**Introduction**

*Mycobacterium avium* subspecies *paratuberculosis*, more conveniently known as MAP, continues to generate heated economic, medical and scientific debate. This is because the organism represents a real threat to the agriculture and dairy food industries, and some believe it to be a potential public health problem for susceptible humans1-5.

MAP is an obligate pathogen of ruminants including cattle, sheep and goats, in which it may cause Johne’s disease, a progressive wasting and usually fatal chronic intestinal infection. It may also occur in some non-ruminants, including primates. MAP appears to be widespread in the environment. It is a hardy bacterium capable of surviving in vegetative, cell wall deficient and dormant states for long periods in soil and water, but unable to multiply outside the host.

MAP may enter the food chain from a variety of sources including faecal shedding from infected animals and contaminated raw meat and raw milk. Because MAP may exhibit low levels of survival after pasteurisation, the possibility arises of contamination of a diversity of dairy-based food products.6 Notwithstanding the huge cost to the dairy industry of animals infected with MAP, it is the zoonotic potential of MAP derived from contaminated foods, particularly milk, which stimulates the most intense controversy.

**The MAP controversy in Crohn’s disease**

Crohn’s disease is a chronic relapsing disorder characterised by transmural inflammation, which may affect any part of the human gastrointestinal tract.7 Few disorders in clinical medicine are associated with as much chronic suffering as Crohn’s disease. With a prevalence of up to 0.1% in developed countries, Crohn’s is a significant burden on public health care resources, affecting young people in a productive phase of life, with much work absenteeism and a requirement for expensive multidisciplinary care.

The pathogenesis of Crohn’s disease involves three main interacting elements: genetic susceptibility, environmental triggers or modifiers such as enteric microbiota, and immune-mediated tissue injury. The molecular nexus of these colliding influences appears to include defects in the innate immune response to microbial challenge from the lumen of the gut. For example, variants of the NOD2/CARD15 gene predispose to Crohn’s disease. This gene codes for the NOD family of cytosolic proteins which recognise bacterial cell wall constituents such as muramyl dipeptide from bacterial peptidoglycan. However, a variant of NOD2/CARD15 is neither necessary nor sufficient to cause Crohn’s disease and accounts for only about 20% of cases. Other genes, particularly those responsible for the innate immune response to bacteria, including defensins8, are likely to be identified in the future that may account for other subsets of patients with Crohn’s disease.

Since the first description of this disease by Dalziel almost a century ago and its re-discovery by Crohn and colleagues, a mycobacterial cause has been a recurrent speculation. This is because the morphology of Crohn’s disease resembles that of intestinal tuberculosis and Johne’s disease, and because MAP has been detected either by culture or by molecular methods with increased frequency over controls from tissues, blood, and even breast milk, from patients with Crohn’s disease.1, 2, 4-6 While such reports have been controversial and are matched with conflicting views and results, several observations can be used in support of an infectious basis for Crohn’s disease.

First, the contribution of environmental factors can be confidently deduced from the concordance rate for Crohn’s disease (less than 50%) in identical twins and from the increasing prevalence of Crohn’s disease in developing countries, which cannot be attributed to a
genetic influence. Second, the heterogeneity of Crohn’s disease as inferred from animal models and from extensive genetic and immunologic evidence allows for the possibility of an infectious cause in at least a subset of patients. Third, historically, most chronic human disorders have turned out to have a microbial contribution to their pathogenesis. Indeed, the relationship between *Helicobacter pylori* and peptic ulcer disease is the most salutary reminder in gastroenterology that the solution to some disorders resides within the microbial environment and not within the host.

Fourth, although the presence of *MAP* in Crohn’s disease is not proof of pathogenicity, there are a few case reports suggesting chronic *MAP* infection can occur in humans. One of these occurred in a 36-year-old man with haemophilia and human immune deficiency virus (HIV)-related acquired immune deficiency syndrome. Another report describes a young boy who developed distal ileitis that was surgically resected, and this occurred five years after being treated for what appeared to be a mixed mycobacterial infection of the cervical lymph nodes. The cervical lymphadenopathy exhibited caseous necrosis with a few detectable acid fast bacilli, but from which *MAP* DNA was detected by molecular amplification. The significance of this report and the relevance of the detection of *MAP* DNA has been questioned; *MAP* is not associated with caseous necrosis.

