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Contents

Cover image: Human foreskin fibroblasts were infected with CMV and stained for CMV immediate-early antigen (red) and MCP-1 (green) at 48 h post-infection. Cell nuclei were counterstained with DAPI (blue). Image courtesy of Zin Naing.

Paul Young

President of ASM

I would like to take this opportunity to once again congratulate the Adelaide LOC and Scientific Committee on the success of the Society's recent annual meeting. We have received excellent informal feedback from delegates on both the scientific program as well as the organisation of the meeting itself. Particular thanks go to Chris Ossowicz as Chair of the LOC and Phil Giffard as Scientific Chair for their tireless efforts in bringing this successful meeting together. The smooth running of the conference owes much to the expert guidance of our new National Office team and PCO, ASN Events – thanks to Maree Overall and Hamish Hill. A post-meeting survey was sent out to all delegates and we will be providing a summary of the feedback in a forthcoming eNews. We now turn our attention to Melbourne in July 2014. The Melbourne organising committee has already put in place a stellar line-up of overseas and local speakers to form the core of what will be an exciting program. We will also be trialling some new initiatives in the format of the meeting including more cross-disciplinary sessions to help foster a little Divisional cross-fertilisation. I look forward to seeing you all there!

There have been a few recent changes on ASM National Executive with Professor John Turnidge, Immediate-Past President rolling off the Committee and Professor Jon Iredell joining us as President-Elect. Farewell John and welcome Jon! The role of President involves a 4-year commitment to the Executive and John's term coincided with substantial upheaval in the Society. His stewardship through those troubled times has been acknowledged previously so I won't elaborate again, but suffice to say that we all owe him and his team a huge debt of gratitude for seeing the Society through to a time where we can again focus on our core business of promoting microbiology. Professor Iredell has already brought fresh enthusiasm to the Executive and I look forward to working with him over the next 2 years. Also new to the Committee is Dr Jack Wang who joins us as the Society's first VP Communications. Welcome Jack! Dr Wang has already brought many new ideas for promoting the Society more broadly among our membership, stakeholders and the wider community so watch this space for developments over the next 6 months or so.

I would like to draw your attention to a number of Society awards that are currently open for applications and which close during the next quarter. These awards are open to our postgraduate and ECR membership and I strongly encourage you to apply. All are to support attendance at overseas microbiology society meetings and to visit a collaborating research laboratory in that country (NZ, the UK and US). Attending meetings such as these and engaging with the overseas research community is a very important part of your early career development. You never know where a casual conversation at one of these events might lead! Please visit the Society web page for further information and instructions on how to apply.

Finally, I would like to share with you some of the thoughts and discussions we have been having on the Executive and Council over the past year with regards to governance of the Society. I outlined much of this at the AGM in Adelaide recently and propose taking a raft of constitution changes to the next AGM in Melbourne in 2014 after a series of further discussions with the wider ASM membership. It is now more than 10 years since the Society underwent a re-structure. The outcome of a Strategic Planning meeting held in Adelaide in 2001 and subsequent consultation with members was the establishment of four Divisions that currently make up the discipline base of our Society. With the Society now on a solid administrative footing, we have the opportunity to reflect on how this structure has worked for us over those 10 years and to consider ways to further functionalise the Divisions. Presently, the role of the Division Chair is as a member of NSAC (Chairs for both the current and next year's meeting sit on the Committee) and to contribute to the development of the scientific program of each annual meeting. The Chairs don't have a say elsewhere, despite being the Society's discipline leaders. Indeed, the Division Chair position itself is not mentioned in either the Society By-Laws or Constitution. Under these Divisions sit the SIGs, although in most cases the Division Chairs don't take any role in SIG oversight. For their part, the SIGs have no role in the governance of the Society at all.

The key assumptions and proposed model for the re-structure were clearly outlined in a position paper by Professor Julian Rood, commissioned by then President, Professor Lyn Gilbert and published in the July 2001 issue of *Microbiology Australia*. It is worth reading this document – you can find it in the online *Microbiology Australia* archive. Many of the targeted objectives have come to pass but some of the outcomes expected of a Divisional structure have not been advanced as much as hoped, for example, the desire for a greater role for ASM in national issues that are impacted by microbiology. One of the major limitations of the current system, at least in the way that it is organisationally framed, is that the Divisions don't have any terms of reference or clearly defined responsibilities within the Society that could help to advance the disciplines they represent. A return to the national appointment of Divisional Chairs and for a longer term would help this process. The absence of Division oversight of the SIGs who comprise their membership is also an anomaly that is worth addressing.

A position paper is being drafted that will outline the proposed changes that meet the original expectations of the Divisional Chairs and goes further in defining a more robust governance structure for the Society. The concepts behind the proposed changes were discussed at Council in February this year and we wish to now open the debate to the membership for wider discussion and input. The intention is for these changes to ensure that the ASM voice is the one that is sought and valued in all national matters dealing with microbiology. We are hoping to engage as many members as possible in this process so expect to hear from us soon.

Microbes and chronic disease

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Infectious disease is merely a disagreeable instance of a widely prevalent tendency of all living creatures to save themselves the bother of building, by their own efforts, the things they require. Whenever they find it possible to take advantage of the constructive labors of others, this is the path of least resistance. The plant does the work with its roots and its green leaves. The cow eats the plant. Man eats both of them; and bacteria (or investment bankers) eat the man. (Hans Zinsser, 1935 from *Rats, Lice and History*)

This edition of *Microbiology Australia* is addressing the issue of microbes and chronic disease. Many of the microbes dealt with in this edition are viruses, partly due to interest, but also due to the nature of viral infection, which is of a host cell parasite, often of limited genomic means. Viruses only replicate by infecting cells, and the essence of viral infection of the cell is manipulation of the cellular processes, presumably in order to increase viral replication and allow viral persistence for the duration of infection. Even viruses with shorter genomes (such as RNA viruses typically with genomes in the order of seven to 10 kilobases in length) have considerable parts of their genome dedicated to non-structural, and in some cases structural, proteins that alter cellular processes. Through encoding different messenger RNAs, and different proteins, virus families have been able to occupy distinct ecological niches, allowing them to infect the human host.

The nature of chronic disease induced by viruses, bacteria, and other organisms often appears as a side-effect, or by product of the infection. It may be that the association of human rhinoviruses with asthma exacerbations is purely fortuitous. Conversely, it appears that in other cases the chronic disease may enhance acute infection and persistence through manipulating specific cells to allow a suitable environment for the viruses, bacteria or other microbes persistence. A good example of this is the herpes virus EBV (discussed by Professor Rajiv Khanna) where infection of the B-cell lineage can result in tumorigenesis, and where infection of such cells is a necessary part of the viral life cycle. In the case of EBV, B cell infection is critical for persistence, where the virus persists in a latent, non-replicating phase. It is harder to see the importance of bacteria in inflammatory bowel disease from the microbes point of view, discussed by Professor Stephen Riordan. However, it is important to keep an open mind, as there may be as yet unclear ecological advantages for the organism.

Microbes are evolving continuously, and in many ways at a globally faster rate than high eukaryotes such as humans. This edition provides an insight into how such co-evolution of microbes with humans has resulted in what are in many cases unexpected untoward effects. Although some of these may seem unrelated, it is likely that better understanding of the organism/host relationship that results in these chronic diseases will further our understanding of both the human disease and the microbe. Through such enhanced understanding, we hope to improve our means of controlling, and perhaps curing, some of these diseases.

Biography

Professor William Rawlinson is a Medical Virologist and is head of the Division of Virology, in the Department of Microbiology SEALS, with a conjoint position in the Department of Infectious Diseases, Prince of Wales Hospital. He holds a conjoint academic position as Professor in the School of Medical Science and the School of Biotechnology and Biomolecular Sciences at The University of New South Wales, currently supervising PhD, Masters and science Honours students. His major research interest is in human cytomegalovirus (CMV) infection of mothers and babies, particularly mechanisms of transplacental virus transmission. The research group that he heads studies congenital infections, enteroviruses, hepatitis viruses, new antivirals and antiviral resistance of herpesviruses.

Epstein–Barr virus-associated malignancies: pathobiology and emerging therapeutic options

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Epstein–Barr virus (EBV) was first identified in malignant Burkitt lymphoma cells in 1964. Since then, EBV has been associated with a number of other malignancies of either lymphocytic origin, including both B cell and NK/T cell cancers, or epithelial origin, predominantly nasopharyngeal and gastric cancers. While a complete understanding of the relationship between EBV-mediated cellular transformation and the oncogenic events that lead to uncontrolled malignant cell growth remains to be determined for a number of these cancers, it is clear in all of these settings that a breakdown in the immune surveillance of virally infected cells contributes to the survival of EBV-bearing malignant cells.

The lifecycle of EBV infection

As with most members of the Herpesvirus family, evolutionary adaptation over millions of years has allowed EBV to establish a unique biological niche in humans that allows it to maintain persistent infection for life with typically limited complications for the host and an efficient capacity to infect a new host. As a consequence of this efficient lifecycle, EBV is ubiquitous in the community, infecting 90–95% of the world population[1]. EBV infection typically occurs via the mucosal surfaces of the oropharynx. Primary lytic infection is followed by the establishment of latent infection of B lymphocytes[2,3]. Following transition of the linear EBV genome to the nucleus of the B cell, the EBV chromosome is circularised. Latent gene expression is then initiated from the W promoter, inducing the expression of the EBV nuclear antigens (EBNA) 2 and 5, which then induce the expression of other EBNA proteins, including EBNA1, 3, 4 and 6, and the latent membrane proteins (LMP) 1, 2a and 2b. While all of the latent cycle genes play important roles in latent transformation, EBNA1 is indispensible for viral latency and functions by binding to the origin of replication, oriP, on the viral episome. EBNA1 tethers the viral episome to the host chromosome, promoting maintenance of the latent EBV episome in daughter cells. Intermittent lytic replication in the upper respiratory tract promotes viral shedding and spread to a new host. This unique aspect of EBV biology, and that of the related gamma-herpesviruses, distinguishes it from other herpesviruses by promoting B cell proliferation without the need for active viral replication. However, it is also this capacity to efficiently transform B cells and induce lymphoproliferation, which leads to both the malignant and lymphoproliferative disorders, that are associated with EBV-infected cells[4–6]. A summary of common EBV-associated malignancies and their viral gene expression is shown in Table 1.

Post-transplant lymphoproliferative disorders

Post-transplant lymphoproliferative disorders (PTLD) arise in immunocompromised patients who have undergone either solid organ (SOT) or haematopoietic stem cell transplantation (HSCT) and are almost universally associated with EBV infection[7]. Under normal immunological conditions EBV-infected B cells are controlled by EBV-specific cytotoxic T lymphocytes, which efficiently clear these cells predominantly via the recognition of peptide epitopes encoded by EBNA3-6[8]. However, the immunosuppressive environment associated with SOT and HSCT disrupts this immunological balance and can lead to uncontrolled proliferation of these EBV-infected cells that can typically be characterised with a latency III profile and the expression of the full array of latent genes[9] (Table 1). PTLD in SOT patients is usually of recipient origin and is most prevalent in seronegative transplant recipients as a

Table 1. Common Epstein–Barr virus (EBV)-associated malignancies, viral gene expression and emerging immunological therapies.

EBV malignancy	Latency profile	Association with EBV	Viral gene expression	EBV-related treatment in clinical trials
Post-transplant lymphomas (early and late onset)	Latency III	Early: predominantly EBV+ Late: less frequently EBV+	Early: EBNA 1-6, LMP1-2 Late: EBNA1, LMP2, EBNA2-6 (+/-), LMP1 (+/-)	Therapeutic/prophylactic administration of autologous, donor-derived or third party EBV-specific T cells
Burkitt lymphoma	Latency I	Almost 100% in endemic regions	EBNA1	None tested
Hodgkin lymphoma	Latency II	30–50%	EBNA1, LMP1-2	Therapeutic administration of EBNA1/LMP1-2 T cells. Therapeutic administration of EBNA1/LMP vaccine
NK/T cell lymphomas	Latency II	Almost 100% in endemic regions	EBNA1, LMP2 LMP1 (+/-)	None tested
Nasopharyngeal carcinoma	Latency II	Almost 100% in endemic regions	EBNA1, LMP1-2	Therapeutic administration of EBNA1/LMP1-2 T cells. Therapeutic administration of EBNA1/LMP vaccine
Gastric carcinoma	Latency I/II	10%	EBNA1, LMP2	None tested

consequence of primary infection following transplant of organs containing EBV-infected cells. However, PTLD can occur in seropositive recipients as a consequence of the immunosuppressive regime used to prevent organ rejection. In Australia, PTLD is typically diagnosed in 1–10% of SOT recipients and is more highly prevalent in children due to an increased likelihood of seronegative status prior to transplant[10]. PTLD in HSCT patients is usually of donor origin and is most prevalent in seropositive recipients who receive a transplant from a seronegative donor. This is typically facilitated by the reactivation of EBV of recipient origin and subsequent infection of B cells of donor origin and the concomitant immunosuppression of the induction of an EBV-specific cellular immune response. However, PTLD does occur following transplant from seropositive donors and increased risks are also associated with T cell depleted HSCT, commonly used to reduce the risk of graft-versus-host disease and due to HLA mismatch between donor and recipient[7].

A reduction in the immunosuppressive regime is usually the first option for treatment of PTLD in both SOT and HSCT patients in order to restore/promote EBV-specific cellular immunity. Rituximab, an antibody that recognises CD20 on the surface of most B cells, is also used to treat PTLD in both SOT and HSCT patients, often in combination with standard chemotherapy used to treat other B cell lymphomas[11]. In addition, adoptive cellular therapy is emerging as a powerful tool for the treatment of EBV-associated PTLD, particularly in the context of HSCT. Pioneered by Rooney and colleagues at the Baylor College of Medicine in Texas, the transfer of EBV-specific T cells, which classically involves the use of *in vitro*-generated EBV-infected lymphoblastoid cell lines (LCL) to stimulate donor memory T cells, have been used effectively both therapeutically and prophylactically to treat or prevent PTLD in HSCT patients[12–14]. However, in the context of PTLD in SOT patients or following a seronegative HSCT transplant, the success of EBV-specific cellular therapy is dependent on the generation of recipient-derived T cells[15] (Table 1). While this has been used successfully to resolve PTLD in SOT patients, an emerging approach for the treatment of PTLD in these instances is the use of a bank of third-party T cells[16–19]. These T cells are generated from another EBV-seropositive healthy donor who shares HLA alleles with the patient. Initial reservations surrounding the potential risk of inducing graft-versus-host disease or organ rejection have proven to be unfounded following the administration of third-party T cells and they have proven effective in the treatment of PTLD in both SOT and HSCT patients[7,20].

EBV-associated Hodgkin and non-Hodgkin lymphomas

In Australia, Europe and North America, 30–50% of Hodgkin lymphoma (HL) cases are associated with EBV. In contrast, EBV positivity in HL cases reaches as high as 100% in certain regions of Asia, Africa and Latin America[21]. A unique characteristic of HL is the large inflammatory infiltrate that is associated with lymphoid organs in HL patients. The malignant Reed–Sternberg cells in HL comprise less than 1% of the cellular mass in inflamed lymphoid organs and the nature of the infiltrate is used histologically to characterise the HL

subtype[21]. While associated with all HL subtypes, EBV infection is most prevalent in classical HL. EBV-infected Reed–Sternberg cells express a latency II gene expression pattern, which is restricted to EBNA1 and LMP1&2 (Table 1), both of which play important roles in the transformation of infected B cells[22,23]. In addition to their important role in the maintenance and transformation of EBV-infected B cells, EBNA1 and LMP1&2 are poorly immunogenic, relative to the other EBV-latent molecules. T cells specific for EBNA1&LMP1 display a loss of function during acute HL and an increased susceptibility to immunomodulatory molecules generated by HL cells, such as galectin-1[24–26]. These immune evasion strategies are likely important for the maintenance of EBV-infected B cells in immunocompetent hosts, but also provide mechanisms for immune evasion by malignant Reed–Sternberg cells[27].

