presenting new lymphoma, which seemed to be distributed according to temperature and rainfall patterns, he was barely able to contain himself. "Sitting there, that immediately conjured up the idea that there must be some biological cause involved, and I thought this could be a tumor-inducing virus," Epstein recalls. "As he was talking, I decided to stop doing everything I'd been doing and try to find a virus in Burkitt's lymphoma."

With the help of one of the first commercially available electron microscopes (currently residing in the Manchester Museum of Science and Industry), Epstein spent the next two years trying to isolate viral material from the lymphoma biopsies that Burkitt sent once a week on a night flight from the Ugandan capital of Kampala to London's Heathrow airport, with no success. With his career teetering precariously on the brink of failure, Epstein decided to change tack and tried instead to culture the tumor samples away from the host's immune defenses in an effort to reactivate any latent virus. This time, he recruited Yvonne Barr

Taking a shot: Graham Taylor (center) and his lab. Preceding page image: cells in Burkitt's lymphoma.

and Bert Achong to the cause. Barr already had some experience with tissue culture, whereas Achong quickly became an expert in the use of the electron microscope, and together the trio set about trying to coax the Burkitt's lymphoma samples into culture. Again, they resolutely refused to grow, until, at last, fate intervened.

On Thursday 5 December 1963, the flight from Kampala was diverted to Manchester owing to bad weather.

"We didn't get the damn sample until late on a Friday afternoon," Epstein recalls gruffly. Worse, the fluid in the bottles was cloudy, suggesting bacterial contamination. "It was time to go home, but instead of throwing the sample away I actually put a drop of this stuff on to a slide as a wet preparation and shoved it under the light microscope—no bacteria!" Epstein says. Instead, he saw "huge numbers of free-floating, viable-looking tumor cells that had been shaken off the cut edges of the sample on the long journey."

Sensing a breakthrough, Epstein and Barr now tried culturing suspensions of single cells, and, miraculously, they grew. "There was great excitement," Barr (who now goes by her married name, Balding) told attendees of the fiftieth anniversary conference via a video address (see <https://www.youtube.com/watch?v=8MXFUHBRQjo>).

"The thing was to get enough ready for electron microscopy," Epstein says the rest followed quickly: "As soon as we had enough of this precious material growing, we pelleted it, sectioned it and looked at it with the electron microscope, and the very first grid square I looked at had a cell with virus in it," he says triumphantly. "As soon as I saw that first cell in the first grid square, I thought 'this is it', and I became elated, and also extremely frightened. The electron beam can sometimes burn the specimen up, and I thought I might never see this virus again. So I switched off and I walked round the

block in the snow without a coat, and when I came back rather calmer I switched on the microscope and took some pictures straight away."

Epstein instantly recognized the particle as a herpes virus unlike any known at the time, but he faced a wall of skepticism from the British virology community. That soon crumbled in the face of an avalanche of evidence confirming the existence of the virus, notably from the husband-and-wife team Werner and Gertrude Henle at the Children's Hospital of Philadelphia. But it took longer to prove the fact that this new virus—by now dubbed Epstein-Barr virus

"As soon as I saw that first cell in the first grid square, I thought 'this is it'."

Viral enthusiasm: Sir Anthony Epstein.

Graham Taylor

(EBV) after the lab shorthand for it stuck—caused Burkitt's lymphoma.

"I believed personally that it was [the cause]," Epstein recalls, "but what I actually wrote at the time, being very careful, was that this may be a wild goose, but it is a wild goose which has to be chased." Decades of painstaking work, including an enormous seven-year prospective study in Uganda of 35,000 children by the International Agency for Research on Cancer (IARC), proved the association beyond any reasonable doubt, and EBV was declared as a group 1 carcinogen by the IARC and the World Health Organization in the late 1990s².

Search and destroy

The true implications of the discovery of EBV are still unfolding, as links between the virus and new diseases continue to be made. Around 90% of the world's population carries a latent form of the virus; most people are exposed as children and never develop clinical symptoms, but EBV can cause infectious mononucleosis (known as glandular fever in the UK) when the infection is picked up later in childhood or adolescence. Once the initial phase of infection has been brought under control, the virus takes refuge in B cells and epithelial cells, turning off almost all of its

genes to evade immune surveillance.