The case may represent an atypical mycobacterial infection unrelated to *MAP* and unrelated to subsequent development of classical Crohn’s disease. More recently, evidence for *MAP* infection was described in a 21-year-old male with severe Crohn’s disease who was genotyped as having a NOD2/CARD15 variant. This raises the possibility that a NOD2/CARD15 gene which predisposes to Crohn’s disease might also predispose to persistent intracellular infection with an organism such as *MAP* in this case. However, the evidence that *MAP* was the cause of the disease was based on three inconclusive pieces of evidence:

- i molecular detection of *MAP* DNA by PCR, but not by culture, from a mesenteric lymph node;
- ii the finding of increased message for γ-interferon in the patient’s lymphocytes after stimulation with both paratuberculin and tuberculin compared with unstimulated cells; and
- iii a therapeutic response but not remission to anti-*MAP* treatment with clarithromycin and rifabutin. Thus, the significance of *MAP* detection in this case is unproven.

Unanswered questions and alternative explanation

Several published point-counterpoint debates on the *MAP* controversy seem to have polarised the research community into enthusiasts and sceptics. The latter point to deficits in study design and conflicting findings in reports of *MAP* as a putative cause of Crohn’s disease. Some questions remain unanswered and seem difficult to reconcile with an infectious cause. For example, environmental conditions such as poor sanitation and overcrowding which should promote transmission of an infection, appear instead to protect against Crohn’s disease. Similarly, conditions with greater expected exposure to *MAP*, like residence within a rural area or an occupation such as farming, appear to be associated with lower rather than enhanced risk of Crohn’s disease.

Another apparent paradox is the sustained clinical remission of the disease in response to immunosuppressive drugs and to anti-tumor necrosis factor-alpha (anti-TNF-α). This is difficult to explain in the context of a putative chronic *MAP* infection, particularly since other mycobacterial infections deteriorate or disseminate when infected patients are immunosuppressed. In addition, if *MAP* drives the intense inflammatory reaction that characterises Crohn’s disease, a vigorous
cellular or serologic reactivity against MAP should be demonstrable in these patients, but this has not been reproducibly shown.

If the detection of MAP in tissues from patients with Crohn’s disease has the pathogenic significance suggested by some proponents, it is reasonable to ask about the significance of the same organism when found in healthy subjects or in disease controls. Thus, several reports describe the presence of MAP in controls, albeit at lower frequency than in Crohn’s disease. Detection of other forms of mycobacteria in human tissue would require explanation.

In addition to the controversy surrounding the significance of MAP detection in Crohn’s and controls, there is a more compelling reason to question the specificity of the finding. Thus, bacterial DNA in the granulomas of intestinal Crohn’s disease is not specific to MAP; other forms of bacterial DNA are also present. This may reflect disturbed host-flora interactions in patients with Crohn’s disease due to defects in the innate mucosal immune response, and is consistent with other observations of increased mucosal bacteria in Crohn’s disease.

Conclusion

It seems likely that MAP may cause opportunistic chronic infection in susceptible humans, but in contrast to other mycobacteria, this appears to be uncommon and probably rare in immunocompetent individuals. However, heterogeneous disorders such as Crohn’s disease involve a complex interplay between the microbial environment and the host immune response. In genetically susceptible individuals, MAP and other microbes which are usually harmless, might drive the host immune response toward excessive or misplaced reactivity with collateral tissue damage.

The potential significance of MAP as a putative cause of human disease, particularly if derived from contaminated milk and other foods, is highlighted by several commissioned reports from expert groups around the world. None has concluded a direct causative link between MAP and Crohn’s disease. Predictably, the consensus was a call for more research. What then needs to be done?

