Professor Ann M Moormann is leading a study which aims to shed light on endemic Burkitt lymphoma. Here, she talks about her studies to date and her hopes for the future of her research.

To begin, could you provide an explanation of endemic Burkitt lymphoma (eBL) and summarise your project’s main aims and ambitions?

eBL is the most prevalent childhood cancer in Equatorial Africa and has also been found in Papua New Guinea. It was first described in 1958 by Denis Burkitt, and can occur in children between two and 14 years of age, most commonly those aged five to nine. From an eBL tumour, Tony Epstein, Yvonne Barr and Bert Ach‘ong discovered a virus which was named Epstein-Barr Virus (EBV). Since then EBV has also been associated with other cancers such as nasopharyngeal carcinoma (prevalent in China) and Hodgkin lymphoma.

EBV was the first virus associated with a human cancer. The virus is transmitted by saliva, and in places like the US and Europe, people may not become infected until adolescence, when acute infectious mononucleosis – which resolves after a few months – can occur. In Kenya, nearly 100 per cent of children are infected with EBV by two years of age, yet have no symptoms when the infection occurs. However, this virus is always found in the B cells that comprise eBL tumours. Our project aims to determine why early-age EBV infection is a risk factor for eBL.

Could you explain the link between eBL and malaria?

Denis Burkitt and others also set out to map eBL, and found a striking correlation which linked high rainfall to intense (holoendemic) malaria transmission. Plasmodium falciparum malaria is a parasitic disease transmitted by mosquitoes and is considered a common life-threatening illness for all African children. Yet most children who survive after repeated malaria infections do not go on to develop eBL. My research aims to understand relationship between malaria and EBV and how these co-infections during early childhood can lead to cancer. Our hope is that if we understand how these two common childhood infections lead to cancer, we can learn how to prevent it.

What led you to this field of research and what continues to hold your interest?

I entered this field because of my interest in malaria and global health research. I lived in Senegal for a year during my undergraduate education at the University of Minnesota, learning that not only perception of illness but also care received at the clinic was important for prompt diagnosis, treatment and childhood survival. During my graduate work at the University of Michigan, I conducted in-depth immunologic studies into placental malaria in Malawian women. My postgraduate studies at Case Western Reserve University then shifted to understanding the development and maintenance of immunologic memory to malaria in infants and young children in Kenya, in collaboration with the Kenya Medical Research Institute.

As an epidemiologist, studying the aetiology of eBL has brought my global health research experience full circle; we interview parents who live far from cancer care about the obstacles they face when their children develop tumours. Increasing paediatric cancer awareness and advocating for prompt diagnosis and treatment goes hand-in-hand with research to discover why some children develop this cancer while others are spared.

Could you highlight some of the reasons for conducting your examinations in Africa?

Most eBL patients are concentrated in Africa and just by being there and studying it firsthand we are creating awareness within the community and within the country. Meeting the children who have this cancer and seeing them cured makes it worth all the challenges and setbacks. The other benefit is working with Kenyan scientists, who are highly motivated to study eBL aetiology since this disease affects children in their country.

Ultimately, what outcomes and impact do you expect from your research?

I would like our research to contribute to our understanding of human immunity. Elegant animal models have been used to discover immune signals and pathways involved in protection from infectious diseases and cancer, but there is an increased emphasis on conducting translational research, which means taking what we have learned in the lab and returning to the bedside (or the community) and determining ways of preventing and curing diseases in humans.

Completely preventing any disease is a lofty goal, but the incidence of this cancer has not changed in almost 50 years. I think with advances in molecular, genetic and immunologic technologies, we will be able to find the combination and sequence of events which puts these children at risk of eBL, thereby enabling us to focus our efforts on ways to prevent the disease.
The role of malaria and EBV co-infection

A Kenyan field study based at the **Kenya Medical Research Institute** in collaboration with the **University of Massachusetts Medical School**, US, is demystifying the mechanisms by which co-infection with Epstein-Barr Virus and *Plasmodium falciparum* malaria can lead to endemic Burkitt lymphoma found in children.

**ENDEMIC BURKITT LYMPHOMA** (eBL) is the most common paediatric cancer in equatorial Africa. Since it was first described over 50 years ago, epidemiologic studies have established a strong link between the development of the disease and co-infection with Epstein-Barr Virus (EBV) and *Plasmodium falciparum* malaria. Whilst the causal link between EBV and eBL has been well studied – with EBV present in nearly 100 per cent of tumours – less is known about the mechanisms by which *P. falciparum* malaria becomes an active agent in the process of eBL's development. It is this area of research which a study based at the University of Massachusetts Medical School is hoping to shed light on. Led by **Ann M Moormann**, Associate Professor of Paediatric Immunology and Infectious Diseases and Epidemiology of Chronic Diseases and Vulnerable Populations, the research aims to eventually find methods of preventing eBL.

