Wolfson Institute of Preventive Medicine

Queen Mary University of London

Report of the Wolfson Institute Workshop on Epstein Barr Virus Infection and Multiple Sclerosis Prevention

Synopsis

There is strong evidence that Epstein Barr Virus (EBV) infection is a cause of Multiple Sclerosis. Immunisation against EBV could in future prevent not only Infectious Mononucleosis (glandular fever) but also Multiple Sclerosis and other EBV caused conditions. The global community will benefit from achieving such gains as rapidly as possible. Next steps include evaluating the burden of potentially avoidable disease imposed by Infectious Mononucleosis in order to inform policies and create support for the development of EBV vaccination as an important public health research objective.

Context and objectives

Multiple sclerosis (MS) was identified as a distinct disease about 150 years ago by the French neurologist Jean-Martin Charcot. It is one of the most common severely disabling conditions affecting young adults in developed countries. Incidence and prevalence rates are lowest in equatorial locations, and highest at latitudes towards the North and South Poles – see Figure 1 (page 2). The disease is more common in women than in men, and also in individuals who have had infectious mononucleosis (IM).

Globally, MS affects approximately 2.5 million people (MSIF, 2013). It is a demyelinating auto-immune disease that damages neurones in the central nervous systems of those who contract it. In the last decade an increasing volume of epidemiological evidence linking MS with Epstein Barr Virus (EBV) infection has emerged (Olsson et al, 2017; Ascherio et al, 2012). Against this background Professor Sir Nicholas Wald convened a Workshop on the topic of Epstein Barr Virus (EBV) Infection and multiple sclerosis, which was held at the Wolfson Institute of Preventive Medicine in London on October 5th and 6th 2016. Its membership is shown at the Appendix on page 7.

The main objective of the Workshop was to produce a report on the evidence that EBV infection is a cause of MS and whether there is a sound basis for carrying out an EBV vaccination trial to determine its efficacy in preventing MS and to assess its safety.

The specific questions that Professor Wald and his colleagues sought to address during the course of the Workshop included *'is a suitable Epstein Barr Virus vaccine available for use in a trial?'; 'is the science sufficiently sound to justify forming a trial development committee?*'; and *'what would be the approximate size, design and cost of a trial and who might be the main funders?'*.

The Workshop concluded that:

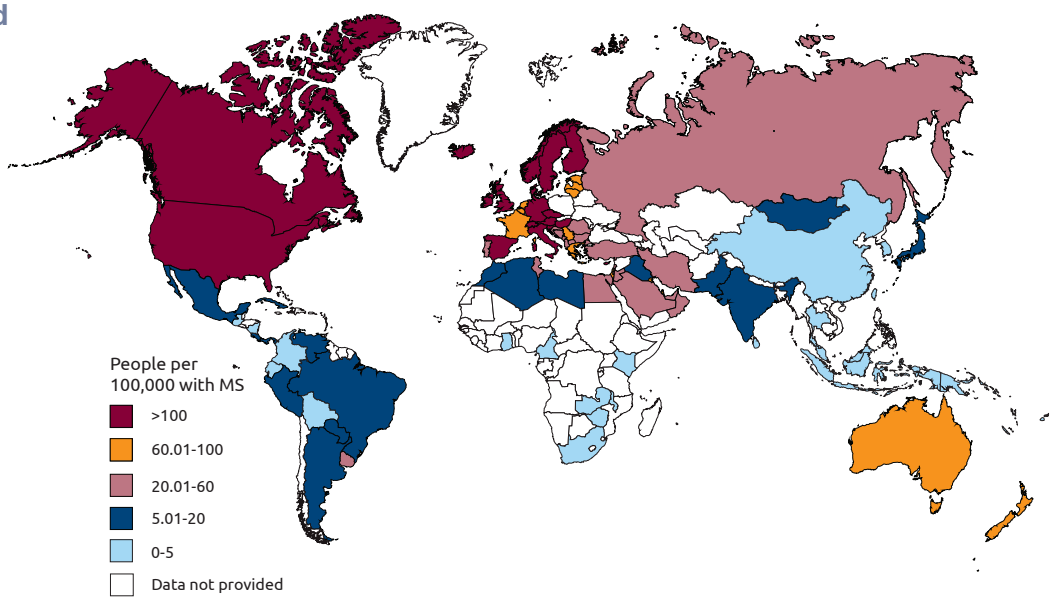
I) there is now strong evidence that EBV infection is a cause of multiple sclerosis in that MS is a rare complication of the infection the expression of which depends on interactions with other causes, both genetic and environmental;