The incidence of HL is bimodal in nature, with a peak in incidence in children under 10 years and in adults over 50 years. An increased risk of developing EBV-associated HL has also been linked to infectious mononucleosis during primary EBV infection[28]. HL is highly amenable to current chemotherapeutic regimes, with 5-year survival rates approaching 90%. However, EBV infection is associated with more rapid progression in HL patients and reduced overall survival[29]. The current chemotherapeutic regimes used to treat HL are also associated with an increased risk of the development of secondary cancers and strategies to reduce the dependence of the current level of chemotherapy are under development, particularly for children[30]. EBV-associated HL, often when refractory to chemotherapy, has also been successfully treated with adoptive cellular therapy[29,31–36]. While initial studies focussed on the LCL-based approach used to treat PTLD, more recent approaches have specifically targeted the induction of T cells specific for EBNA1 and LMP1&2[37]. These approaches have proved to be successful in phase 1 clinical studies and are currently being evaluated in phase II studies[31].

EBV infection is also associated with a number of non-Hodgkin's lymphomas (NHL), including Burkitt lymphoma (BL) and diffuse large B cell lymphoma (DLBCL)[38]. Although rare in Australia, EBV-associated BL is endemic in regions of Africa and Papua New Guinea[22]. It arises primarily in young children and its incidence is closely associated with malaria endemic regions, which has been attributed to a loss of functionality in EBV-specific T cells in children with co-exposure to malaria[39,40]. Burkitt lymphoma cells typically display an EBV latency I profile, characterised by the expression of only EBNA1 (Table 1). In association with reduced expression of surface HLA molecules, this renders BL cells highly immunoevasive[41–43]. Burkitt lymphoma is also characterised by the translocation of the gene encoding the oncogenic c-MYC protein[44].

Nasopharyngeal carcinoma

EBV infection is associated with the majority of undifferentiated nasopharyngeal carcinoma (NPC)[45–47]. While rare in the Australian population, with an incidence of less than 1 in 100,000, EBV-associated NPC is endemic in regions of south-east Asia and North Africa. It reaches its highest prevalence in southern China with incidence rates of 15–50 cases per 100,000 within some communities[48]. An increased incidence of NPC is also evident in Australia in immigrant populations from these regions; however, this incidence is reduced in first generation Australians, indicative of the role both genetics and environment play in the development of NPC.

Similar to HL, EBV gene expression in malignant NPC cells is restricted to EBNA1 and LMP1&2. Current treatment options for NPC typically involve surgical intervention, radiotherapy and/or chemotherapy[49]. Standard treatment options have seen response rates in primary disease improve to greater than 90%. Despite this, a significant proportion of patients, particularly those who initially present with late stage disease, will relapse, which often leads to distance metastases that are refractory to conventional therapy[49]. EBV-targeted treatment options are therefore in development as adjunct approaches to current radio/chemotherapeutic regimes. While some strategies focus on the induction of lytic viral reactivation as a potential mechanism of enhancing cellular immunity and inducing lysis of malignant cells, the majority of these approaches focus on using EBV-specific immunotherapy to treat NPC[33] (Table 1). Augmentation of cellular immunity to EBNA1 and the LMP antigens have emerged as potential adjunct or alternative approach to chemo/radiotherapy. A number of phase I clinical studies treating NPC with a range of immunotherapeutic strategies have provided evidence that these approaches are safe and might have some clinical benefit, particularly in patients with locoregional disease[33,50]. Current efforts are being placed on validating these observations in larger cohorts of patients.

Other EBV-associated malignancies

EBV-infection has also been associated with a number of other malignancies of both lymphocytic and epithelial cell origin, including a range of NK/T cell lymphomas, lymphoepithelial-like carcinomas and gastric carcinomas. While not considered a normal target for EBV infection, EBV is associated with a subset of lymphomas of NK and T cell origin[51]. The most prevalent of these lymphomas are the nasal NK/T lymphomas that are rare in Australia, but more common in parts of south-east Asia. EBV-associated NK/T cell lymphomas have a very poor prognosis, with current 5-year survival rates of less than 50%[51]. EBV infection in NK/T cell lymphomas is characterised with a latency I/II gene expression profile,

characterised predominantly by the expression of EBNA1 and LMP2[52] (Table 1). Despite this, due to the lack of an *in vitro* model, the role EBV and these latent antigens play in the tumorigenesis of NK/T cell lymphomas is not clear.

The EBV-associated lymphoepithelial-like carcinomas share features with NPC but are found in a range of other anatomical sites, including the stomach[53,54]. These cancers also show a distinct geographical distribution and are endemic in regions of Asia and indigenous communities in America, further evidence for the influence of environment and genetics on the incidence of EBV-associated carcinoma. EBV-infection is also associated with a small percentage of non-lymphoepithelial-like gastric carcinoma cases[55]. While the incidence of gastric carcinoma has reduced significantly in most developed countries, the incidence remains high in developing countries and in some developed countries, including Japan and Korea[56]. EBV infection in gastric carcinoma cells is associated with a latency I/II gene expression profile, characterised by the expression of EBNA1 and in some instances LMP2A[57,58]. Although no aetiological relationship has been established between EBV infection and the transformation of gastric carcinoma cells, it will be important to explore therapeutic options that specifically target EBV to improve clinical outcome[59].

Concluding remarks

Over the past two decades considerable knowledge has been accumulated on EBV pathogenesis in different malignancies. This knowledge has recently been successfully exploited to develop new diagnostic and therapeutic tools that have significantly impacted on the clinical management of patients with EBV-associated diseases.

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Biographies

Dr Corey Smith completed his PhD in 2004 at the University of Melbourne and then took up a research position in the Tumour Immunology Laboratory at the Queensland Institute of Medical Research where his work focuses on the development of immunotherapeutic approaches to treat cancers associated with viral infection. This work led to a phase I clinical study in Hong Kong, using a novel immunotherapeutic approach to treat Epstein–Barr virus associated nasopharyngeal carcinoma. His work has also focused on understanding the mechanisms that influence the efficient induction of T cell responses to persistent human viral infections and the role immune evasion strategies developed by virally associated proteins play in promoting the survival of infected malignant cells.

Professor Rajiv Khanna obtained his doctorate degree from India and undertook his post-doctoral training at the Queensland Institute of Medical Research (QIMR), Brisbane, Australia. He is currently appointed as the Coordinator of the Centre for Immunotherapy and Vaccine Development at QIMR and also holds Senior Principal Research Fellowship from the National Health and Medical Research Council of Australia. The major goals of his research group are to obtain a deeper understanding of the mechanisms by which an immune response to human herpesviruses-associated diseases might be generated, augmented and applied to control these diseases. Over the past few years, Professor Khanna has successfully translated his research towards the development of novel immune-based therapeutic strategies for the treatment of patients with herpesvirus-associated complications and is currently lead investigator on five clinical trials. He has published more than 150 scientific papers in leading journals and holds numerous international patents on EBV and human cytomegalovirus (CMV) and has successfully co-developed a diagnostic kit (QuantiFERON-CMV) in collaboration with Cellestis Ltd (now QIAGEN). QuantiFERON-CMV has been CE marked in Europe for diagnostic application and is expected to be launched in the US in late 2013. Currently, he is also collaborating with Intercell AG (now Valneva SE) to develop a new prophylactic vaccine against CMV.

Sneezing leads to wheezing: microorganisms important in asthma

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Asthma is a common, chronic disease of the airways. Asthmatics can suffer exacerbations (worsening symptoms) due to a range of environmental and occupational factors. At least half of all asthma exacerbations are caused by respiratory viruses. In this article we examine some of the microbiological causes of asthma development and exacerbations.

What is asthma?

Asthma is a chronic inflammatory condition of the airways. It is a heterogeneous disease with several clinical phenotypes described as mild, moderate or severe, although definition can be difficult[1]. Asthma is often difficult to diagnose as the symptoms (wheeze, difficulty breathing) are common to other illnesses such as respiratory tract infections and obesity[2]. 'Viral-induced wheeze' and cough are common in young children with respiratory infections, but are not necessarily caused by asthma. The disease is characterised by episodes of wheezing, breathlessness and chest tightness due to widespread narrowing of the airways in the lungs, known as exacerbations. Exacerbations can vary from mild to severe and result in periods of incapacity, emergency hospital admissions and, rarely, death.

Exacerbations can be triggered by a number of environmental and occupational allergens[3] including:

- viral infections (which cause the majority of exacerbations in children and adults[4])
- exercise
- cold weather
- exposure to specific allergens such as:
  - house dust mites
  - pollens
  - mould spores
  - animal dander
- irritants such as:
  - tobacco smoke
  - pollution (such as nitrogen dioxide (NO_2) from the combustion of natural gas and motor fuel)
  - some food additives
- occupational exposure to:
  - specific allergens
  - irritants including dust and fumes.

One in 10 Australians suffer from asthma[5]. The prevalence of asthma in Australian children increased between 1982 and 1992[2], but has now declined in children since 2001 and is stabilised in adults[5,6]. Asthma is a significant health problem in Australia, and in 2006–2007 the number of Australians hospitalised due to this disease reached well over 36,000 people. It has been predicted that over the next two decades, asthma will continue to rank as one of the major causes of disease burden in Australia[7]. With this there is also great cost. The most recent data show that from 2004–2005 $606 million was spent on asthma (1.2% of all health expenditure in that year)[5].

While there is currently no cure for asthma, inhaled corticosteroids and other medications are available to control the disease and prevent exacerbations[8]. The underlying causes of asthma are not yet well understood, but it appears that a combination of predisposing genetic factors and certain environmental factors cause an individual to develop chronic asthma[9,10].

Asthma and respiratory viruses

Respiratory viruses cause up to 90% of asthma exacerbations in children[11]. The most common viruses responsible are human rhinoviruses (HRVs), respiratory syncytial virus (RSV), human metapneumovirus (hMPV) and influenza viruses (Table 1).

Table 1. Common respiratory viruses and asthma exacerbations.

Virus	Disease	Importance in asthma	Reference
Human rhinoviruses (HRVs)	'Common cold'. Rarely complications.	Most common virus associated with asthma exacerbations. HRV-A and HRV-C associated with more severe effects. HRV-C associated with worse exacerbations. HRV-A associated with longer duration of symptoms.	26
Respiratory syncytial virus (RSV)	Upper respiratory tract infections (URTI) but can cause pneumonia/ bronchiolitis in young children (inflammation of the bronchioles making breathing difficult)	Commonly associated with asthma and wheezing in children (~20% of viral-induced wheeze in young children is caused by RSV)	11
Influenza viruses	URTI	Influenza viruses are more often associated with asthma exacerbations in adults (20–25%) than in children (4%). Recent evidence on H1N1 2009 and asthma showed people with asthma had reduced ICU stay and hospitalisation compared with non-asthmatics. Probably due to prior corticosteroid use and earlier hospital admission.	27,28
Human metapneumovirus	Similar to RSV and indicated in URTI, severe bronchiolitis and pneumonia in children	Has been detected in a small number of asthma exacerbations in young children	29–32
Parainfluenza viruses	Can cause lower respiratory tract infection in young children	Recent systematic review failed to find an association between parainfluenza and asthma exacerbations	33
Human coronavirus	URTI	Seems to be more important in adult asthma exacerbations, but probably plays only a minor role overall	33
Human bocavirus	Clinical relevance unknown. More common as co-infections with other respiratory viruses.	Has been detected in a small number of exacerbations in asthmatic children that were negative for other respiratory viruses	26,34

HRVs are the most frequently detected respiratory pathogen, and typically cause the common cold[12,13]. Annually, HRVs infect billions of people worldwide[14] and cost billions in healthcare dollars[15]. HRVs were discovered as the common cold pathogen over 50 years ago, with the first classical strain discovered in 1956[16,17]. There are approximately 160 types and together they cause a wide range of clinical outcomes infecting both upper and lower respiratory tracts[13–15]. In addition to the common cold, infections with HRV can be asymptomatic but can also cause severe lower respiratory illnesses such as exacerbations of asthma and even pneumonia[14]. HRVs are a large group of genetically diverse RNA viruses and are classified into three different species (A, B and C). The classical serotypes are found within species A and B with another 50 additional strains recently identified as being part of species C[13,14]. They are part of the *Picornaviridae* family, have a 7200-nucleotide mRNA positive sense genome and are classified due to their sequence variations with HRV-C showing substantial sequence divergence from the other classified species[13,18,19]. The development of more sensitive molecular techniques has enabled scientists to detect more HRVs and other respiratory virus infections[20] and gain a greater appreciation of the broad range of clinical illnesses caused by HRVs[13,14]. By using these techniques, viral respiratory infections have been detected in up to 85% of asthma exacerbations in children and approximately 50% in adults[13]. Of these infections, approximately two-thirds are caused by HRVs. Of the three species, HRV-A and HRV-C are more common in infections and exacerbations. Miller *et al*. showed that almost half of all hospitalisations due to HRV infections were associated with HRV-C suggesting that this group causes a substantial burden of paediatric disease[21].

Multiple strains of HRV circulate at any one time during a season[13,22], with children having several HRV infections per year[23]. HRVs are present year-round with all three species (HRV-A, HRV-B and HRV-C) being represented[24,25]. In temperate climates there are peaks of HRV infections in autumn and spring, coinciding with return to school after holidays. This is known as 'the back-to-school effect' and it is seen globally[23]. Exacerbations of asthma and hospital admissions for asthma also show distinct peaks in autumn and

spring, suggesting that viral infections could be major contributors to seasonal asthma morbidity[13]. The seasonal prevalence of different HRV subtypes has also been examined with the proportion of respiratory infections in which HRV-B and HRV-C were detected being the lowest in summer, and more common in autumn[21,25]. It also appears that with this seasonal variation, HRV-C seems to exchange its dominance with HRV-A.

Evidence for early life viral infections and development of asthma

Approximately one-third of infants who have an acute viral wheezing illness will go on to develop more common wheezing events[13]; however, most wheezing illnesses in infancy will resolve with no long-term effects. A number of birth cohort studies have shown that viral respiratory illnesses early on in life might promote asthma in some children[13].

Sigurs *et al*.[35] studied 47 children hospitalised due to RSV infection in their first year of life, and 93 age- and gender-matched controls, prospectively for 18 years. The cohort infected with RSV had high prevalence of early onset allergy-associated wheeze, increased airway hyper-responsiveness (AHR) and reduced airway function at 18 years of age[35]. The RSV group had a higher incidence of parental asthma/atopy compared with the control group (indicating the children were at 'high-risk' of developing asthma), but the difference was not significant.

Jackson *et al*.[11] enrolled 289 genetically 'high-risk' children at birth into the NIH-funded Childhood Origins of ASThma study (COAST) based on one or both of their parents having asthma/allergies. Children who had a wheezing respiratory illness caused by HRV in the first 3 years of life had a 10-fold increase in asthma risk at age 6 years. HRV infection without wheeze was not associated with an increased asthma diagnosis at age 6 years. RSV wheezing illness was associated with a smaller increase in asthma risk at 6 years. An Australian birth cohort study has also found that more wheezing illness in infants is caused by HRV than RSV[36].

But do early life infections with HRV or RSV necessarily cause asthma? It is the age-old question: Which came first, the chicken or the egg? Whether it is causal or genetic factors linking HRV and RSV to asthma later in life is still not known and much debated. Kuehni and colleagues[37] argued that existing evidence shows it is more likely that a genetic predisposition for asthma renders an individual more susceptible to worse illness caused by RSV in early life, rather than RSV being the cause of asthma development. It might be that, as hypothesised by Jackson *et al.*[11], in genetically high-risk individuals, HRV and RSV infections cause pathological changes in the lungs that have lasting effects and lead to asthma. However, not all infants who wheeze with a viral infection will go on to develop asthma. Further studies are needed to resolve this question.

Asthma and other microorganisms

Respiratory viruses are important in children with asthma, but bacteria and fungi can also cause exacerbations of asthma and are more important in older children and adults[1].