Crucially, the few viral proteins that are expressed during latency can eventually lead to the emergence of B cells able to escape the normal process of programmed cell death and proliferate indefinitely. In equatorial Africa, where exposure to the virus typically occurs earlier in childhood than in the West, usually by around 18 months of age, the already weak immune surveillance of EBV-infected infants is often

further compromised by coincident infections, which enable immortalized B cells to proliferate unchecked, increasing the risk of mutations that give rise to Burkitt's and other lymphomas.

On either side of the Atlantic, teams led by two of Epstein's former protégés have been working on two different but potentially

complementary approaches to treating EBV-associated cancers. Epstein moved to the University of Bristol in 1968 and recruited Alan Rickinson in the early 1970s, who was shortly followed by Cliona Rooney. When Rickinson left to take lead of the School of Cancer Sciences at the University of Birmingham in 1983, Rooney followed as a postdoc before heading to the US, eventually to the Baylor College of

Medicine in Houston, to pioneer the use of EBV-specific T cells to treat EBV-associated lymphoma in patients who had undergone bone marrow transplantation, a technique known as adoptive T cell therapy.

EBV-associated lymphoma is a particular risk for patients who undergo bone marrow transplantation because they are severely immunosuppressed and thus extremely vulnerable to infection, including by latent viruses such as EBV. "You can protect patients by keeping them away from infections, but you can't protect them from their own endogenous viruses," Rooney explains. And until the approval of rituximab, a monoclonal antibody that destroys B cells, many patients died from EBV-related lymphomas caused by the unchecked proliferation of B cells.

In an effort to rapidly reboot transplant patients' immune surveillance to prevent a resurgence of latent EBV after they had undergone their procedure, Rooney and her colleagues started to collect and culture EBV-specific cytotoxic T cells (CTLs) from bone marrow donors before the transplant operation and then infuse large numbers of the primed T cells into the bone marrow recipients after transplantation. The results were dramatic: none of the patients who have so far received CTLs have gone on to develop EBV-related disease, and, equally impressively, CTLs have also shown promise in a nontransplant setting in patients with established disease, with the latest trial showing a sustained complete response in about 50% of patients with relapsed or resistant EBV-related lymphomas who received this treatment³.

Rooney's results have been "absolutely first class," says Epstein. "But as a practical proposition on any kind of scale, adoptive T cell therapy doesn't seem to me to be feasible," he adds. The technology needed to produce the T cells for adoptive therapies is expensive, Rooney concedes, but she contends that concerns about the cost of cell therapies have been overblown. "If you have a good manufacturing practice unit, the production of one CTL line probably costs about \$10,000," she says. "One night in a hospital in the US costs about \$10,000, and rituximab, which is currently used to treat lymphoma, costs around \$40,000."

What's more, the evidence so far suggests that T cell therapies might have important advantages over current treatments. "With T cell therapies, these are self-replicating drugs that have memory," says Bruce Levine, a specialist in cancer gene therapy at the Abramson Cancer Center of the University

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Cell culture: Cliona Rooney (second from left on stairs) and her team.

Early adopter: Alan Rickinson came early to EBV.

of Pennsylvania in Philadelphia. “These cells can persist from months to many years, so they have an effect that lasts much longer than a small-molecule drug or a more targeted drug, because you are reconstituting immunity and providing sentinel immunity for the potential of a recurrence,” he says.

The same type of long-lasting immunity to EBV-related disease could, of course, also be conferred by an effective therapeutic vaccine, which is where Rickinson’s Birmingham-based team comes in. “Instead of adoptively transferring T cells, you could use a vaccine to try to educate the T cell system against the viral antigens that are expressed in the tumor and elicit or reactivate the patient’s own T cell response against those antigens,” Rickinson explains. That is exactly what his team has been working on since 2001.