II) there are robust public health reasons for investing in research relating to whether an immunisation based strategy could reduce the occurrence of MS, or at some future point come close to eliminating it;

III) a single antigen vaccine has been used in a trial that demonstrated 75 per cent efficacy in preventing infectious mononucleosis. A multivalent vaccine which promises greater efficacy is now in development; and

IV) although the new vaccine is not ready for use in a large scale trial of its capacity to protect against IM and/or EBV infection sequelae such as MS, preparations for such a trial – including the production of materials to inform policy makers and facilitate the initial introduction of immunisation programmes – should now be made.

Figure 1. Reported MS prevalence by country (2013)



Source: www.msif.org/wp-content/uploads/2014/09/Atlas-of-MS.pdf

Specific issues

The nature and consequences of EBV infection

The Workshop began with a presentation by Professor Münz. He outlined the wide range of clinical conditions associated with EBV infection and discussed the immunological and other mechanisms involved in their pathogenesis (Taylor et al, 2015). EB virus infection is a recognised cause of a range of conditions, including:

I) infectious mononucleosis (IM – also known as glandular fever);

II) various lymphomas, including Burkitt's lymphoma, Hodgkin's disease, post-transplant lymphoproliferative disorder (PTLD), and HIV related immunoblastic lymphomas;

III) nasopharyngeal cancer; and

IV) gastric cancers.

EBV is a DNA virus. It is also known as human herpes virus 4. It causes both acute and latent (life-long) infections and is primarily transmitted via salivary exchanges, initially infecting the oropharyngeal mucosa. The EBV genome codes for a range of oncogenic proteins. They include EBV nuclear antigen 2 (EBNA 2) and latent membrane protein 1 (LMP 1). The age at which individuals are infected, along with genetic and environmental factors, determines its impacts.

With regard to its role in causing MS, two mechanisms could be involved:

I) autoimmunity associated with 'simple' molecular mimicry, in which an antibody or T cell response to an agent such as EBV may coincidentally attack myelin (or cells that produce myelin); and

II) autoimmunity that may occur when EBV infected B cells act as antigen presenting cells (Münz et al, 2009).

In the latter instance B cell infection could be the facilitator of a T cell attack on the oligodendrocytes responsible for the production of myelin (Pender and Burrows, 2014). An episode of IM on average results in a two fold increase in an individual's subsequent risk of developing MS, as compared with that observed in people without a history of diagnosed IM (Handel et al, 2010; Sundqvist et al, 2012). The majority of the latter will nevertheless have had a non-symptomatic EBV infection.

People who become seropositive for EBV without developing IM subsequently have an at least 4 fold greater risk of developing MS than seronegative subjects. In addition, Human leukocyte antigen (HLA) type variations (HLA proteins regulate immune responses involving T cells) can synergise with IM to result in an up to twenty fold difference in the incidence of MS (Nielsen et al, 2009; Sawcer et al, 2011; Brynedal et al, 2007; Beecham et al, 2013; Moutsianas et al, 2015).

Elevated antibodies against the nuclear antigen 1 of EBV (EBNA 1) and in particular against its amino acid sequence 385 to 420 also confer a four-fold increase in the risk of developing MS (Sundqvist et al, 2012; Strautins et al, 2014). Given the difficulties involved in accurately assessing serological status it may be that 'true EBV sero-negatives' do not develop MS.

Epstein Barr Virus infection as a cause of MS – epidemiological evidence

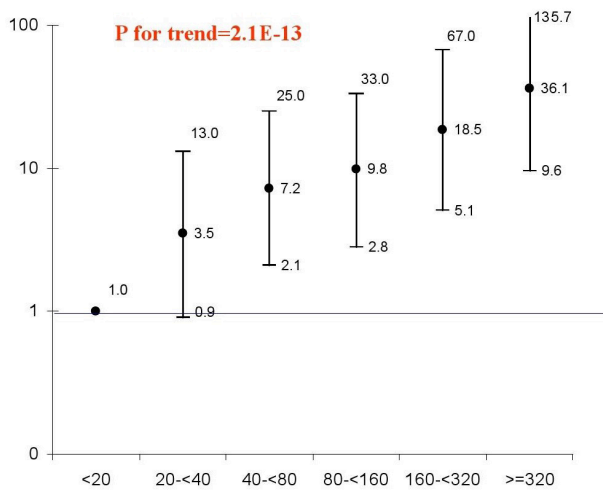
Professor Wald detailed the results of a study published in a paper by Munger, Ascherio and colleagues on EBV antibodies as serological markers for MS. This cohort study involved analysing the records of 8 million US military recruits with a mean age of 23 years at the time at which their serological status was determined. In a nested case-controlled analysis within this cohort the antibody status of 222 members of this population who were subsequently diagnosed with MS was compared with that of 444 matched controls (Munger et al, 2011).