Recent studies of *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Legionella pneumophila* have looked at the associations of these atypical bacteria with both acute exacerbations and chronic cases of asthma[38]. Evidence from human studies have linked both *M. pneumoniae* and *C. pneumoniae* to cases of prevalent asthma and even new cases of wheezing indicating they could play possible roles in promoting airway inflammation[38]. Gil *et al*. found that *M. pneumoniae* colonised at a higher rate in patients with asthma (24.7%) than those without (5.7%), possibly inducing the wheezing events[39]. Qasem and colleagues, in a study of both asthmatic and non-asthmatic patients, found that *M. pneumoniae* was more common in asthmatic patients and was also related to the exacerbation of asthma symptoms (patients with suspected viral infections were excluded from this study)[38]. In a study conducted in France of children with known asthma and children with a new diagnosis of asthma, Biscardi *et al*. found that *M. pneumoniae* and *C. pneumoniae* were found in both groups of children, but at higher infection rates in those children with newly diagnosed asthma (50% of newly diagnosed patients had *M. pneumoniae* infection compared with 5.2% of stable asthma patients). Along with the different bacteria, respiratory viruses were tested for within the participants; however, major comparisons were not made and coinfections were not looked at[40]. Further to this, of those children infected with both bacteria and who were experiencing their first attack, 62% had asthma recurrences compared with only 27% who were not infected[40].

Some individuals with asthma (approximately 2.5%[41]) can become chronically infected with the fungus *Aspergillus fumigatus*, causing the diseases allergic bronchopulmonary aspergillosis (ABPA). ABPA can cause bronchiecstasis (chronic inflammation of the airways, decreased mucus clearance, leading to chronic lung infection) and sometimes death. *A. fumigatus* acts as an allergen and pathogen and also sensitises the sufferer to several other fungal pathogens.

Conclusions

Clinical data now indicate microorganisms are the main cause of asthma exacerbations, with many cases of wheeze in children

<1 year linked to asthma later in life. However, many aspects of the interaction between asthma and microorganisms are still not well understood. Why do some children with wheezing HRV/RSV infections go on to develop asthma and some do not? Why do some children with viral respiratory infections have more severe exacerbations of their asthma (leading to hospitalisations) and some do not? Further studies are needed on the severity of these infections and resulting impact on asthma symptoms. The likely benefits of such studies are enormous in terms of reducing the clinical impact of asthma.

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Biographies

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Chronic viral hepatitis and hepatitis B virus infection: stop the cancer

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The World Health Organization (WHO) estimates that 2 billion people have been infected with hepatitis B virus (HBV). Despite an efficacious vaccine having been available since 1982, there are currently 350 million people, predominantly in Asia, suffering from chronic HBV infection. Of these chronically infected individuals, approximately 25% will develop liver cancer, which is the fifth most common cancer worldwide, ranking third as a cause of cancer mortality. In Australia, by 2007 liver cancer had become the 11th most common cause of cancer mortality as well as the fastest increasing cause of cancer mortality, reflecting the increasing prevalence of viral hepatitis[1].

Hepatitis is the term describing inflammation of the liver and viral hepatitis means the liver inflammation is caused by a virus. The five most common causative agents of viral hepatitis are designated hepatitis A virus, HBV, hepatitis C virus (HCV), hepatitis D virus and hepatitis E virus. While infection with any of these viruses can lead to the classic hepatitis symptoms of dark urine, yellowing of the eyes and skin (jaundice) and anorexia, the viruses are otherwise unrelated, using different replication strategies that divide them into separate taxonomic families.

Unlike many viral infections where recovery is associated with immunity and life-long protection, a proportion of those infected with HBV or HCV develop chronic infection in which the virus continues to replicate in the liver. This chronic carriage of HBV or HCV can lead to progressive liver disease, which after many years can result in cirrhosis and liver cancer. Around 25% of people infected with HCV will clear the virus spontaneously but the remainder develops chronic infection. For HBV, the age at which the infection is acquired is the major factor that determines clearance or chronicity. Chronic infection is the predominant outcome if infected early in life (>90%), whereas healthy adults are unlikely to become chronically infected (<5%). In many developing countries, HBV is transmitted from chronically infected mothers to their babies, who in turn become chronically infected, perpetuating the cycle of transmission. It had been proposed that the ability of the virus to persist in the newborn and infants is due to an immune tolerance caused by the secreted hepatitis B 'e' antigen (HBeAg), which can cross the placenta. Recent work has confirmed that HBeAg can modulate the innate immune pathway by suppression of Toll-like receptors[2].

Diagnosis of HBV infection relies on interpretation of serological assays designed to detect viral antigens and their corresponding antibodies. The most useful marker is serum hepatitis B surface antigen (HBsAg), a non-infectious mosaic of the viral surface glycoproteins that circulate in the blood in great excess during the course of infection. This was the so-called Australia antigen, originally discovered by Blumberg and colleagues in the blood of an Aboriginal Australian when investigating immunological variation of complement proteins[3]. It was some time before the association of this protein with hepatitis B was established but its public health significance was subsequently recognised. Blumberg was awarded a Nobel Prize in 1976 and further acknowledged recently when 28 July was declared World Hepatitis Day, the date of Blumberg's birthday. Detection of HBsAg is now carried out by automated immunoassays and the persistence of HBsAg for longer than 6 months defines chronic HBV infection.

The advent of molecular testing for HBV DNA has also shown the importance of this marker of viral replication. Quantification of HBV DNA is required to determine patient eligibility for treatment and for monitoring the efficacy of treatment. In large population studies carried out in Taiwan, the HBV DNA load has been shown to be independently associated with the incidence of cirrhosis and liver cancer with a positive correlation between viral load and the development of liver disease[4]. The corollary of this is that antiviral therapy that successfully reduces HBV DNA load will reduce the incidence of liver injury. Indeed, antiviral therapy with nucleotide or nucleoside analogues that inhibit virus replication has been shown to significantly reduce the frequency of liver cancer[5].

Recent decreases in the incidence of liver cancer in regions endemic for HBV have been reported that may be related to an increased uptake of vaccine and greater awareness of viral transmission routes.

However, cases of liver cancer are increasing in low incidence regions such as North America, France and Australia[6]. The burden of chronic HBV infection in Australia has been very recently evaluated and for 2011 the number of people living with chronic HBV infection was estimated to be 218,000, for a population prevalence of 1%[7]. The majority of those with chronic infection were born overseas in endemic area of the Asia Pacific; Aboriginal and Torres Strait Islander people were another group disproportionately represented[7].

Expert advocacy has propelled viral hepatitis into public awareness internationally. The Global Burden of Disease Study 2010 estimated that the total number of deaths attributable to HBV infection was 786,000 and when combined with 499,000 deaths from HCV infection, viral hepatitis ranks as one of the most frequent causes of human mortality[8]. The World Health Assembly adopted a resolution in 2010 calling for a wide-ranging approach to the prevention and control of viral hepatitis[9], resulting in the establishment of the inaugural WHO Global Hepatitis Program. In Australia, the first National Hepatitis B strategy (2010–2013) has been developed, as has a National HBV Testing policy. The Cancer Council of Australia has provided a chapter devoted to liver cancer in its National Cancer Prevention Policy and healthcare workers can access online information and advice in the investigation and management of people with HBV infection (HepBHelp.org.au). Further promotion and implementation of the strategies outlined in many of these documents will be of great benefit to public health and help us redress the damage caused by this insidious virus.

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Biography

Scott Bowden is the Head of the Molecular Microbiology Laboratory at the Victorian Infectious Diseases Reference Laboratory and also is an adjunct Associate Professor in the Department of Microbiology at Monash University. He has served on Government committees developing the National Testing Strategies for Hepatitis B and Hepatitis C as well as the National HBV Testing policy.

Measles and SSPE: occurrence and pathogenesis

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Measles is an acute febrile exanthematous condition that is usually a self-limiting disease, but it can be associated with several complications, one of which is subacute sclerosing panencephalitis (SSPE). It is a rare delayed complication of measles due to persistence of the virus in the central nervous system. All of the genetic analyses of viral material derived from brain tissue of SSPE patients have revealed sequences of wild-type measles virus (MV). There is no evidence that measles vaccine can cause SSPE. Several mutations have been described in genes coding for proteins in SSPE strains of MV. Several host cell modifications, mechanisms of virus reactivation and immunopathology in pathogenesis of SSPE have been explained recently, broadening the understanding of this fatal disease.

Measles is a highly contagious disease caused by the measles virus and is one of the most devastating infectious diseases in humans. Usually it is a self-limiting acute febrile exanthematous condition, but up to 40% of patients can have complications. Common complications mainly occur in the respiratory tract, with pneumonia, laryngotracheobronchitis (croup) and otitis media[1]. Rare but serious complications of measles usually involve the central nervous system (CNS). Encephalomyelitis occurs within 2 weeks of the onset of rash. Other CNS complications that occur months to years after acute infection are measles inclusion body encephalitis and SSPE, both of which are caused by persistent measles virus infection[2].

From 1990 to 2010, there has been a decrease in measles incidence in Australia, but the incidence increased in 2011 and 2012, with several imported and local clusters of measles in several territories of Australia (Table 1)[3].

Subacute sclerosing panencephalitis

The incidence of SSPE varies greatly from approximately 0.2 to 40 cases per million population per year, depending on the country and the time at which the data were collected. Data analyses in the UK and, more recently, the USA have shown the true incidence of SSPE to be approximately 4–11 cases of SSPE per 100,000 cases of measles[4]. In the nations of India and Eastern Europe the incidence of SSPE remains high[5]. A study suggests an incidence of 0.02/100,000 per annum on the basis of four cases in Australian children for the period 1995–1998[6]. Measles vaccination programmes have led to a dramatic reduction in the incidence of SSPE[7].

Initial symptoms of SSPE typically occur some years after natural measles infection and are usually subtle, with intellectual decline and behavioural changes. Most patients proceed over months or years to generalised convulsions, dementia, coma and death. Death usually occurs within 1–3 years. There is also a higher incidence among males than females, with a ratio of three to one. SSPE is confirmed when there is a recognised clinical course accompanied by one or more of the following: measles antibody detected in the cerebrospinal fluid; a characteristic pattern on electroencephalography; typical histological findings in brain biopsy material or tissue obtained by post-mortem examination[4]. There is currently no effective treatment for SSPE, although many therapies have been tried. Two case reports have suggested slight improvement of clinical condition with intravenous administration of high-dose ribavirin combined with intraventricular administration of IFN-α. Management largely depends on supportive care[8].

Measles virus proteins and SSPE virus strains

Measles virus is composed of six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin (H) and large protein (L). The N, P and L proteins are essential for viral replication and transcription. Sequences of viral genomes of SSPE cases are typically not related to circulating wild-type viruses when patients developed SSPE, but instead to those in circulation when patients developed an acute MV infection some years back. This is consistent with other evidence that SSPE is caused by persistent MV infection and that this is partly dependent on the infecting strain[9]. Genetic analyses have also revealed that persistent MV derived from SSPE cases (SSPE virus strains, SSPEV) contain numerous mutations. The M gene of SSPEV appears to be particularly vulnerable to mutation and its expression is restricted. Other changes in SSPEV structural proteins have been found in the F

Table 1. Measles incidence in Australia.

Year	Measles incidence per 100,000 population
1990	5.14
2000	0.56
2008	0.30
2009	0.47
2010	0.31
2011	0.83
2012	0.83

and H proteins. The base pairs difference in N, P, M, F and H genes of the SSPE strains from standard Edmonston measles strain are 2.3, 3.3, 2.1, 3.3 and 2.5% respectively[10].

There is no evidence that measles vaccine can cause SSPE. Sequence analyses of 57 SSPE viral strains derived from brain tissue of SSPE patients from 1955–1998 have revealed sequences of wild-type measles virus (genotype C1, C2, D1, D3, D5, E and F) never vaccine virus (genotype A)[4,11].

Pathogenesis

Several host cell modifications, mechanisms of virus reactivation and immunopathology in pathogenesis of SSPE have been explained recently, broadening the understanding of the disease.

Host cell modifications in MV persistence

Modulation of gene expression patterns in MV-infected dendritic and other CNS cells that upregulate certain cytokines (e.g. interferon α) have been reported[12]. Alterations in molecules (e.g. NF-κB transcription factors) in post-transcription of MV-infected cells might be involved in SSPE pathogenesis. NF-κB is also implicated in susceptibility to multiple sclerosis. Glial cells appear to be vulnerable to endoplasmic reticulum (ER) stress, altered expression of the above molecules involved in ER stress can perturb myelination[13]. Myelination is a complex process that requires a precise stoichiometry for gene dosage, along with protein and lipid synthesis. Alterations in lipid metabolism, such as decreased cholesterol synthesis and impaired β-oxidation are associated with MV persistence[14]. An alteration in lipid metabolism during persistent MV infection would affect the maintenance of myelin in the CNS (Figure 1B).

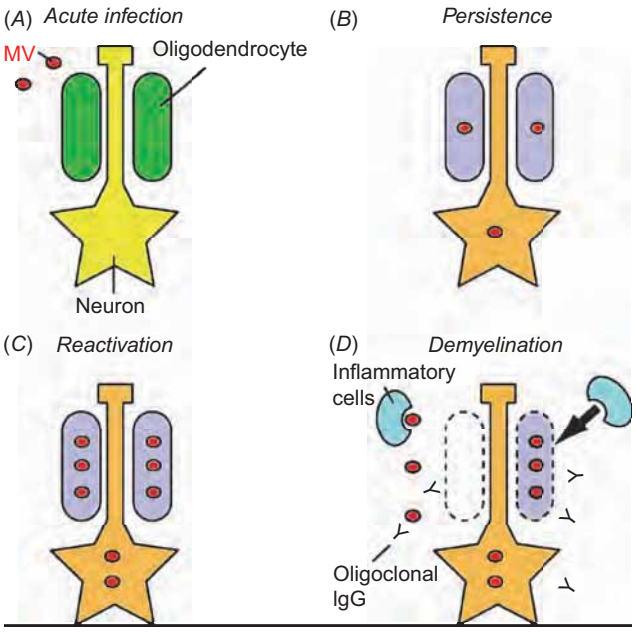

Figure 1. (A) Acute infection. Measles virus (MV) enters the central nervous system (CNS) and infects neurons and oligodendrocytes. (B) Persistent infection. MV establishes a persistent infection in the CNS. MV replication is attuned to the host cells, with minor or reversible modifications of the cells. Minor or reversible modifications, such as alterations in lipid metabolism, in MV-infected cells might be involved in a progressive infection. (C) Reactivation. Some reactivation events stimulate the latent MV. (D) Demyelination. Re-activated MV destroys host cells, including oligodendrocytes, and drives damaging inflammatory responses. [Reproduced from Honda *et al*.[13].]

Reactivation mechanisms of persistent MV

It is known that persistent MV infection is asymptomatic, but can eventually result in SSPE. The latent MV should be reactivated at the onset of disease, resulting in clinical signs of SSPE (Figure 1C). Several molecules and cellular mechanisms have been implicated on reactivation recently. Potential molecules involved in MV reactivation in SSPE are heat shock protein 72 and peroxiredoxin 1. Age-related modifications such as hyperoxidisation might explain why it takes several years after an acute MV infection for the first symptoms of SSPE to appear[15].

Pathogenesis of persistent MV infection

The immune system appears to be involved in SSPE pathogenesis (Figure 1D). Three mechanisms have been explained in immunopathology of SSPE: direct cytopathic effects, autoantigen and superantigen.

Direct cytopathic effects: Persistent MV infection might destroy infected cells, including oligodendrocytes, and damage inflammatory responses, thereby resulting in demyelination. Consistent with this idea, there is a strong correlation among the extent of viral fusion activity, cytopathic effects of MV and severity of neurovirulence in a hamster model[16].

Autoantigen: Autoimmune responses to myelin proteins are considered to be possible causes of some demyelinating diseases including SSPE. It has also been suggested that autoimmunity could arise as a result of cross-reactivity between viral and myelin antigens[17]. Myelin basic protein (MBP)-homologous sequences in the N and C proteins in measles might account not only for encephalomyelitis in humans, but also for cross-reactions as detected by delayed skin tests with MBP in measles-sensitised guinea pigs[18] (Figure 1D).