**MUTUALLY COMPATIBLE THEORIES**

In order to explain the relationship between EBV, *P. falciparum* malaria and eBL, two working theories are currently under consideration by Moormann and her team. The first suggests that *P. falciparum* malaria is responsible for inducing polyclonal B cell expansion and lytic EBV reaction, which allows EBV to take up residence in more B cells (referred to as latent EBV infection). “Once the number of latently infected B cells reaches a certain level, the chances of a c-myc translocation (ie. a breaking and re-joining within human chromosomes that activate the growth gene, c-myc), which can be found in all eBL tumours, is greatly increased,” explains Moormann. The second, mutually compatible, theory argues that EBV specific T cell immunity deteriorates during co-infection with *P. falciparum* malaria. This could either be as a cause or consequence of an increase in EBV replication, and in either case, leads to a loss of viral control. The researchers are currently working to determine the relative (and temporal) contribution of these theories for the development of eBL. While it appears both processes may be involved in eBL aetiology, greater understanding is needed about the interaction between malaria and B cell expansion and T cell dysfunction – incremental steps towards increasing eBL risk.

**INFECTION IN EARLY CHILDHOOD**

The geographical link between *P. falciparum* malaria and eBL, first witnessed in the early 1970s has been well demonstrated. More recently, Moormann and her team have further detailed the consequences of early infection with EBV in Kenyan children. Their studies show that whilst children commonly contract EBV early on – often before a year old – it is very rare for them to develop eBL before the age of two. This suggests that there is a temporal relationship between co-infection with EBV and *P. falciparum* malaria and the development of eBL: “We have found that EBV-specific T cell immunity appears soon after primary EBV infections, but for children residing in malaria holoendemic areas where they experience repeated malaria infections each year, T cell immunity to EBV diminishes over time,” notes Moormann. The deterioration of T cell immunity appears to coincide with the peak age-incidence of eBL – between five and nine years of age; years after primary EBV infection.

Moormann’s studies have also found that children who have been diagnosed with eBL have robust immune responses to malaria, yet lack T cell immunity to EBNA1, the only EBV antigen expressed by eBL tumour cells. This could have important implications for immunotherapy aimed at EBV-infected B cells, with the possibility that they could be targeted to attack only eBL tumour cells, as Moormann elaborates: “Our studies suggest that these children had immunity to EBNA1 but then lost it, perhaps through T cell exhaustion and clonal deletion after repeated malaria infections”. In response to these findings, Moormann is currently in the process of investigating the mechanisms by which immune exhaustion and the loss of T cells might occur in humans. Her group has already discovered that EBV-specific T cells appear more exhausted and typically have a shorter lifespan in children who live in malaria holoendemic areas; whilst appearing functional, the cells are in fact phenotypically naïve, reducing their ability to receive signals and develop into T cells capable of controlling EBV (referred to as immunologic memory). It is hoped this research will generate a better understanding of immune deficits that increase the risk of eBL and will provide clues as to the best time to intervene in order to prevent it. Malaria and EBV are common paediatric infections in Africa therefore completely preventing these infections may not be possible. However, only a few children go on to develop eBL, providing a window of opportunity to prevent this cancer if informative risk factors can be identified.

**POTENTIAL VACCINES**

The researchers have found some evidence that the quality of EBV-specific CD8 T cell immunity could be dictated at the time of primary EBV exposure. If this proves to be correct, future efforts should focus on preventing children from contracting EBV infection at an early age, or on implementing a vaccine that could reduce the viral load during times of reactivation. An EBV vaccine is under development, but since evident disease resulting from the virus is rare in most populations, the target group for such a vaccine would be fairly small. However, Moormann still argues for the importance of a vaccine: “In a malaria endemic area with a high incidence of eBL, an EBV vaccine trial could be justified, using EBV viral load and the maintenance of functional EBV specific T cell immunity as surrogate markers for decreased eBL risk.” Alternatively, an anti-malarial vaccine, such as the one which is currently in Phase III trials, could also result in a decreased incidence of eBL. Since the incidence of eBL is so low, however – two in 100,000 children annually – population anti-malarial vaccine coverage sustained over many years would be required in order to see a reduction in incidence. “In western Kenya, the original site of the insecticide treated bednet trials and an area undergoing aggressive efforts by Kenyan malaria control programmes, we have observed a gradual decrease in eBL cases presented to the Nyanza Provincial General Hospital (renamed the Jaramogi Oginga Odinga Teaching and Referral Hospital in 2012), and cautiously speculate that the incidence of eBL...”
Since it was first described over 50 years ago, epidemiologic studies have established a strong link between the development of the disease and co-infection with Epstein-Barr Virus and Plasmodium falciparum malaria.

family clusters of eBL are rare, and any genetic component is likely to be a common or complex trait which controls immunity to infectious diseases.

Another study area close to Moormann’s heart is improving the long-term survival for children diagnosed with eBL. “Since 2003, we have enrolled over 600 children with eBL at the hospital in Kisumu; with an in-hospital survival rate of 67 per cent… but we can do better,” she reflects. In order to improve survival rates it is essential to have better education, earlier diagnosis, alternative treatment for tumours that do not respond to conventional therapies, and improved post-discharge care. Whilst eBL treatment is expensive – treating one child with eBL costs around the same as treating 1,000 children with malaria – Moormann remains hopeful that the costs involved will decrease, and that she and her research team can help determine the most promising treatment for eBL patients in Africa, to give them the best chance of survival.