The relative risk of MS was strongly and positively correlated with the level of anti-EBV nuclear antigen complex titer. The observed incidence of the disease was higher at each raised titer level (Figure 2). For those with the highest titer the relative risk was 36. Professor Wald concluded that the size of this association and the existence of a clear 'dose response' constituted strong evidence of a causal relationship between EBV infection and the development of MS. From a mathematical perspective confounding is improbable because any possible confounding factor would need to near-perfectly mirror the association with both the exposure to EBV and the incidence of MS. This study also showed the temporal relationship requisite for causality.

In epidemiology relative risks of 30 or more are extremely unlikely to be non-causal except when the disease causes the change in the risk factor under study, rather than the risk factor causing the disease (ie reverse causality). Workshop discussions emphasised the difficulties involved in accurate antibody level measurement. This source of error will tend to lead to understatements of the strength of the measured relationship between EBV infection and MS.

Figure 2. The relative risk for developing MS against anti-EBNA complex titers

Sourse: Munger et al, 2011



Further epidemiological observations on a causal relationship between EBV infection and MS

Professor Ascherio presented additional data highlighting the fact that EBV negative individuals have a very low risk of developing MS. He discussed research amongst paediatric populations which, with other information, also indicates that confounding due to genetic factors or serological testing errors is improbable. Importantly, he presented evidence from a large longitudinal study within the military population noted above, showing that young individuals who at the beginning of the study were EBV negative only developed MS after being infected with EBV. In all cases the onset of MS followed EBV infection after a minimum incubation period of several months. These observations support the view that EBV is a cause of MS.

Professor Ascherio pointed to evidence that the risk of developing MS is not increased by immunosuppression (unlike that for post-transplant lymphoproliferative disorder/PTLD, which is known to be caused by EBV infection) and that overall viral load is only modestly linked to a raised MS relative risk ratio. By contrast there is a strong association with the presence of EBNA antibodies. There is evidence that the risk of MS is persistently increased for 30 or more years after infectious mononucleosis, and that it does not appear to be affected by the experienced severity of the condition (Nielsen et al, 2007).

The hypothesis that B cell infection linked to the activation of a dually specific (EBV and myelin) T cell compartment underpins MS is supported by evidence that Rituximab (a chimeric monoclonal antibody targeted against the pan B cell marker CD20) and similar medicines can control Relapsing Remitting MS and to a lesser degree, recent trials indicate, Primary Progressive MS (Montalban et al, 2017). However, evidence of environmental factors that have effects that may be independent from that of EBV infection was also referred to, as was the possibility that some EBV strains are more likely to cause MS than others.

There is evidence that the risk of females developing MS has in certain countries, including the US and the UK, risen over the course of the past century, while recorded male MS incidence rates have been more or less static (Harbo et al, 2013). Women tend to become seropositive

for EBV two to three years before men, and in countries with good national registries the recorded female:male MS incidence rate ratio is presently in the order of 3:1 (Koch-Henriksen and Soelberg Sorensen, 2010). There is an approximate 10 fold increase in risk for MS seen in the first degree relatives of people diagnosed with MS compared to other members of the population (see, for instance, Robertson et al, 1996). If one monozygotic twin has MS the chance that the other twin will develop MS is high. One study estimated a concordance rate of 26% (7/27) (95% confidence interval (11% – 46%)). (Ebers GC et al, 1986).

As already noted, the incidence of MS rises with increased latitude. So too, the available data suggest, does the likelihood of monozygotic twins both developing the disease. Migrant studies also indicate that MS risks are linked with the location of childhood residence (Compston and Coles, 2008).