Superantigen: A whole class of T lymphocyte cells can activate by superantigens (which might produce certain bacteria, mycoplasma or viruses) in a distinctive mode irrespective of antigen specificity. Activated T lymphocyte cells can cross the blood–brain barrier, enter the brain parenchyma and initiate inflammatory lesions. The permeability of the blood–brain barrier increases, leading to an influx of soluble factors, such as tumor necrosis factor, into the CNS, which will result in extensive CNS lesions[19].

Conclusions

Several host cell modifications, mechanisms of virus reactivation and immunopathology in pathogenesis of SSPE have been explained recently broadening our understanding of the disease. However, there could be unidentified mechanisms involved in disease progression during measles virus persistence and pathogenicity. Future research should focus on these aspects and address on early markers of disease, possible novel therapeutic agents in prevention and treating this fatal condition.

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Biography

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Is cytomegalovirus infection causative for coronary heart disease?

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Coronary heart disease (CHD) is the leading cause of death in Australia, representing 16% of all deaths registered in 2009. Atherosclerosis is the major pathological process involved in CHD that usually leads to a clinical condition such as acute myocardial infarction or angina. Epidemiological studies have indicated cigarette smoking, family history, diabetes, hypercholesterolemia and hypertension as independent risk factors for CHD; however, a considerable proportion of patients do not have these classical risk factors. Chronic infections with bacteria and viruses, including cytomegalovirus (CMV), have been proposed to play causative roles in the pathogenesis of CHD. Detection of CMV in atherosclerotic plaques, seroepidemiological data and *in vitro* and animal experiments suggests evidence for a direct role (viral presence in atherosclerotic plaques, increased LDL uptake and proliferative activity in CMV-infected arterial smooth muscle cells, upregulation of inflammatory cytokines in vascular endothelial cells), as well as an indirect role (viral presence in uninvolved aortic tissue of surgical patients, systemic upregulation of cytokines) for CMV infection in the development of CHD.

Several lines of evidence exist that implicate microbial agents, including CMV, in the pathogenesis of CHD. CMV is a highly prevalent herpes virus, with 40–80% of Australians seropositive for the virus, and the prevalence of seropositivity increases with age[1]. Once acquired, CMV can establish a latent infection in the host that persists for a lifetime and may undergo periodic reactivation, especially in immunocompromised individuals[2].

Evidence for CMV as a potential atherogenic agent came from the detection of CMV antigens and virus-specific nucleic acid sequences in cells derived from atherogenic plaques of patients with severe arterial disease[3,4]. This observation was supported by higher prevalence of CMV genome in the coronary arteries of patients with severe atherosclerosis (90%) compared with specimens taken from patients with mild or no atherosclerosis (50%)[5]. Furthermore, CMV DNA was detected in early lesions of atherosclerosis in young trauma victims without symptomatic disease[6]. These observations suggest the human arterial wall might be the site of latency for CMV, and reactivation of the virus could have direct effects on the components of the vascular wall and subsequent development of atherosclerotic lesions. There is supportive evidence for an indirect role of CMV infection in CHD since viral DNA was also detected in uninvolved aortic tissues of patients undergoing surgery for symptomatic atherosclerotic disease as well as in matched controls[7,8]. More recently, Liu and colleagues investigated the coronary plaques of acute CHD patients using CMV-specific immunohistochemical staining and observed a significantly higher number of CMV-positive cells in specimens from patients with severe CHD, compared with patients with stable angina[9], indicating the contribution of CMV infection to the severity of the disease. However, other studies have reported lack of evidence for CMV involvement in atherosclerotic tissue[10,11]. Kol *et al.*[12] assessed the expression of CMV MIE gene mRNA as an early marker for viral replication in coronary atherectomy specimens, but reported the absence of active CMV replication within these tissues, suggesting CMV might be localised within the vessel wall without being involved in the pathogenic process of CHD.

Seroepidemiological studies also suggest that CMV seropositivity is associated with an increased risk of CHD. Danesh and colleagues reviewed several seroepidemiological studies and reported a positive correlation between CMV antibodies and cardiovascular disease[13]. However, evidence for the contribution of CMV to cardiovascular disease in these studies is weakened by small sample sizes, incomplete adjustment for known confounders, as well as focusing mainly on restenosis and transplant atherosclerosis rather than classic CHD. Recent meta-analysis involving six prospective and 49 retrospective case-control studies found that CMV infection was associated with an increased risk for CHD in both prospective and retrospective studies, as well as in Asian and non-Asian

populations[14]. Furthermore, evidence also exists for the measurement of high titre CMV antibodies as a risk factor for increased intimal-medial thickening[15] and increased incidence of coronary artery disease and myocardial infarction[16,17]. Large-scale prospective studies, with careful consideration of confounding factors (other risk factors for CHD) should be carried out to confirm the significance of these earlier findings.

In vitro experiments involving vascular smooth muscle cell (SMC) cultures showed CMV infection was associated with increased uptake of oxidised LDL[18]. Also, CMV infection of human coronary artery SMCs resulted in increased expression of platelet-derived growth factor receptors and increased proliferative activity of SMCs, which could lead to the development of atherosclerotic lesions[19]. In addition, CMV can prevent p53-mediated apoptosis of coronary artery SMCs, leading to accumulation of SMCs[20]. Furthermore, CMV-induced upregulation of intercellular adhesion molecule-1 was demonstrated in human coronary artery endothelial cells, which could facilitate adhesion and migration of macrophages into the vessel wall[21]. CMV infection has also been demonstrated to induce inflammatory cytokines (TNF-α, IL-1β, MCP-1) and trigger procoagulant activity in vascular endothelial cells[22], thereby contributing to proatherosclerotic effects. Elevated cytokine expression, specifically TNF-α, has been well demonstrated to play an important role in reactivation of latent CMV[23], and therefore might lead to increased reactivated virus in the circulation as well as continual reactivation of the virus in the vascular wall for accelerated progression of CHD.

In experiments involving animal models, ApoE knockout mice infected with murine CMV (MCMV) showed larger area lesions in the aortic arch (approximately 2.5 fold at 20 weeks post-infection) compared with mock-infected mice[24]. In addition, increased vascular wall and plasma cytokine (TNF-α, IFN-γ) levels were detected in the infected mice[24–26], which might contribute to the accelerated progression of atherosclerotic process. Induction of local and systemic immune response by MCMV also suggests an indirect effect of the virus on the atherosclerotic process could be equally important as a direct effect observed. In another mouse model, feeding with atherogenic diet and infecting with MCMV resulted in a significant increase in arterial blood pressure, which was independent of atherosclerotic plaque formation in the aorta[27]. Interestingly, Vliegen *et al*. demonstrated that injection of UV-inactivated MCMV was sufficient to aggravate atherosclerosis in hypercholesterolemic mice[28], indicating inflammatory response to viral envelope components rather than active viral replication might contribute to observed atherosclerotic effects. This observation also supports earlier findings that CMV presence in the vascular might be enough to trigger acceleration of atherosclerotic process, without necessarily requiring active viral replication. Taken together, *in vitro* and animal experiments suggest strong molecular evidence for the role of CMV in the major phases of atherosclerosis and CHD. However, the ubiquitous nature of CMV infection means careful interpretation is needed for epidemiological and serological studies in particular. Given the potential impact of CMV on the development of CHD, molecular and pathogenesis research will be of significant interest.

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Biography

Zin Naing joined the Virology Research Lab (VRL) in 2007 and completed a year of honours with research into determination of viral and host factors influencing the outcome of human cytomegalovirus (CMV) infection in liver transplant recipients. After completion of his degree, he worked as a full-time research assistant for 3 years at VRL until commencing his PhD. Zin's research is focusing on the role of CMV genetic variation on infection of the human placenta and alteration of immune response within placental tissue. This research will (i) determine the role CMV cellular tropism genes on infection of placental trophoblast cells, and (ii) determine the role of CMV (immediate-early, early, late) gene products on induction of inflammatory cytokines during infection.

Subversion of immunity by schistosomes

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Chronic parasitic disease affects millions of people worldwide. Helminth infections are seldom fatal but cause chronic disease that may range from asymptomatic to debilitating. One of the hallmarks of the immune response to parasitic infections is the tendency to suppress inflammation and control tissue damage. The overall effect of this is not only to reduce or inactivate the activities of the parasite, but even to skew immune responses to other infectious agents. This article discusses the state of our understanding of the complex regulation and control of immune responses induced by schistosomes.

Schistosoma: an important neglected pathogen

With an estimated 1 billion people infected with one or more helminths in the developing world[1], the global burden of helminthic diseases exceeds that of better known infectious diseases like HIV/AIDS (34 million)[2], tuberculosis (9 million)[3] or malaria (225 million)[4]. However, many helminthic diseases are neglected and difficult to control, as available drugs are not always effective and do not provide protection from reinfection. Since 30 January 2012, the London Declaration on Neglected Tropical Diseases[5] represents a new, coordinated programme aiming to control or eliminate 10 neglected diseases by the end of the year 2020.

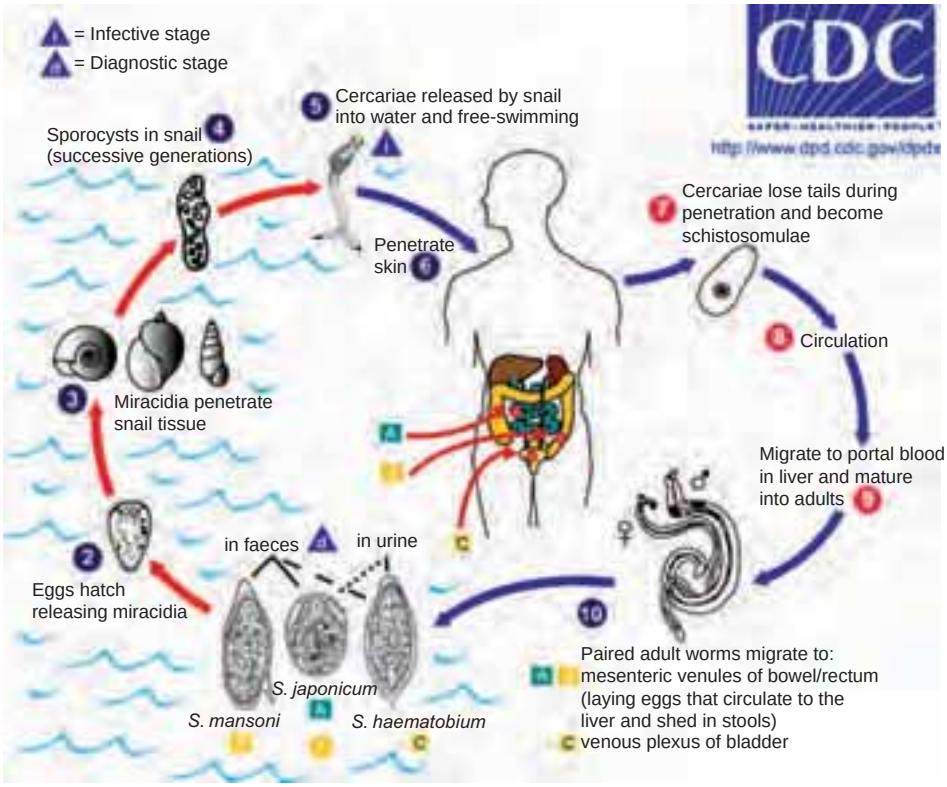

Figure 1. Schistosome life cycle. Eggs are eliminated with faeces or urine (1). Under optimal conditions the eggs hatch and release miracidia (2), which swim and penetrate specific snail intermediate hosts (3). The stages in the snail include two generations of sporocysts (4) and the production of cercariae (5). Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host (6), and shed their tail, becoming schistosomulae (7). The schistosomulae migrate through several tissues and stages to their residence in the veins (8, 9). Adult worms in humans reside in the mesenteric venules in various locations, which are specific for each species (10). *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine (A), and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine (B). *S. haematobium* most often occurs in the venous plexus of bladder (C), but can also be found in the rectal venules. The female deposits eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*) and are eliminated with faeces or urine, respectively (1).

Schistosomiasis ranks fourth among the most prevalent helminthic diseases[1], and is included in the London Declaration. Schistosomiasis (bilharziosis) is caused by blood flukes of the genus *Schistosoma* (Figure 1) and is one of the most severe and chronic parasitic diseases worldwide, affecting ~250 million people with illness, disfigurement or death. The disease is endemic in 76 countries and more than 280,000 deaths occur annually due to schistosomiasis, mainly in rural areas of the developing world, which is testimony to its public health significance[1,6]. The infection is not endemic in Australia despite the fact that infected travellers and immigrants enter the country each year. The absence of an appropriate snail vector in Australia means that the life cycle cannot be completed, and effectively limits its transmission beyond an infected individual. In endemic countries, the overwhelming burden of disease is on children.

Pathogenesis of schistosomiasis

One of the most intriguing aspects of schistosome biology is the interaction of the parasites with the host immune system. The immune response enables the host to survive while infected, and to eventually develop resistance or immunity to subsequent reinfection. However, it is also central to the development of disease due to its role in orchestrating granuloma formation around tissue-trapped parasite eggs. While schistosomes have clearly evolved mechanisms for evading host immune responses, their normal development is, paradoxically, also dependent on the presence of a normal immune system[7]. It been shown that worms in experimental animals that lack T helper cells do not develop normally[8]. Further, inflammation is required to permit access of eggs to the gastrointestinal tract to enable the parasite to complete the lifecycle[7].

Immune responses to schistosome infection in humans (and animal models) differ between the acute and chronic stage of the disease[9,10]. The early immune response is stimulated by cercariae migrating through the skin and schistosomulae migrating through the lungs. This induces innate (macrophage) and T-cell mediated inflammatory immune responses (termed TH1 responses). The symptoms are also known as Katayama syndrome, emerge from 14 days after infection[11,12] and may include fever, coughing, fatigue, diarrhoea and anorexia.

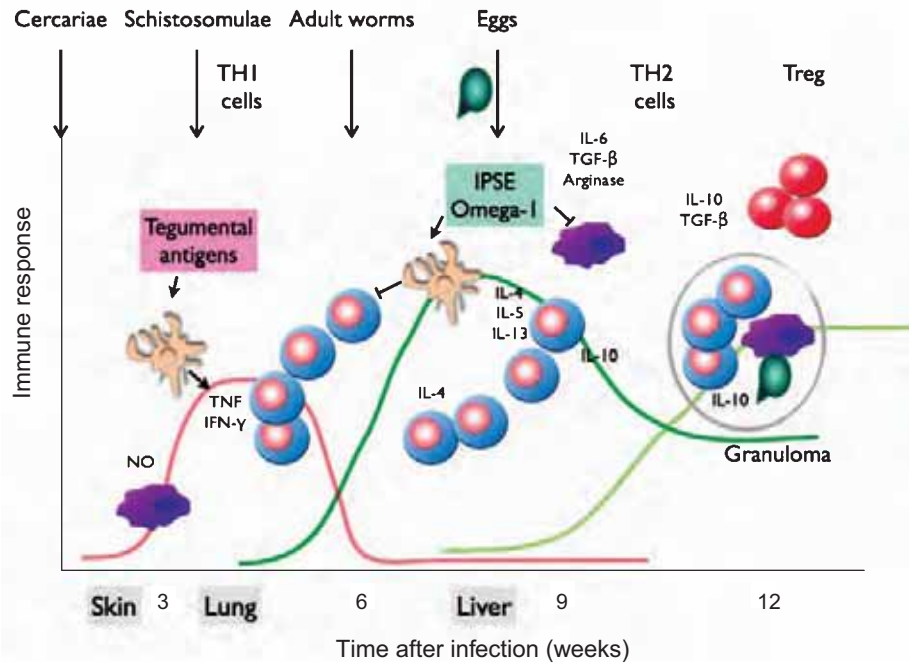

Figure 2. Immune response to schistosomes and immunoregulation by egg-secreted proteins. In early infection, the immune response against cercariae, schistosomulae and adults is dominated by a Th1 response (red line) and the production of cytokines like TNF and INF-γ. With the onset of egg deposition and secretion by the eggs of omega-1 and IPSE, this Th1 response is dramatically shifted to a Th2 response (dark green line). Omega-1 is internalised by DCs after binding to the Mannose receptor. In the DC, omega-1 can block gene expression induced by activation of pattern recognition receptors that abrogates production of IL-12 and subsequently inhibits Th1 polarisation. In turn, DCs unable to respond to Th1 polarising signals promote the development of Th2 responses characterised by IL-4, IL-5 and IL-13 secreting T cells. IPSE can also interact with DC via C-type lectin receptors and induce degranulation of basophils and secretion of IL-4 and IL-13. The cytokine milieu leads to recruitment of granulocytes and mast cells into the affected tissues and, subsequently, the formation of granulomata surrounding the egg. The strong Th2 profile is downregulated by Treg through secretion of the regulatory cytokines IL-10 and TGF-β (light green line). The presence of IL-13, IL-5 and TGF-β causes deposition of excess collagen into the tissues resulting in fibrosis.