Graham Taylor joined the Rickinson lab at the same time and currently leads its therapeutic vaccine development efforts. He’s guardedly optimistic about the potential of the team’s candidate therapeutic vaccine. Targeted against the nuclear antigen EBNA1 and the latent membrane protein LMP2, the vaccine has already proved to be safe and well tolerated and to elicit an immune response in a phase 1 dose-finding trial in Hong Kong in patients with nasopharyngeal carcinoma⁴, and the team is now in the midst of a UK-based phase 1 trial with funding from Cancer Research UK and a Hong Kong-based phase 2 trial looking for clinical benefit in patients with nasopharyngeal carcinoma. “We are seeing encouraging signs,” says Taylor.

A therapeutic vaccine, says Epstein, has the advantage of being easily scalable for use on a large population and would be inexpensive enough to be used in resource-poor settings, but there are some doubts about the potential for a therapeutic vaccine

to overcome the battery of defenses that tumors deploy against immune detection. “If you think of cancers in an evolutionary context, those that succeed have evolved ways to hide from or suppress the immune system,” notes Levine. “If you’re going to go in and vaccinate cancer patients, but they have high levels of regulatory suppressor T cells, or their T cells are suppressed in other ways, then one could argue that the efficacy of a therapeutic vaccine approach may be compromised by that fact,” he says.

Intriguingly, the two teams have floated the possibility of working together to combine their therapeutic approaches, which could be particularly beneficial when treating bulky, established EBV malignancies. “One advantage of using the vaccine is that you can use it to increase the frequency and improve the function of the T cells of the patient, and then it’s much easier to actually make the T cell lines and then infuse the T cells and revaccinate to further boost them,” says Rooney. “My personal feeling is that if you have advanced

disease you may need to employ multiple routes to achieve your aim; both adoptive T cell therapy and therapeutic vaccination have their strengths and weaknesses, and this would combine the strengths of both,” says Taylor. “We would need to get funding for a new batch of vaccine to make it a reality, but we’re interested in it, and we’re looking to combine forces,” he says. A similar approach has already been used to good effect by Levine’s group in patients who had undergone autologous stem cell transplants during treatment for myeloma. “We certainly appreciate the rationale of that combination of therapy,” Levine says.

A prophylactic vaccine

In the longer term, all of the therapies in the works from teams such as Rooney’s and Rickinson’s would be unnecessary if doctors had a vaccine that prevented people from getting infected with EBV in the first place. The only phase 2 trial of a prophylactic vaccine to date (which reported in 2004) showed that an EBV subunit glycoprotein 350 vaccine failed to prevent infection by EBV in 181 young adults in Belgium⁵. However, the vaccine *was* effective at preventing the development of mononucleosis induced by

EBV infection, leading the study’s authors to conclude that there was a strong clinical case for using the vaccine to control the disease. Nevertheless, the trial’s funder, GlaxoSmithKline, still shelved the program.

So far there has been little interest from drug companies in resurrecting the vaccine. “There is a perception by many that EBV infectious mononucleosis is a very mild disease,” says Jeffrey Cohen, chief of the Laboratory of Infectious Diseases at the US National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland. That’s a big mistake, Cohen adds. “In fact,” he explains, “10% of people with infectious mononucleosis have fatigue that persists for six or more months, and 1% have more severe complications including hepatitis or neurologic problems.”

Cohen has spoken out repeatedly about the needs and challenges of developing a prophylactic EBV vaccine, and he has support from friends in equally high places. In 2011, Cohen coauthored a position paper with top leaders at the US National

Institutes of Health, setting out a road map to a prophylactic EBV vaccine⁶. Meanwhile other initiatives, including a collaboration between Taylor’s team and Paul Kellam’s research group at the Wellcome Trust Sanger Centre in Cambridge, UK, are in the early stages of developing a second-generation prophylactic vaccine.

“With the NCI giving it a big kick, it’s going to go forward,” says Epstein, but he’s keen to stress that each approach, be it therapeutic or prophylactic, has its merits. “Everything that works is going to be a winner,” he says, though he won’t be drawn out on which approach might win out first. “It’s not for me to say at this stage in my life,” he laughs.

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