EBV vaccine development

Professor Cohen described his work with NIH colleagues in developing an EBV vaccine using gp350-ferritin together with additional glycoprotein(s).This new vaccine is likely to be available for Phase 1 trialling in 2018. He thought that if this work progresses a Phase 2 trial of the new vaccine could commence in about 2022. Key points made by Professor Cohen (who referred to a paper by Professor Balfour, who was unable to attend the Workshop) included:

- a gp350 vaccine made by GSK has been tested and found to reduce the risk of IM by over 75 per cent. The gp350 viral protein is abundant in the EBV envelope and in virus-infected cell membranes. Antibodies to EBV neutralise the virus and reduce infection of cells in culture. However, this vaccine does not confer sterilising immunity, defined as immunity that prevents people from becoming infected with EBV. Further work on the development of the monovalent gp350 vaccine has reportedly been discontinued;

- an adjuvant is likely to be needed to optimise the immunogenicity of the multivalent NIH vaccine and work on the choice of adjuvant is in progress. This requirement may extend the time needed to conduct the Phase 1 and 2 safety and proof of therapeutic concept trials needed to permit experimental use in paediatric populations; and

- the amount of money and expertise needed to establish the quality and consistency of vaccine production needed for regulators to permit a Phase 3 trial in children is such that 'big pharma' involvement is likely to be vital.

In anticipation of a more effective vaccine against EBV Professor Cohen suggested conducting a trial of its efficacy in preventing IM in college-aged adults, or in the prevention of post-transplant lymphoproliferative disorder (PTLD) in seronegative individuals prior to their receiving organ or haematopoietic stem cell transplants. Workshop members recognised that a vaccine capable of protecting against MS might also reduce the incidence of other diseases caused by EBV infection, including Hodgkin's disease, Burkitt's lymphoma and nasopharyngeal cancer (Cohen, 2015).

Professor Almond said that given the high overall cost of vaccine development (which may be in the order of $800 million on a fully capitalised basis – see Waye et al, 2013) it would be necessary to attract industry funding. In his view any experimental EBV vaccine would need to demonstrate a capacity to completely prevent virus persistence (defined here as conferring sterilising immunity) as it is possible that even a low level of persistence could lead to the development of MS.

The single GP350 antigen experimental vaccine (see above) is no longer in development. The new NIH vaccine should be more effective. Professor Almond thought that the most likely scenario is that an EBV vaccine will initially be developed to prevent IM. He considered it unlikely that industrial investment in a vaccine for preventing MS would be made without the intermediate step of licensing a vaccine for the prevention of IM. Following the marketing and use of the latter, research could be conducted with the goal of obtaining a secondary licensed indication for MS prevention.

In the current situation very significant State or charitable funding would be needed to progress an IM/MS vaccine project. It would be useful to investigate the value to health care systems and societies such as the US of preventing IM in order to strengthen the case for funding the development of an effective vaccination programme.

A possible Phase 3 trial

Professor Wald outlined a design for a fifteen year trial powered to show vaccine efficacy against IM with high level of confidence, and with lower but adequate levels in the MS and Hodgkins Disease contexts. It would involve vaccinating 100,000 girls aged 12-14 at the HPV vaccination turnstile (some 80 per cent of whom would be seropositive for EBV at immunisation) and following both them and 100,000 control subjects. Identifying disease would probably require robust national disease registers to be in place. Costs could be limited by storing the sera samples given by all 200,000 girls and only carrying out detailed sera testing amongst those

developing conditions such as IM and MS and in an equivalent number of matched controls. This would require only about 5 per cent of the total number of sera samples taken to be retrospectively analysed.

However, Professor Wald thought that the evidence on factors such as the strength of the familial relationship in relation to diagnosed MS rates that had emerged during the Workshop suggested an alternative trial design. This would involve randomly allocating the daughters (and perhaps sons) of patients with MS to either an EBV vaccination group or a control group. Alternatively, it might as suggested by Professors Cohen and Almond be more practical to introduce a vaccine with a licensed IM prevention indication and then seek to demonstrate the viability of an EBV immunisation based strategy for reducing, or coming close to eliminating, the incidence of multiple sclerosis – see the conclusions below.

Regulatory issues

Professor Miller described the regulatory requirements relating to the organisation of immunisation trials in this country and elsewhere in the world. She noted, for example, that if children were to be involved in an EBV vaccine trial evidence would be needed to assure regulators that there would be no identifiable risk of increasing the future occurrence of conditions such as MS amongst participants.

She thought that, given the time required to complete Phase 1 and 2 candidate vaccine trials, ten years might well be needed before a large scale Phase 3 trial of the NIH product could commence. Her remarks together with the observations offered by other Workshop participants indicated that even if the pursuit of an EBV vaccine were judged a public health priority it is unlikely that its mass use for the prevention of IM could be introduced before the end of the 2020s.