As the disease develops, the immune response shifts toward the production of cytokines that reduce inflammation and promote the formation of granulomas. This anti-inflammatory response (termed TH2) is actively induced by antigens secreted by the eggs that start to be released by the adult worms in the portal circulation (Figure 2). This shift to TH2 type responses is orchestrated by the secretion of IL-4 and later IL-5 and IL-13[13]. Following primary infection with schistosomes, an IgE-mediated hypersensitivity response can also develop against penetrating cercariae (cercarial dermatitis), which is often seen in visitors to endemic areas. The TH2 response, especially the presence of IL-4, leads to the formation of granulomata surrounding the eggs particularly in the liver, but also in the lung, pancreas and lymph nodes[14–16]. Developing granulomata consist predominantly of eosinophils, granulocytes, T cells and macrophages[10]. TH2 cells also make IL-13, and this cytokine stimulates myofibroblasts to make collagen, which can lead to excessive fibrosis associated with granuloma formation and resolution[17,18].

As the infection enters the chronic phase, the TH2 response is downregulated and modulated largely by IL-10 that results in reduced cellular responsiveness to schistosome antigens (Figure 2). This regulation keeps tissue damage in check and is mediated by regulatory T-cells (Treg). As a result, most chronically infected patients remain asymptomatic[14]. However, in chronically infected and untreated patients the severe persistent granulomatous inflammation against deposited eggs results in excessive collagen deposition in affected tissues and therefore leads to fibrosis[11,12,14,19]. This is especially severe where eggs are trapped in liver sinusoids causing periportal fibrosis and occlusion of the portal veins, which, in turn, results in portal hypertension, hepato-splenomegaly, ascites and gastrointestinal bleeding[12].

Schistosome eggs are remarkable for their ability to polarize the immune response. Given the importance in disease progression during schistosome infection, a major focus has been on the investigation of the schistosome egg and its components that might be involved in the induction and modulation of immune responses.

Eggs as a major driver of immune suppression

Proteins of schistosome eggs have been classified into two groups: soluble egg antigens (SEA) and egg secretory proteins (ESP). ESP consists solely of secreted proteins from mature eggs containing live larvae and, therefore, represents the 'secretome' of the egg. A proteomic analysis[20] has revealed that ESP comprises at least 188 proteins. The predominant components of this mixture are omega-1

and the 'IL-4-inducing principle' of *S. mansoni* (IPSE). These two molecules have been characterised on the molecular level and described as being heavily glycosylated proteins that are exclusively expressed by the sub-shell area of mature eggs.

Omega-1: a hepatoxin that drives TH2 activation in dendritic cells. In an early paper, Doenhoff and colleagues[21] reported that T-cell-deprived and immuno-suppressed mice suffered from severe tissue damage during *S. mansoni* infections that could be prevented by monospecific antibodies against a highly immunoreactive egg antigen. Interestingly, the presence of cytotoxic products in *S. mansoni* eggs indicates that granuloma formation protects the host against tissue damage[21]. This hepatotoxic protein was isolated from ESP and named omega-1. Further investigation revealed that omega-1 is a member of the RNase T2 family with ribonuclease activity[22]. Later studies demonstrate that omega-1 is one of the major molecules that induces/drives TH2 responses on activation of DCs, with similar characteristics as whole ESP *in vitro* and *in vivo*[23,24], as depletion of ESP of omega-1 decreases TH2 inducing capacity *in vitro* substantially (by 70%)[23].

IPSE: a selective inducer of IL-4. Stimulation studies using human basophils demonstrated that IPSE is a bioactive protein in ESP, leading to activation of basophils and expression of IL-4 and, more importantly, IL-13[25]. The IL-4 inducing capacity of IPSE strongly suggests a role in initiating TH2 responses, whereas IL-13 has been emphasised as a key mediator in the progression of hepatic fibrosis.

As our understanding of the role of these proteins in immune modulation expands, another interesting possibility is raised – could such proteins be used to treat other inflammatory conditions?

Parasite antigens as potential modulators for immune-mediated disease

To date, little work has been done to investigate the possible applications of these immunomodulatory proteins in other systems. However, in a recent study IPSE was found to skew immune responses in a xenograft tumour model and was proposed as a potential approach to prevent transplant rejection[26].

However, the immunomodulatory effects of infections with other helminth parasites have been explored in more detail and an interesting application of this idea has been the successful use of infection with nematodes to treat Crohn's disease (a severe chronic inflammatory disease of the colonic mucosa that is intractable to other therapy). Several studies have been reported where patients who were infected with embryonated eggs of the pig whipworm (*Trichiuris suis*) displayed significant improvements in symptoms[27]. Given the encouraging results using the IPSE protein, schistosoma egg antigens might also have potential as therapeutics for inflammatory diseases.

Schistosomiasis and co-infections

In areas where schistosomiasis is endemic, other infectious diseases are also of serious concern. There are surprisingly few data on the impact of co-infections; however, there is evidence that in infections with schistosomes concurrent with malaria, for example, synergies exist and the severity of effects such as anaemia and low birth weight are increased[28]. A study by Florey *et al.* investigating the parasite burdens in a rural Kenyan population revealed that not only were children 9.3 times more likely to be co-infected with *S. haematobium* and malaria, but also increased malaria infection intensity correlated with increased schistosome infection[29]. This study highlights the need for further studies on the interactions between parasitic and other co-infections.

Chronic infection is also an important consideration for vaccination campaigns and may explain why some vaccines are less effective in the field than predicted. The immunological basis for this may also relate to immunomodulation. For example, a recent study showed that antibody responses in mice to a hepatitis B vaccine were significantly reduced in mice infected with *S. japonicum*. However, this effect was reversed when mice were treated with anthelmintics to cure the infection[30].

Concluding remarks

Schistosomes and other helminth parasites manipulate the immune response of the host, a necessity that has evolved to ensure their survival and reproductive success in long-term infections. With the development of modern techniques and the availability of genome data for many of these parasites we are now beginning to understand the molecular mechanisms that underlie this complex relationship, which might lead to new therapeutic approaches.

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Biographies

Bernd Kalinna is a Principal Research Fellow at the Centre for Animal Biotechnology, Faculty of Veterinary Science, The University of Melbourne. He has had a long-standing interest in schistosome infections. With the recent completion of the *S. mansoni* genome project and emerging abundance of molecular information, his group is focussed on functional genomics in schistosomes to discover novel genes and biochemical pathways involved in pathology, growth, reproduction and survival of the parasite as potential intervention targets. Bernd is also Editor-In-Chief of *Experimental Parasitology (Elsevier),* one of the leading parasitology journals.

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Fatigue after infection: aetiology and pathophysiology

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Individuals suffering from acute infections typically experience systemic symptoms, including fever and musculoskeletal pain, as well as fatigue. Acute infections are also accompanied by increased slow wave sleep and stereotyped behavioural responses, including reduced motor activity, social withdrawal and anorexia. These manifestations are collectively termed the 'acute sickness response'[1]. The action of pro-inflammatory cytokines, such as interleukin (IL)-1 and IL-6 on the central nervous system mediate the key features of the response[2]. In the great majority of cases, these constitutional symptoms, including fatigue, resolve in parallel with the fever. However, in a minority, a disabling fatigue state may persist for weeks, months or (rarely) years. Many of the features of the acute sickness response remain evident in the prolonged fatigue state.

Following acute Q fever, Epstein–Barr virus (EBV) infection or Ross River virus (RRV) infection approximately 30% of individuals experience severe post-infective fatigue and other symptoms lasting 3 months, and 10% of cases meet diagnostic criteria for chronic fatigue syndrome (CFS) when evaluated by a physician and psychiatrist at 6 months following the infection[3]. Infection with several other viral and non-viral pathogens have been linked to a subsequent prolonged fatigue state (i.e. lasting 1 month or more), including cytomegalovirus (CMV), influenza, toxoplasmosis, brucellosis and leptospirosis[4–6]. In contrast, non-specific viral infections (upper respiratory tract infections and gastroenteritis) have been specifically shown not to be associated with an increased incidence of prolonged fatigue[7]. In addition, inappropriate attribution of chronic fatigue to a coincidental preceding infection is common. For instance, reported prevalence rates of persistent fatigue obtained from surveys taken 12–24 months following RRV infection are considerably higher than those identified in prospective studies[8].

Evaluation of a patient with post-infective fatigue relies on a thorough history, careful physical examination and judicious laboratory investigations. The assessment should include a review of the accuracy of the original infective diagnosis, both on clinical and epidemiological grounds, and the laboratory investigations conducted at the time. A characteristic feature of the fatigue state is a prolonged exacerbation triggered by relatively minor physical or even cognitive activities. This should be differentiated from muscle weakness (neuromuscular disease), dyspnoea (cardiac or respiratory disease), somnolence (primary sleep disorders) and loss of motivation and anhedonia (major depression). This assessment is important as a documented infection might have triggered the onset, but other factors may perpetuate the illness, such as depression or sleep–wake cycle disorder[9].

The physical examination should include a careful assessment for signs of persisting infection with the triggering pathogen, as chronic infection might provide an alternative explanation for the fatigue state. The most important infections in this regard are the non-viral pathogens, such as Q fever, in which chronic, localised infection might occur in the form of endocarditis or hepatitis, which in turn might cause a prominent fatigue syndrome. The remainder of the physical examination is aimed at identifying signs indicative of an alternative medical diagnosis.

Laboratory investigations are primarily intended to exclude alternative medical diagnoses and rule out classical features of infection or inflammation (Table 1)[9]. Further specific investigations might be warranted, such as: phase I anti-Q fever antibody testing and PCR for suspected Q fever endocarditis; sexually transmitted infection (STI) screening in those with sexual exposure; or computed tomography (CT) scan of the sinuses if chronic sinusitis is suspected.

Given the association with infection at onset, many studies have sought evidence of persistence of the pathogen as a driver for ongoing symptoms in post-infective fatigue, and in chronic fatigue syndrome. These investigations have been both comprehensive and uniformly negative[5]. A notable recent example has been the flurry of interest in relation to the report in *Science* describing the detection of sequences of xenotropic murine leukemia virus-related virus

Table 1. Recommended investigations for chronic fatigue after infection.

Full blood count, differential white cell count and film
Erythrocyte sedimentation rate
Urea, creatinine and electrolytes
Calcium and phosphate levels
Liver function tests
Thyroid function tests
Random blood glucose
Midstream urine for microscopy and culture
Other tests to exclude active infection or autoimmune disorder, if indicated

(XMRV), as well as putative infectious virus in the blood of the majority of patients with chronic fatigue syndrome (67%) compared with a small proportion (3.7%) of healthy individuals[10]. Multiple subsequent studies failed to replicate the finding[11–15], which was ultimately shown to be laboratory contamination with murine genomic DNA[16].

Similarly, in Australia there has been ongoing interest in the possibility of locally acquired Lyme borreliosis as a cause for both acute manifestations and protracted fatigue[17,18]. However, these reports are largely based on serological testing, including western blot based on IgM positivity, which is recognised to largely reflect false positives when sampling is undertaken outside the acute phase, or IgG detection but with insufficient IgG bands to meet Centers for Disease Control criteria for a positive result[19]. Very few cases in non-travellers have been supported by PCR evidence of Borrelia sequences, and none have been independently validated (to exclude laboratory contamination). These data are juxtaposed against a thorough survey by microscopy, immunohistochemistry and PCR of approximately 12,000 ticks collected in coastal areas of New South Wales[20]. No evidence of *Borrelia burgdorferi* or any other spirochaete was detected in likely tick vectors. Furthermore, in patients with well-diagnosed acute Lyme disease followed by a post-infective fatigue syndrome there is no evidence of persistence of the pathogen[21] or clear response to antimicrobial therapy[22,23].

In addition to the data arguing against the possibility of a unique pathogen or abnormal persistence of a recognised microbe, several studies have examined the possibility of an exaggerated or protracted immune response as a driver of post-infective fatigue, including excessive pro-inflammatory cytokine production. Although cytokine levels correlate with fatigue and other symptoms during the acute, febrile phase[2], case-control studies in the post-infective phase do not show a similar association with fatigue[24,25]. In combination, these findings are consistent with the most widely accepted hypothesis for the pathogenesis of post-infective fatigue, which is a central nervous system disorder triggered by acute infection associated with sensitisation to normal physiological signals from the body, including fatigue and pain[5].

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Biography

Professor Andrew Lloyd AM, BBS, MD, FRACP is an infectious diseases physician and immunology researcher. He leads a multidisciplinary team of clinicians and scientists in research studying the biological basis of inflammation in human infectious diseases, including studies of hepatitis C infection and post-infective fatigue states. He is a NHMRC Practitioner Fellow and his research is supported by a NHMRC Program, Partnership and Project Grants. He is the Director of Infection and Inflammation Research Centre (IIRC) in the School of Medical Sciences at the University of New South Wales. Professor Lloyd was awarded an Australia Medal (AM) for his research achievements in infectious diseases and for his work in establishing the hepatitis service in the NSW prisons.

Do pathogens contribute to multiple sclerosis aetiology?

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Multiple sclerosis (MS) is a common neurological disease characterised by sclerotic plaques of dead and dying oligodendrocytes and neurons in the central nervous system, usually with evidence of accompanying inflammatory activity. Infectious agents might cause or contribute to this cell death, but none have yet been established as doing so. Instead, MS is regarded as predominantly an autoimmune disease, responsive to immunomodulators. However, there is compelling epidemiological and empirical evidence implicating Epstein–Barr virus (EBV), with minor support for other viruses, as contributing to the pathogenic immune response in MS. Now the recent dramatic advances in identifying the genetic risk factors for MS have provided some tantalising leads to microbe involvement. If conclusive evidence is found, vaccines, antivirals/antibiotics or pathogens themselves might prove useful therapeutics.

Do pathogens contribute to MS aetiology?

Multiple sclerosis is one of the most common neurological diseases of young adults. It is primarily an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin and axons. It is triggered by environmental factors, especially in individuals with genetic susceptibility[1]. Many of the genetic variants increasing risk have now been identified[2,3], and these point to variation in cell mediated immunity as driving susceptibility. These genes also potentially point to specific pathogens.

There is empirical and epidemiological evidence that pathogens could be environmental triggers, but the distinction between association and causation is problematic. Two likely mechanisms for causation have been described: bystander activation, where self-reactive T cells are activated due to tissue damage by a pathogen; and molecular mimicry, where T cells that recognize pathogen epitopes are also self-reactive and are activated by the pathogens (Figure 1). Alternatively, pathogens might be protective in autoimmune disease, by inducing tolerance[4].

The sudden arrival and subsequent epidemics of MS in the Faroe Islands during and after WWII was most easily explained as facilitated by the arrival of British troops and accompanying pathogens in 1943[5]. But if MS here and elsewhere was caused by an infection, the pathogen has remained elusive. From the geographic and ethnic distribution, the pathogen is likely to be ubiquitous in western

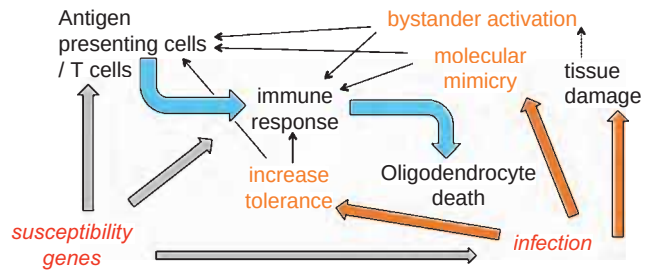

Figure 1. Multiple sclerosis (MS) is considered to be caused by immune system mediated killing of the oligodendrocytes (OD) in the central nervous system. Genes affecting susceptibility are mainly expressed in antigen-presenting cells and T cells, but also in other immune cells. These genes might also affect response to infections. Infections could cause an immune response targeted on OD through bystander activation, an indiscriminate activation including to ODs; or molecular mimicry, a mistaken activation, where infectious agent and OD share similar epitopes. Infection might also tolerise immune responses.

countries, but also in Asia and elsewhere (because MS is described worldwide), infect children (because MS risk reflects childhood environment), cause chronic infection (because the disease is usually late onset and persistent) and is more likely in the higher latitudes (because MS prevalence increases with latitude).

The strongest candidate to date is EBV, which fulfils all these criteria[6]. Although typically 90% of the general population carry EBV, virtually 100% of people with MS do. Notably, though, other common chronic viruses, such as HHV6, chicken pox, measles and cytomegalovirus have also been described as more prevalent in MS[7], such that exposure to these viruses might also render people susceptible to MS and the virus is not causative but associative[8]. More compellingly, late infection or severe infection with EBV increases risk of MS by up to 20-fold[9], an increase not yet found for other pathogens. People with MS have poor CD8 responses to EBV[6], T cells recognizing myelin proteins can be activated by EBV[10], high titres of EBV antibodies are associated with relapses and paediatric MS[11]. A prospective study of 915 individuals found the 10 who were EBV negative and later developed MS were all EBV positive before they developed MS, whereas of the 28 EBV negative controls only 35% became EBV positive over the same time period[12]. High EBV antibody titres, in combination with the first MS susceptibility gene, and variant of largest effect yet known, HLA DRB1*1501, increases the risk of MS, additively and independently, not interactively[13]. Oligoclonal bands found in the CSF of many with MS can be reactive to EBV and other herpesviruses[14].

EBV infects B cells through CD21[6], and possibly CD35[15]. Both of these genes are not among those known to affect susceptibility to MS. The infected B cells proliferate through signaling through EBVs own analogue of human CD40. Strikingly, genetic variants of CD40 are associated with MS, and the genotype with higher expression of CD40 decreases MS risk[16]. Because CD40 is a costimulatory molecule required for T cell activation, it would be expected that higher expression would increase the risk of MS. A potential explanation for this paradox is that low host CD40 expression on B cells favours proliferation/survival of EBV infected B cells, using their EBV CD40. In support of this, anti-CD20 B-cell-depleting drugs such as Rituximab, which are used as a therapies to reduce EBV infection[17], are effective in MS, but those targeting terminally differentiated B cells are not[18]. The effect of more specific depletion of EBV infected B cells on MS needs to be investigated.

Genome wide analysis studies have implicated specific viruses for some autoimmune conditions. Individuals homozygous for the Crohn's diseases risk allele rs601338 of FUC2, the receptor for noroviruses, are protected against norovirus infection[19], but are more likely to develop Crohn's and other autoimmune diseases such as type 1 diabetes and inflammatory bowel disease[20], consistent with the 'hygiene hypothesis'[4]. The MS risk gene TNFSF14 (also known as herpesvirus entry mediator ligand or LIGHT)[2,3] is an entry receptor for Herpes simplex 1 (HSV1)[21], a neurotropic virus. A ligand for TNFSF14, TNFRSF6B, is also a MS risk gene. SLAM family proteins are used by morbilliviruses to facilitate cell entry[22]. These viruses then circumvent the interferon response by blocking Tyk2 signalling[23]. Both SLAMF7 and TYK2 are risk genes for MS[3]. Measles and canine distemper virus, both implicated in the Faroe Island MS 'epidemics', are morbilliviruses. Further viral receptors and genes important in their infection might be in the list of MS genes. But as TNFSF14/TNFRSF6B and SLAMF7/TYK2 also have roles affecting other aspects of the immune response, further implication of a HSV1/morbillivirus contribution to MS might follow if the MS susceptibility genotype can be shown to increase tissue damage or other infectious consequences due to these viruses.

The genes affecting MS susceptibility support a role for immune response in MS, but although they, as yet, provide no smoking gun for particular viruses, the evidence that most of these genes are under recent positive selection[24] is consistent with the genetic variants affecting selection against pathogens. Most of these genes are also predominantly expressed in T cells or antigen-presenting cells, consistent with a role for molecular mimicry or bystander activation contributing to disease aetiology, but also with excessive reaction to self antigen independently of pathogen effect.

A 'smoking gun' for one environmental trigger was found. This is vitamin D. Low vitamin D has been implicated as contributing to MS development and progression from epidemiological and empirical studies[25]. It now seems that genetic variants of the two genes regulating its activation in antigen presenting cells, CYP27B1

and CYP24A1, affect MS risk[2]. As well as regulating DC tolerisation, vitamin D can be antimicrobial, through its upregulation of cathellicidin[6,26].

Current successful therapies for MS alter leukocyte survival, modulation or trafficking[18]. Further manipulation of the immune response might be beneficial if a microbial effect on MS aetiology is defined. Vaccination might be a useful strategy whether viruses such as EBV increase susceptibility (especially if late infection) or protect (especially if early infection) against MS[27]. This might include use of the pathogen itself[28]. To support such approaches, experimental studies are needed to determine if genes associated with MS function through their effect on infections. Novel approaches to establishing if viruses are pathogenic include sequencing of non-host DNA/RNA, especially in plaques, and amplified endogenous retroviruses. Such studies could use historic samples from the Faroe islands and elsewhere. If pathogens contribute to aetiology, even if in only a subset of patients, it is highly likely that their identification will provide significant clinical benefits.

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Biography

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Peptic ulcer disease: current notions

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***Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs (NSAIDs) are the two most important aetiological factors in peptic ulcer disease (PUD). While host genetic factors and dietary factors might also play a role, to date there is insufficient data to implicate these in the aetiology of PUD. A range of *H. pylori* virulence factors, including the cytotoxin associated gene A and the vacuolating cytotoxin A, have been linked with both increased levels of inflammation and PUD. A further virulence factor known as the duodenal-ulcer promoting gene A has been specifically associated with an increased risk of PUD worldwide. Although increasing reports of *H. pylori* negative PUD has led some to question the role of *H. pylori* in PUD, investigations of this issue suggest that *H. pylori* negative PUDs are mainly due to NSAIDs usage and to false negative results related to diagnostic methods.**

Prior to the discovery of *Helicobacter pylori,* peptic ulcer disease (PUD), which encompasses both gastric (GU) and duodenal (DU) ulcers, was believed to be due to gastric acid, the common dictum being 'No acid, no ulcer'. The discovery and initial isolation of *H. pylori* by Robin Warren and Barry Marshall, and their subsequent fulfilment of Koch's postulates showing a pivotal role for *H. pylori* in gastritis[1], the underlying pathology associated with PUD and gastric cancer, not only revolutionised our understanding of gastroduodenal disease but lead to antibiotic therapies that could cure PUD. The global impact and importance of their discovery was recognised in 2005 when Marshall and Warren received the Nobel Prize in Physiology or Medicine.

Currently, extensive scientific evidence shows that the two major aetiological factors involved in PUD are infection with *H. pylori* and ingestion of non-steroidal anti-inflammatory drugs (NSAIDs)[2,3]. Although gastric acid hyper-secretion is still considered to be a necessary factor, it is not a sufficient aetiological factor.

Each year PUD affects 4 million people worldwide with complications reported in 10–20% of these patients, and ulcer perforation in 2–14% of cases[4]. The annual incidence of PUD ranges 0.10–0.19% and 0.03–0.17% for physician-diagnosed and in-hospital-diagnosed PUD, respectively[5]. Over recent decades, the incidence of PUD has decreased significantly, which is believed to be due to the decrease in *H. pylori* infection, particularly in developed countries[5]. Currently, GU is more commonly associated with use of NSAIDs, especially in older patients and those with comorbidities, in whom widespread prescription of NSAIDS and suboptimal adherence to gastroprotective therapy is common[6].

Pathogenesis of peptic ulcer disease

Helicobacter pylori and peptic ulcer disease

In all those infected with *H. pylori* the outcome of colonisation is a chronic active gastritis. However, the gastric distribution of *H. pylori* and the severity of the chronic inflammatory response might differ according to the colonising strain, host genetics and immune response, diet, and the level of acid production. While the majority of those infected develop no other complications and are often free of any obvious clinical symptoms, approximately 10% of those infected will develop PUD[7].

An important predisposing factor related to PUD is an individual's level of gastric acid secretion, as this determines the location of gastritis[7]. In subjects with reduced levels of gastric acid, a pangastritis results, which predisposes to GU, whereas in subjects in whom gastric acid secretion is increased, an antral-predominant gastritis develops, which predisposes to DU[7].

H. pylori infection impairs negative feedback of acid secretion by impairing somatostatin release and its subsequent inhibitory control of gastrin release from G cells, leading to functional disruption of antral-fundic neural connections[8]. The immunopathogenic aspect of ulcerogenesis is primarily driven by the influx of neutrophils and macrophages into the gastric mucosa in response to *H. pylori* colonisation, with the release of lysosomal enzymes, leukotrienes and reactive oxygen species that impair mucosal defence[9].

H. pylori virulence factors, including adaptive enzymes, toxins and mediators of inflammation, lead to bacterial persistence in the stomach and disruption of the gastric mucosal barrier. Production of alkaline ammonia by *H. pylori* urease prevents D cells in the antral glands from sensing the true level of acidity, leading to inappropriate release of somatostatin[8]. It has been suggested that *H. pylori* strains from ulcer patients produce higher amounts of this enzyme than do those from people without ulcers[10].

A wide range of *H. pylori* virulence factors have been associated with more serious disease outcome. Of these, the cag pathogenicity island (*cag*PAI), the vacuolating cytotoxin A (VacA) and the duodenal ulcer promoting gene A (*dupA*) have received the most scrutiny in relation to PUD[11–16].

The *H. pylori cag*PAI is a 40 kb DNA insertion element that encodes up to 32 genes including the cytoxin gene A (*cagA)* and genes encoding a type IV secretion system (T4SS). The T4SS has been shown to translocate the CagA protein into host gastric epithelial cells where it is tyrosine phosphorylated at the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs by the cellular kinases Src[17] and Abl[18]. Four classes of EPIYA motifs, designated EPIYA-A to -D, as determined by the 32–40 amino-acid sequence flanking the core Glu-Pro-Ile-Tyr-Ala motif[19] have been identified. The EPIYA-C (predominantly found in Western strains) and -D motifs (predominantly found in Eastern strains) are the major sites of tyrosine phosphorylation within CagA and are not only necessary for further interaction between CagA and SHP-2, but determine the strength of this interaction, the extent of SHP-2 deregulation, and the extent of hummingbird phenotype induction *in vitro*[11]. Further, translocated CagA activates nuclear factor κβ resulting in expression of interleukin (IL)-8 that leads to increased inflammation[11]. In both Western and Eastern populations, *cagA* positive strains of *H. pylori* have been associated with an increased risk of PUD[12,20,21]. While *H. pylori* strains carrying CagA with multiple EPIYA-C motifs or an EPIYA-D motif have been linked with gastric cancer (GC) development, the majority of studies have failed to show an association with PUD[11,15,22]. However, a recent study reported a significant association between an increased number of EPIYA-C motifs and GU[12].

The *vacA* gene is present in all *H. pylori* strains. While the majority of strains produce a VacA protein, only approximately 40% make the most active form of the toxin[23]. The production and activity of VacA has been shown to relate to the presence of polymorphisms in three main regions of the *vacA* gene, the signal (s1 and s2), mid (m1 and m2) and intermediate (i1 and i2) regions[24]. While *vacA* s1/i1/m1 strains are fully active, s2/i2/m2 strains are inactive. Recent association studies have shown that subjects infected with *H. pylori vacA* s1/i1/m1 strains develop more severe corpus inflammation and more severe antral and corpus neutrophil activity[15,23], and that *H. pylori vacA* s1/i1/m1 and s1/i1/m2 forms are associated with an increased risk of PUD and GC[15,16].

In 2005, the first disease-specific *H. pylori* virulence factor was identified and named *dupA* by Lu *et al*.[25], who showed *dupA* to increase the risk of DU (odds ratio (OR): 3.1, 95% confidence intervals (CI): 1.7–5.7) but protect against gastric atrophy, intestinal metaplasia and GC. Although the association between *dupA* and DU has been supported by a number of subsequent reports[26–28], many studies have failed to show an association between *dupA* and DU[27,29–30]. However, two recent global meta-analyses by Hussein *et al*.[13] and Shiota *et al*.[14], which included 2358 and 2466 individuals, respectively, concluded that worldwide *dupA*-positive subjects had indeed an increased risk of DU (OR: 1.4, 95% CI: 1.1–1.7 and OR: 1.41, 95% CI: 1.12–1.76, respectively). However, in both studies distinct geographical variations in the prevalence of the *dupA* gene, and the association between *dupA* and DU, were observed[13,14]. Given this, a recent study by Jung *et al*.[31] investigated if genetic differences in the makeup of the *dupA* gene may explain this disparity. In this study, the presence of a complete *dupA* cluster rather than *dupA* alone was found to be associated with the development of DU and increased IL-8 production, suggesting that the complete *dupA* cluster is required for full expression of this virulence factor[31].

Nonsteroidal anti-inflammatory drugs and peptic ulcer disease

NSAIDs have been shown to inhibit the synthesis of protective prostanoids in the gastric and duodenal mucosa, leaving the mucosa susceptible for subsequent ulceration by gastric acid[32]. While animal studies have shown that neutrophil adherence to gastric microcirculation plays a critical role in initiation of NSAID damage[33], NSAID-related gastropathy in humans is characterised by an absence of inflammatory cells unless there is a concomitant *H. pylori* infection[10]. However, it is not known if neutrophils can initiate NSAID damage in humans.

NSAIDs have also been shown to increase the risk of PUD complications. For example, the use of low-dose acetylsialicylic acid (ASA) increases the risk of ulcer bleeding by 2–3-fold compared with non-users[34]. This has been mainly attributed to ASA antiplatelet action on a pre-existing *H. pylori*-related ulcer, rather than its ulcerogenic effect as an NSAID[35].

Other factors and peptic ulcer disease

It has been suggested that mucosal resistance to the effect of acid might be a key factor in PUD pathogenesis. For example, the presence or absence of protective substances in staple diets including certain phospholipids, sterols, and sterol ester fractions in lipids, which protect the gastric mucosa, has been related to geographical differences in the prevalence of duodenal ulceration[36]. These substances have been shown to exert protective activities against both gastric and duodenal ulceration, including NSAIDs-associated ulceration, and also promote healing of ulceration[36].

Although smoking is not a primary cause of PUD, it has been reported to regulate aggressive and protective factors in the gastric mucosa, and therefore, it might be still considered an important contributor to the pathogenesis of PUD[37].

Studies examining the possible role of host genetic factors in ulcerogenesis have reported genetic polymorphisms in the nucleotide-binding oligomerisation domain-containing protein (NOD1), the metabolizing enzyme cytochome P450 2C19 (CYP2C19) and IL-8 to be associated with an increased risk of PUD[38–40]. In contrast, specific polymorphisms in IL-1β, toll like receptor (TLR) 4 and TLR1 have been negatively correlated with the disease[38,41–42].

Conclusions

H. pylori infection and NSAIDs are the two most important aetiological factors in PUD. In addition to the *H. pylori* virulence factors *cagA* and *vacA*, a novel biomarker known as *dupA* increases the risk of DU worldwide. Further investigations assessing the role of factors such as diet and host genetic polymorphisms in PUD pathogenesis need to be conducted. *H. pylori* eradication results in long-term cure of *H. pylori*-related PUD, thus, it is recommended in patients with PUD who are naive NSAIDs users.