Professor Miller said that it would be important to develop the economic case to support public and other (private industry or philanthropic) investment in an EBV vaccine trial. Those seeking to encourage the use of a vaccine to prevent EBV infection might first seek to develop cases for reducing the burden of disease caused by IM in the context of the US and Canadian markets and others in relatively 'high value' settings, like Germany and Scandinavia. A secular reduction in MS incidence could then be sought.

Trial development and governance

Professor Cuzick spoke briefly to the topic of trial design and governance. His contribution highlighted the experience and expertise of WIPM colleagues in relation to this field, and his willingness to support Professor Cohen's work and the future development of EBV vaccination in ways consistent with Professor Cohen's preferences and the requirements of the NIH.

MS treatment issues, including the role of MS registers

Professor Giovannoni noted the inadequacy of MS registration in England compared with Sweden, Canada and Australia. Efforts are being made to build on Scotland's attempts to create a comprehensive record of MS incidence in order to establish a register in England. Future evaluations of EBV immunisation as a means of MS prevention will require good disease registration systems. They should be of value both epidemiologically and clinically.

Newly licensed medicines can slow progression of the disease. However, some more effective MS treatments can have serious unwanted side effects, such as JC (John Cunningham) polyomavirus associated progressive multifocal leukoencephalopathy (PML). Using sophisticated immunotherapies can in addition be very costly. Such concerns strengthen the case for prioritising primary prevention.

Professor Giovannoni commented on the value of early diagnosis and treatment of MS. He pointed to avoidable delays of up to 10 years in the diagnosis of MS in the NHS. Better use of risk profiling and brain imaging techniques could in future reduce treatment delays. Nevertheless, MS is likely to remain an important cause of disability. The possibility that the global burden of disease due to EBV infection may rise along with the average age of initial infection was identified as a potential public health risk. The world-wide case for developing and using an effective form of immunisation against IM and the other consequences of EBV may therefore be stronger than is realised, even amongst those already aware of this potential medical and social advance.

Conclusions – next steps

At the end of the Workshop Professor Wald suggested that a second Workshop should be held. One locational option for this event is Bethesda USA, where Professor Cohen and his team work. A possible title for the project as it moves forward could be *The Wolfson EBV Collaboration.*

With regard to the question "*is there a sound basis for carrying out an EBV vaccination trial to determine its efficacy in preventing MS and assess its safety"* the final conclusion of the first Workshop meeting was that at present the most realistic way forward, as and when appropriate opportunities arise, would be to support the development of the new NIH vaccine for IM prevention and to facilitate its timely use in the US and/or elsewhere. Following this, it will also be in the global public's interest to ascertain the effectiveness of an EBV immunisation based approach to preventing MS and other EBV associated disorders as quickly as possible.

Lives will be needlessly impaired or lost if there are avoidable delays in putting knowledge of the overall impacts of EBV infection into optimal practical use for public health protection. A strategy paper based on this finding should be available in time for the second Workshop meeting. The members of the first Workshop thanked Professor Wald and the WIPM, and agreed that:

I) there is now strong evidence that EBV infection is a cause of multiple sclerosis;

II) the development of an EBV vaccine should now be seen as a public health priority;

III) sero-epidemiological studies to estimate the incidence of EBV infection by age and sex would be useful;

IV) it would be useful to undertake an economic burden of disease study for IM in countries where the costs of this condition (and those of MS) are highest, and the affordability of an immunisation programme for IM prevention is most likely to be acceptable; and

V) it would be useful to prepare and publish a review on the public health impacts of EBV infection with special reference to IM and MS. This would help decision makers in government, the pharmaceutical industry, and the charitable and health care sectors to understand the health improvement opportunities available, and to create a supportive environment for the introduction of EBV immunisation.

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Rapporteur

References

Ascherio, A., Munger K. L. et al. (2012). "The initiation and prevention of multiple sclerosis." *Nat Rev Neurol* **8**(11): 602-612.

Beecham, A. H., Patsopoulos N. A. et al. (2013). "Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis." *Nat Genet* **45**(11): 1353-1360.

Brynedal, B., Duvefelt K. et al. (2007). "HLA-A confers an HLA-DRB1 independent influence on the risk of multiple sclerosis." *PLoS One* **2**(7): e664.