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Biographies

Dr Natalia Castaño-Rodriguez (MD, MPhil) has worked in the fields of molecular biology, microbiology, biostatistics and bioinformatics related to genetics of cancer and immunogenetics. For the past 4 years, she has been working extensively on *Helicobacter pylori*-related gastric cancer, initially as a postgraduate student completing a MPhil program, and currently as a PhD candidate in Biochemistry and Molecular Genetics at The University of New South Wales.

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Commensal gut microbiota, genetic and epigenetic factors and susceptibility to inflammatory bowel disease

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The incidence of inflammatory bowel disease, an often debilitating disorder, is increasing. Recent data indicate that complex interactions between the commensal gut microbiota, genetic and epigenetic factors and mucosal immunity are important in pathogenesis. Ongoing studies into these interactions will continue to advance understanding of processes responsible for the development of inflammatory bowel disease, as well as inform new and more effective approaches to management.

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is an often debilitating disorder marked by chronic and relapsing intestinal inflammation that has a high incidence in Australia[1]. Incidences have increased over the past five or so decades both in children and in adults and in both low-incidence and high-incidence geographic locations[2]. Studies into factors responsible for intestinal inflammation in IBD implicate a disturbance in the otherwise symbiotic relationship between the host's intestinal mucosal immune system and the commensal gut microbiota, with genetic predisposition, environmental influences and dysbiosis of the gut microbiota each likely contributing.

It is well established that genetic factors contribute to susceptibility to IBD and much recent work has centred on identifying specific loci involved. A recent meta-analysis of Crohn's disease and ulcerative colitis genome-wide association studies, followed by validation of significant findings in 75,000 patients and controls, identified a total of 163 IBD loci, more than that reported for any other complex disease to date[3]. Over two-thirds of these susceptibility loci are associated with both Crohn's disease and ulcerative colitis, suggesting that shared genetic mechanisms contribute to their pathogenesis. Exceptions are particular risk alleles at two Crohn's disease loci, namely nucleotide oligomerisation domain 2 (NOD2) and PTPN22, which demonstrate significant protective effects in ulcerative colitis[3]. Many of the DNA polymorphisms and haplotypes shown to be associated with predisposition to IBD participate in the host's innate and adaptive immune responses to microbial organisms, pointing to a key role for perturbation in the mucosal immune response to commensal gut bacteria in disease pathogenesis[3,4].

Despite the large number of genetic loci linked to IBD, however, only a minority of the variance in disease risk for both Crohn's disease and ulcerative colitis can be explained on a currently identified genetic basis[3], indicative that environmental factors likely contribute substantially to aetiopathology. Indeed, evidence of disease concordance rates in monozygotic twins of only 50% and 20% for Crohn's disease and ulcerative colitis, respectively[5], along with the documented increased incidence of IBD among migrants from low-incidence to high-incidence areas, within the same generation[6], suggest an important environmental influence.

Recent data suggest that a complex interplay between commensal gut microbiota and epigenetics, defined as hereditable changes in gene expression due to mechanisms other than variations in DNA sequence, such as DNA methylation and post-translational histone modifications, may be responsible for at least some of the apparent effects of environment on IBD pathogenesis[7]. For example, expression by intestinal epithelial cells of the pattern recognition receptors, Toll-like receptor (TLR) 2 and TLR4 is regulated by DNA methylation and histone deacetylation[8,9], which, in turn, depend on the presence of commensal gut microbiota[10]. Furthermore, the expression of the chemokine receptor ligand, CXCL16, in a murine model of IBD is regulated by DNA hydroxymethylation, which also is critically dependent on exposure to commensal gut bacteria, especially early in life[11]. It is well established in various genetically susceptible animal models that IBD does not occur in a commensal gut bacteria-free environment[12].

Analyses of mucosa-associated and faecal bacteria have demonstrated that patients with established IBD have both qualitative and quantitative changes in the composition of the commensal gut microbiota, with a reduction in diversity of faecal bacteria and an

increase in number of mucosa-adherent bacteria[13]. Reduced numbers of bacteria with anti-inflammatory properties, such as *Faecalibacterium prausnitzii* and *Bifidobacterium* spp and an increased number of pro-inflammatory Enterobacteriaceae, especially *Escherichia coli*, have been reported in those with established Crohn's disease, while alterations in the composition of Gram-negative bacterial species and under-representation of *Lactobacillus* spp and *Akkermansia muciniphila* have been reported in ulcerative colitis[14–16]. The likelihood of penetration of the intestinal mucus layer by gut flora is 10-fold higher in patients with IBD compared to healthy controls[17], in concert with an increase number of mucolytic bacteria such as *Ruminococcus gnavus* and *Ruminococcus torques* demonstrated in this setting[18]. Recent data suggest that microbial function is also disturbed in IBD, with perturbations in oxidative stress pathways particularly affected[19].

Given that the composition of the commensal gut micobiota may be disturbed by inflammation[20], an important issue, for which little data are currently available, is whether the dysbiosis so far identified in established IBD is present at disease onset - and hence may play a role in initiation of the disorder – or, rather, develops as a consequence. A recent analysis performed in the paediatric IBD setting at diagnosis, limiting the likelihood that microbial changes may have evolved as a consequence of the disease, is noteworthy in demonstrating a significant reduction in bacterial diversity early in the clinical course of Crohn's disease. However, no such trend was apparent in children with ulcerative colitis. Furthermore, an increase in *Faecalibacterium prausnitzii* was evident in the early stages of Crohn's disease, suggesting a more complex gut microbial dynamic in IBD than previously considered[21].

Ongoing studies into the complex relationships between the commensal gut micobiota, genetic and epigenetic factors and mucosal immunity will continue to advance understanding of the pathogenesis of IBD, as well as inform new and more effective approaches to management of this condition.

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Biography

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Enterovirus infection, β-cell apoptosis and type 1 diabetes



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Type 1 diabetes (T1D) results from a complex interplay between genetic and environmental factors, leading to chronic immune mediated destruction of pancreatic β-cells. The inflammatory process is initiated by one or more environmental triggers, such as a viral infection, stimulating release of autoantigens, inflammatory mediators including cytokines and chemokines, and death effectors, with resultant β-cell loss. While multiple enterovirus (EV) serotypes demonstrate β-cell tropism, most studies support a role for the coxsackievirus B (CVB) group in the pathogenesis of T1D. Experimental studies using animal models, insulin-producing cell lines and human islets indicate that the major mechanism of EV-induced β-cell destruction is apoptosis.

Human EV infection has long been implicated in the pathogenesis of T1D. Many epidemiological and cross-sectional studies have demonstrated higher rates of EV infection among individuals with T1D compared with non-diabetic controls[1,2]. In our meta-analysis of 26 studies using molecular methods for virus detection, there were significant associations between EVs and initiation of autoimmunity (OR 3.7, 95% CI 2.1–6.8) as well as development of T1D (OR 9.8, 95% CI 5.5–17.4)[3]. The detection of EV particles in pancreatic tissue from people with recent onset and long-standing T1D, with prominent islet tropism[4–6], provides direct evidence for the involvement of EVs in T1D. Furthermore, EVs isolated at T1D onset effectively destroyed human islets in culture and the pancreas of experimental animals, supporting a potential causal role for specific EV serotypes in the pathogenesis of T1D[4,7,8]. Recently, the EV capsid protein VP1 was detected by immunostaining in 44/72 (61%) of pancreatic autopsy specimens from patients with recent onset T1D. The capsid protein was detected in multiple islets and immunostaining was specific to β-cells, with no involvement of other pancreatic cell types[5,6]. These studies provide some of the most compelling evidence in support of an aetiological role for EVs in at least some cases of T1D.

While pancreatic autopsy specimens provide *in vivo* data, we and others have shown that a range of EV genotypes, including CVBs

and several enteric cytopathic human orphan (ECHO) viruses infect, replicate, impair β-cell function and cause cell death in human islets and insulin producing cells *in vitro*[9–11] (see Figure 1: islets infected with CVB3). *In vitro,* CVB3 and CVB4 can cause persistent infection in human β-cells, with release of infectious particles up to 1 month after infection, without cell lysis[12]. Interestingly, EVs isolated from individuals discordant for development of T1D differed in their capacity to infect β-cells *in vivo*. Four isolates, from a mother and her son diagnosed with T1D on the same day (both infected with CVB5) and from twins (both infected with ECHO 21), one of whom subsequently developed T1D, replicated in human islets and caused slowly progressive β-cell lysis. However, β-cell tropism varied across these isolates, with the least cytolysis manifested by the isolate from the non-diabetic twin[13], suggesting divergence of species between individuals. This suggests that recombination events might have resulted in changes in virulence and/or viral persistence, rather than the host immune response determining whether T1D ensues following an EV infection.

It is generally accepted that activation and infiltration of autoimmune cells within the islets is initiated largely by cytokines and chemokines produced by macrophages and other immune cells[14–16]. Recently, several studies have demonstrated that pancreatic islets are actively involved in signalling immune cells to invade the site of EV infection. CVB4 infection upregulates expression of cytokines such as interleukins (IL) eg; IL-1β, IL-6 and IL-8 and the chemokines; C-C motif ligand-5 (CCL-5) and C-C motif ligand-2 (CCL-2) by human islets[17]. Similarly, CVB5 induces β-cell expression of IL-15, CCL-5 and interferon (IFN)-γ induced protein 10 (IP-10), as we and others have shown[9,18].

The constant activation of pro-inflammatory cytokines leads to the expression of high levels acute inflammatory mediators, such as IL-1β, CCL-2, tumour necrosis factor-α (TNF-α) and IL-8[19,20], with progression to T1D through activation of apoptotic signalling factors. In addition, acute or chronic viral infection activates the innate immune response[21] via interaction of ssRNA with pattern recognition receptors such as toll-like receptor (TLR) 7 and TLR8, activation of signalling pathways and production of antiviral cytokines such as IFN-α[22].

There are two major apoptotic pathways: intrinsic and extrinsic. The intrinsic pathway is regulated by B-cell lymphoma 2 (Bcl-2), which is associated with the outer mitochondrial membrane, while the extrinsic pathway is induced by death receptors such as Fas and TNF receptor-1 (TNF-R1). Either pathway involves activation of MAPK kinase, NF-κB and JAK/STAT pathways triggering, downstream cysteine proteases (caspases) – the final step of apoptosis[23].

Most studies examining mechanisms of EV mediated cell death have utilised cell types other than human β-cells. There is some evidence that the MAPK kinase pathway is involved (Figure 2); for example CVB3 infection in HeLa cells[24,25] and Jurkat T cells[26] phosphorylated p38 MAPK, JNK and ERK1/2. Similarly, c-Jun and p44 MAPK were phosphorylated following EV71 infection in rat brain astrocytes[27].

There is also more limited evidence that EVs induce β-cell death via apoptosis. Following CVB5 and 4 infection of islets derived from human pancreatic progenitor cells, we observed increases in ERK1/2, JNK and p38 (data not shown), confirming activation of the MAPK pathway (Figure 2). Similarly, EV infection in pancreatic islets activated the intrinsic pathway via an increase in Bim and a

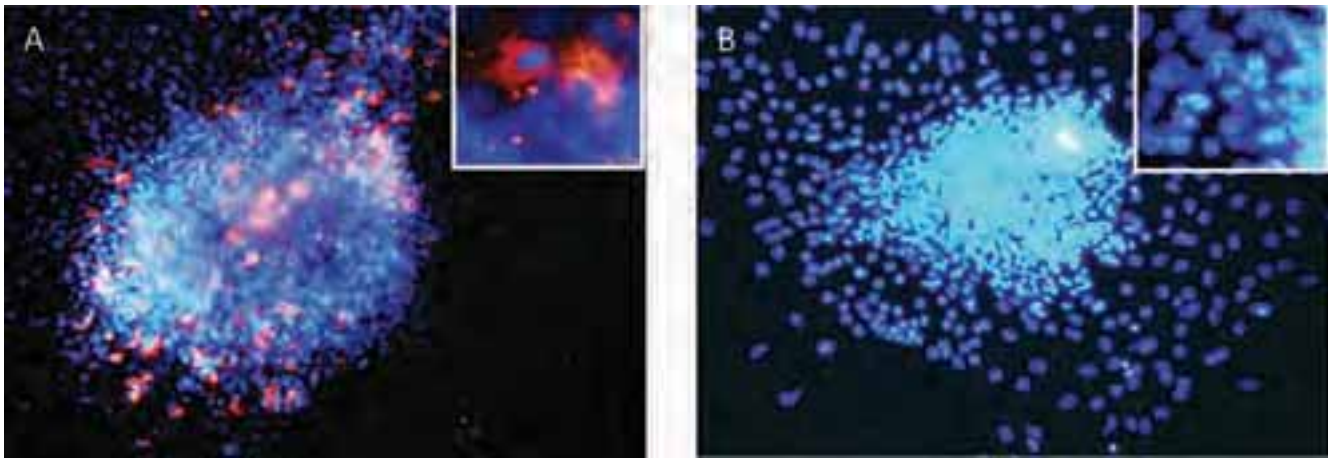

Figure 1. CVB3 infection in human pancreatic islets on day 3 post-infection. (A) CVB3 infected and (B) non-infected control islets were harvested on day 3 post-infection and antibodies to the enterovirus capsid protein (VP1), which stain red, were added. The fluorescent stain 4′,6-diamidino-2-phenylindole (DAPI) was used as a counterstain for DNA content (blue).

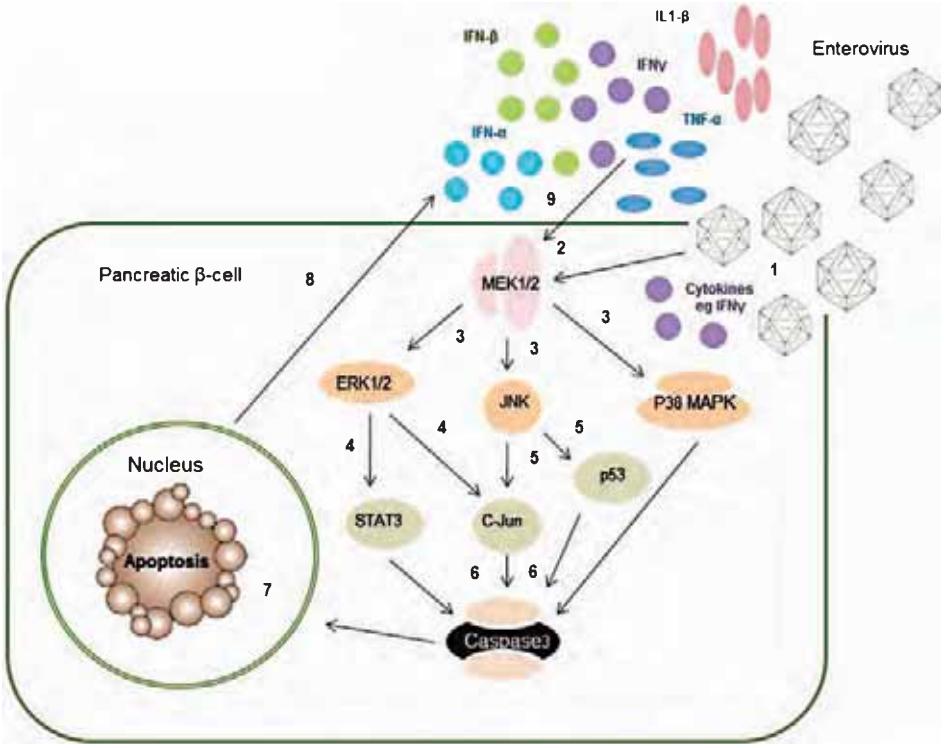

Figure 2. Schematic representation of pathways involved in enterovirus-induced β-cell apoptosis. Enterovirus infection of pancreatic β-cells activates intracellular production of cytokines (1) and signal transduction factors MEK1/2 (2), which in turn activates ERK1/2, JNK and/or p38 MAPK (the MAPK pathway) (3). ERK1/2 activates STAT3 (the JAK-STAT pathway) and c-Jun, (4), while JNK activates c-Jun and p53 (5). This pathway ultimately converges and leads to the activation of caspase 3 (6), which plays a central role in the execution phase of cell apoptosis (7). This induces cytokines and chemokines, which recruit and activate immune cells (8), further activating apoptotic signalling pathways (9).

decrease in induced myeloid leukemia cell differentiation protein (Mcl-1), an anti-apoptotic factor,[6,28] as well as the NF-κB promoter[29]. Collectively, these data indicate that apoptosis is the major mechanism of cell death following EV infection of β-cells. However, the involvement of other signalling pathways has not been investigated.