Cohen J.I. (2015) Epstein-barr virus vaccines. *Clin Transl Immunology* **4**(1) e32 doi 10.1038/ctl.2014.27

Compston A. and Coles A. (2008). Multiple sclerosis. *Lancet* 372:1502–1517

Ebers GC, Bulman DE, et al. (1986). "A population-based study of multiple sclerosis in twins." *NEJM* **315**:1638-42

Handel, A. E., Williamson A. J. et al. (2010). "An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis." *PLoS One* **5**(9). pii.e.12496

Harbo H.F., Gold R. and Tintoré M. (2013). "Sex and gender issues in Multiple Sclerosis". *Ther Adv Neurol Disord.* **6**(4): 237 – 248

Koch-Henriksen N. and Soelberg Sorensen P. (2010) "The changing demographic pattern of multiple sclerosis epidemiology". *Lancet Neurol* **9** 520-532

Montalban X., Houser S.L. et al (2017). Ocrelizumab versus placebo in Primary Progressive Multiple Sclerosis. *N Engl J Med* 376:209-220 DOI: 10.1056/NEJMoa1606468

Moutsianas, L., Jostins L. et al. (2015). "Class II HLA interactions modulate genetic risk for multiple sclerosis." *Nat Genet* **47**(10): 1107-1113.

MS International Foundation (2013). Atlas of Multiple Sclerosis. Avialable at https://www.msif.org/about-us/advocacy/atlas/ Last accessed April 6 2017

Munger K.L., Levin L.I et al (2011). Anti-Epstein-Barr virus antibodies as serological markers of multiple sclerosis: a prospective study among United States military personnel. *Mult Scler* **17**(10):1185-93. DOI: 10.1177/1352458511408991

Münz, C., Lünemann J. D. et al. (2009). "Antiviral immune responses: triggers of or triggered by autoimmunity?" *Nat Rev Immunol* **9**(4): 246-258.

Nielsen T.R., Rostgaard K. et al (2007) "Multiple sclerosis after infectious mononucleosis." *Arch Neurol* **64**(1): 72-75

Nielsen, T., Rostgaard K. et al. (2009). "Effects of infectious mononucleosis and HLA-DRB1*15 in multiple sclerosis." *Mult Scler* **15**(4):431-6.

Olsson, T., Barcellos L. F. et al. (2017). "Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis." *Nat Rev Neurol* **13**(1): 25-36.

Pender M.P and Burrows S.R (2014) Epstein-Barr virus and multiple sclerosis: potential opportunities for immunotherapy. *Clin Transl Immunology* **31**, 3(10) e27 doi: 10.1038/cti.2014.25.

Robertson NP, Fraser M, et al. (1996). "Age-adjusted recurrence risks for relatives of patients with multiple sclerosis." *Brain* **119**:449-455

Sawcer, S., Hellenthal G. et al. (2011). "Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis." *Nature* **476**(7359): 214-219.

Strautins, K., Tschochner M. et al. (2014). "Combining HLA-DR risk alleles and anti-Epstein-Barr virus antibody profiles to stratify multiple sclerosis risk." *Mult Scler* **20**(3): 286-294.

Sundqvist, E., Sundstrom P. et al. (2012). "Epstein-Barr virus and multiple sclerosis: interaction with HLA." *Genes Immun* **13**(1): 14-20.

Taylor, G. S., Long H. M.,et al. (2015). "The immunology of Epstein-Barr virus-induced disease." *Annu Rev Immunol* **33**: 787-821.

Waye A., Jacobs P. and Schryvers A.B. (2013) "Vaccine development costs: a review". *Expert Review of Vaccines* **12**: 1495-1501

Appendix

The Membership of Inaugural WIPM Workshop on Epstein Barr Virus Infection and Multiple Sclerosis Prevention

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Professor Gavin Giovannoni FRCP FRCPath
Professor of Neurology, Queen Mary University of London

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Professor Christian Münz PhD
Professor of Viral Immunobiology, University of Zurich, Switzerland

Professor David Taylor BSc, FRSPH (*Rapporteur)*
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Professor Sir Nicholas Wald FRS FRCP (*Chair)*
Professor of Epidemiology and Preventive Medicine and former and founding Director, Wolfson Institute of Preventive Medicine, Queen Mary University of London

Professor Henry H Balfour Jr MD
Department of Laboratory Medicine and Pathology, University of Minnesota (Professor Balfour was unable to attend and his paper was presented by Professor Cohen)

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