Although EVs are ubiquitous, their contribution to the burden of T1D remains poorly understood. Furthermore, the putative role of other viruses such as rotavirus, rubella and mumps, as initiators and/or accelerators of T1D, is even less studied[30]. While it is essential to identify specific serotypes and molecular characteristics of EVs that infect and destroy β-cells, a better understanding of the mechanisms of β-cell death may provide insights into development of novel strategies for prevention and treatment of T1D. In particular, EV induced β-cell death may be prevented through intervening in the production and/or action of immune mediators and apoptotic pathways. Development of vaccines targeting 'diabetogenic' EVs is another promising approach that might pave the way to reducing the burden of this chronic life-long disease.

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Biographies

Sandhya Nair completed a year of honours with the Virology Research Laboratory at the Prince of Wales Hospital and continued as a PhD student, furthering her research into the mechanism behind type 1 diabetes and enterovirus. Sandhya's current research is examining viral induction of signalling pathways in beta cells infected with different enterovirus subtypes, to further understand the pathogenesis of virus-induced diabetes.

Dr Ammira Akil is a Postdoctoral research fellow in the School of Women's and Children's Health, Faculty of Medicine, University of New South Wales. Her work in the Virology Research Laboratory is focused on the molecular mechanisms for enterovirus-induced pancreatic beta-cell destruction. Currently she is investigating the viral pathogenesis of type 1 diabetes, particularly enteroviruses, aiming to expand the basic understanding of type 1 diabetes progression to allow the development of better therapeutic and prevention strategies by using a range of systems including human and non-human models.

Associate Professor Maria E Craig is a NHMRC Practitioner Fellow and a Staff Specialist in Paediatric Endocrinology at The Children's Hospital at Westmead. After training in paediatric endocrinology, she was awarded a NHMRC Postgraduate Medical Research Scholarship for her PhD studies at the Virology Research Laboratory, investigating the association between enterovirus infection and the onset of type 1 diabetes. She has since been undertaking further larger-scale cohort studies of at-risk children to investigate the link between viruses and diabetes, including several NHMRC-funded project grants.

Cytomegalovirus and ageing of the immune system: a controversial cause of ageing

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Human cytomegalovirus (HCMV) is a herpesvirus that infects 30–90% of the population in developed countries, establishing lifelong latent infection. Infection during foetal development, in immunocompromised and immunosuppressed individuals might cause severe disease, whereas in the adult immunocompetent host HCMV infection is generally considered asymptomatic. However, recent studies suggest that HCMV affects ageing of the immune system in healthy individuals. These recent findings provide a novel area of research into ageing and the current controversy is discussed here.

Human cytomegalovirus

HCMV is a herpesvirus that spreads between individuals by close contact with infected body fluids such as saliva, milk, blood or urine. HCMV infection rates increase gradually with age with 70–80% of individuals older than 60 years being HCMV-seropositive in western countries[1]. After primary infection (first infection in life), HCMV is not cleared from the host but persists throughout life in a latent form. While primary infection in healthy adults rarely elicits symptoms, primary infection during pregnancy can lead to HCMV crossing the placental barrier and infecting the foetus, resulting in congenital HCMV. The prevalence of HCMV infection at birth, worldwide, is ~0.7% and is a leading cause of birth defects and developmental disabilities[2,3]. In addition, congenital HCMV infection has been associated with adverse pregnancy outcomes, including stillbirth[4].

Reactivation of latent HCMV infection is a significant health issue in immunocompromised and immunosuppressed individuals as it is associated with increased risk of morbidity and mortality. Interestingly, recent clinical, epidemiological and immunological studies suggest that HCMV infection might have clinical importance in the immunocompetent, and might affect immune senescence.

Ageing and immune senescence

Immune senescence is described as the age-related alteration and dysfunction of the immune system, which leads to impaired protective immunity. This is likely to be a multifactorial process involving molecular, cellular, genetic and environmental factors. Interestingly, all components of the immune system undergo age-related alterations; however, the T-cell compartment seems to be affected the most. A shift in T-cell subset distributions with a decline in the naïve T-cell population plus an increase in end-differentiated T cells (CD45RA+CD57+CD28- T cells) have been described as biomarkers of human immune senescence[5].

Long-term effects of HCMV on the T-cell compartment

An association of HCMV-seropositivity with an altered distribution of T-cell phenotypes was first reported by Looney and colleagues[6]. Interestingly, they and others observed that HCMV-seropositive individuals have a similar peripheral blood lymphocyte profile as seen in the ageing immune system. This profile consisted of a large population of HCMV-specific CD8+ and CD4+ T cells (with a late-differentiated phenotype), fewer naïve T cells, and a decreased CD4:CD8 T-cell ratio[7] (Figure 1).

These biomarkers of human immune senescence, with the exception of reduced naïve T cells, were later identified as part of an immune risk profile (IRP) in the Swedish OCTO and NONA immune longitudinal studies[8,9]. This IRP was associated with persistent HCMV-infection and shown to be predictive of an increased mortality in Swedish individuals between 86 and 94 years of age. More recently, Strindhall and collegues[10] identified an identical association with HCMV seropositivity in the HEXA longitudinal study of Swedish individuals at 66 years of age, suggesting that HCMV might also be connected to the generation of late-differentiated CD8+ T cells in this age group. Notably, data from the Swedish NONA study at 6-year follow up demonstrated that 80.6% (25 of 31) of individuals between 92 and 101 years of age were CMV-seropositive, although given the ubiquitous nature of CMV infection, this was not surprising[11].

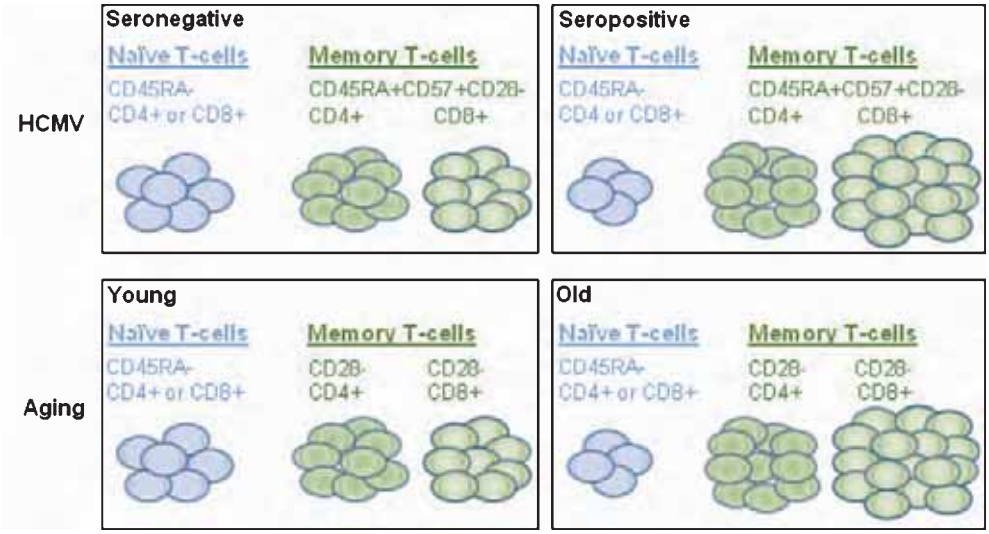

Figure 1. Association of HCMV-seropositivity and biomarkers of immune senescence. The peripheral blood lymphocyte population profile of HCMV-seropositive individuals was observed to consist of fewer naïve T cells, a larger population of HCMV-specific CD8+ and CD4+ T cells and a decreased CD4:CD8 T-cell ratio[7] (upper panels). This profile is similar to that seen in the ageing immune system[5] (lower panels).

The overall findings from the Swedish OCTO and NONA studies have led to the assumption that ageing-related alterations to the cellular composition are detrimental to survival. However, data from the Leiden 85-plus Study suggests that this may not necessarily be true. In this study, Derhovanessian and colleagues[12] observed that low naïve CD8+ T-cell frequencies and an accumulation of late-differentiated effector memory CD8+ T cells in CMV-seropositive individuals correlated significantly with longer survival on 8-year follow up. So the controversy continues.

Long-term effects of HCMV on the immune response

There is a clear evidence for an association of CMV-seropositivity with pronounced changes of cellular components of the immune system. However, the data suggesting a causative role for CMV in immune dysfunction or impaired immunity are highly controversial.

Khan and colleagues[13] observed that HCMV-infection might affect the immune response to other viruses, since lower frequencies of Epstein–Barr virus (EBV)-specific CD8+ T cells were observed in HCMV-seropositive elderly donors. However, others demonstrated that absolute numbers and phenotype of pre-existing EBV-specific memory T cells were not affected by the appearance of HCMV-specific CD8+ T cells on primary HCMV infection[14].

Interestingly, Cicin-Sain and colleagues[15] found that latent/prior murine CMV (MCMV)-infection led to lower CD8+ T-cell responses to influenza virus, herpes simplex virus type I (HSV-1) and West-Nile Virus (WNV)[15]. However, in a rhesus CMV (RhCMV) macaque model specific CD8+ T-cell responses to a super-physiologic RhCMV-infection were equivalent in adult and old monkeys, even though latent RhCMV-infection demonstrated the features of immune senescence found in humans[16].

Furthermore, the findings from Trzonkowski and colleagues[17] suggest that HCMV might interfere with the development of an adequate immune response, since a serological response to influenza vaccine could not be observed in participants with high concentrations of anti-HCMV antibodies. However, these findings could not be confirmed by others[18].

There is also some evidence suggesting that latent HCMV might induce the development of dysfunctional CD8+ T cells. Ouyang and colleagues[19] found that only a small fraction of HCMV-specific CD8+ T cells from elderly individuals were able to mount an immune response on stimulation. However, Gillespie and colleagues[20] showed that stimulated HCMV-specific CD8+ T cells were fully functional as these cells expressed both cytokines and chemokines and were capable of cytotoxicity.

Conclusion

There is mounting evidence indicating an association of HCMV infection and biomarkers of immune senescence; however, a causative role for HCMV is yet to be proven. This controversial area remains an important subject for research, and if CMV does affect immune senescence, then it provides a method for intervening in ageing.

Additional efforts are needed to establish whether, and how, latent HCMV infection might influence immunity. If future studies demonstrate that HCMV is involved in immune senescence, then eliminating or reducing HCMV viral load potentially will not only reduce the risk of morbidity and mortality in immunocompromised and

immunosuppressed individuals, but might also aid in maintaining appropriate immunity.

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Biography

Wendy van Zuijlen is a postdoctoral scientist in the School of Medical Sciences at the University of New South Wales. She recently joined the Virology Research Laboratory at the Prince of Wales Hospital to combine her interests in immunology and virology. Her current research aims to understand the pathogenesis of congenital cytomegalovirus infection.

Report from ASM 2013: A Tour of Microbes

Chris Ossowicz
Adelaide 2013 LOC Chair

This year the annual scientific meeting for the Australian Society for Microbiology was held at the Adelaide Convention Centre during 7–10 July. It attracted approximately 600 delegates from many parts of the world. They were treated to fine winter days, a warm welcome and a diverse scientific program.

As a society we are constantly trying to improve our meetings for delegates and this year was no exception. We continued the shortened format, initiated by Brisbane in 2012, and once again included a meeting APP in an attempt to improve communication and reduce the need for hard copies of the abstract book. Most plenary sessions were also audio recorded and linked to the slides for viewing as you listen. These should be available on the website in due course, along with as many of the posters as possible in PDF format. More significantly and for the first time, we trialled merging symposia with relevant proffered paper presentations in the one session, which by all accounts worked well and was well received.

On Sunday the meeting started with five workshops: Cosmetics and Pharmaceuticals; Culture Media; FASM Applications; BioCyc; and of course the ever popular Antibiotics workshop, which was filled to capacity. A public lecture was held mid-afternoon, which was presented by Forrest Rowher, who spoke on the interactions of macro-organisms, like corals and humans, with their viral and microbial communities. It was a fascinating and entertaining presentation from one of our key plenary speakers who was a real character. After a short break the meeting proper began with the awards ceremony followed by the Bazeley Oration presented by Raman Rao from the Sanofi Pasteur Institute, on the progress of their Dengue Vaccine program. The Fenner Lecture was then given by the recipient of the Frank Fenner Award, Gilda Tachedjian, who spoke on HIV prevention strategies for women. As usual the ever popular welcome mixer was held in the trade hall together with the first of two poster sessions.

Over the next 3 days delegates were treated to another 12 plenary sessions delivered by internationally renowned speakers such as Patrick Murray, Ron Atlas and Juan Lubroth, just to name a few, who delivered the Snowdon Lecture. The Rubbo Oration on Monday night was delivered by Roy Curtis III in a more relaxed format over

ASM 2013 Local Organising Committee: Heather Rickard, Peter Speck, Desley Wilson, Helena Ward, Paul Young, Ryan Taylor, Donna Capurso, Haig Henry, Chris Ossowicz, Phil Giffard, Maria Ginis, Gary Smith, Diana Lagana, Nick Wells, Paul Sideris, Rina Pattullo, Carla Giles. Absent: Sarah Kidd.

dinner followed by a dance to the tunes of the Baker Boys Band who played late into the evening. By all accounts the night was a great success and enjoyed by all who attended. On Wednesday we were joined by the Australasian Mycological Society who held a further 2 days of seminars on Thursday and Friday. Both our societies and delegates alike will always benefit from this type of collaboration and we look forward to joining with them at future national meetings.

A renewed focus on students was undertaken this year by holding, not only the student poster awards but also a networking breakfast so that students were given the opportunity to meet and talk to several of the plenary speakers and local experts in a casual and relaxed forum. It was well attended for a 7.30 am start and finished all too soon, but overall was a great success. A student social night was also held on Tuesday night at a local hotel, with pizza and subsidised drinks on the menu. This was also a huge success and, I believe, carried on into the wee hours of the evening. I must acknowledge the LOC student representative, Carla Giles, for a magnificent effort in organising these very successful activities.

Of course no meeting would be complete without the support of our trade participants and sponsors, especially those who attend the meeting year after year. In particular I thank the Adelaide Convention Bureau and Thermofisher Scientific as our major sponsors for the meeting.

Finally I thank everyone who attended the meeting and hope you are as inspired and motivated as I am after a meeting containing presentations of the quality and depth that we were privy to. I also thank everyone involved in the organisation of the meeting – The National Executive, the National Scientific Advisory Committee, Divisional Chairs, Special Interest Groups, Local Organising Committee and last but certainly not least ASN Events who did a fantastic job in their first year as conference organisers for the society.

The society's flag has now been handed on to Melbourne for next year's meeting and planning is well under way. I hope to see many of you there.

Awardees

Peter Timms: ASM Distinguished Service Award.

Sylvia Kirov: ASM Distinguished Service Award.

Noelene Wilson: FASM.

Helen Cain: ASM Teachers Travel Award.

Iain Gosbell: bioMérieux ASM Identifying Resistance Award.

Gilda Tachedjian: ASM Frank Fenner Award.

BD ASM Student Travel Awardees

Victoria: Snehal Jadhav, Swinburne University
Tasmania: Shi Feng, University of Tasmania
New South Wales: Laura Nolan, University of Technology, Sydney
Queensland: James Marsh, Queensland University of Technology
South Australia: Flinders University

Patrick Murray with Laura Nolan, James Marsh, Snehal Jadhav, Renee Smith and Shi Feng.

ASM Adelaide Student Poster Prize

Angie Jarrad, University of Queensland
Motahareh Nobakht, University of the Sunshine Coast
Zoe Anne Dyson, La Trobe University
Carla Giles, University of South Australia
Victoria Lewis, University of Adelaide

Zoe Anne Dyson, Carla Giles, Paul Young, Motahareh Nobakht and Angie Jarrad.


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