The completion of the genomic sequencing of MAP should facilitate the development and enhance the specificity of diagnostic probes. In addition, the cellular immune response to MAP needs to be definitively explored, and potential defects in the setting of NOD2/CARD15 gene variants should be determined. However, in order to conclusively prove or refute a causal relationship between MAP and Crohn’s disease, we need to examine the impact of complete elimination of MAP on the natural history of the disease. This requires careful, well designed, prospective and long-term controlled trials.

Figure 4. Guernsey cow with clinical signs of Johne’s disease.

Figure 5. Thick, corrugated mucosal surface of the bovine ileum.

An indirect pathogenic role for MAP in Crohn’s disease?

Although MAP may not have a direct cause and effect relationship with Crohn’s disease in most patients, the presence of MAP or DNA from MAP or other bacteria within the gastrointestinal mucosa could have secondary clinical implications. Firstly, bacterial DNA has immunomodulatory activity by signalling via pattern recognition receptors such as toll-like receptor 9 on host epithelial and immune cells. Secondly, there is circumstantial evidence for the possibility of immunologic cross-reactivity between MAP antigens and mimicking peptides of host intestinal origin. Thus, immunologic responses to MAP or other microbes within the gut theoretically could stimulate cross-reactive autoimmunity as the initiator or perpetuator of Crohn’s disease in genetically susceptible individuals.

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Studies will need to document the effectiveness and specificity of the anti-MAP treatment versus a non-specific antibacterial influence. Uncontrolled trials to date have been unconvincing, the sensitivity of MAP to the treatment regimen was not determined in all cases, a multi-centre study of anti-MAP therapy (clarithromycin, rifabutin, and clofazimine) over a 2-year period showed no difference between the treatment and control groups for the primary efficacy criterion.\textsuperscript{17}

The economic impact of MAP on the dairy and agriculture industries should help drive the discovery of more effective measures for clearance of MAP. In the absence of convincing proof of the role of MAP in human disease, it seems prudent to ensure that available measures for eliminating the organism from the food chain are optimised.\textsuperscript{6}

Photographs used to illustrate the paper are courtesy of University of Wisconsin’s School of Veterinary Medicine.

\textbf{References}

3. Anonymous. Follow the map. \textit{The Economist} p56, October 8th, 2005
Infectious Risks Associated with Blood Transfusion

Roger Eglin

Introduction

The National Blood Service (NBS) collects approximately 1.8 million donations per year and this is the dataset which gives rise to the figures for the incidence, prevalence and residual risk of infections. For estimates of the general population, surveys of subsets of the general population are undertaken to establish a similar data set for calculations. For the general population the prevalence of infections is 1:1000 for Hepatitis B Virus (HBV), 1:2000 for Hepatitis C Virus (HCV). Among homosexuals (MSMs) attending sentinel Genito-Urinary Medical (GUM) clinics in 2005, the prevalence of previously undiagnosed Human Immunodeficiency Virus (HIV) infections in those aged under 25 was 1.5% for London and 1.3% outside London. Syphilis, which has been at a low incidence for many years, has been undergoing a widespread outbreak in England for the past 6 years. Blood and body fluids are routes of transmission for these infections. It is logical, by means of the pre-donation questionnaire, to attempt to identify donors who have put themselves at a risk by undertaking a range of activities, life styles or from country of birth, previous geographical residence or holidays. Use of the donor questionnaire reduces the risk of these infections in new donors to prevalence of around 1:100,000 donations, i.e. by a factor of around 100 fold.

The second risk is one of bacterial contamination of the collected blood unit, which may occur at the time of collection, during processing into the components or at the time of transfusion. Measures currently in place to reduce these bacterial risks are discussed below.

Outcomes of transmission of the blood borne viruses may vary depending on the immune competence of the recipient. Routine blood screening tests undertaken as follow up of recipients during their routine medical care will identify potential virus transmissions. Transmission of bacteria as a contaminant may have more dramatic and almost immediate effects, e.g. systemic shock, caused by the transfusion of a platelet unit containing a significant number of bacteria.

The approach taken by the Blood Service to minimising the risks of transferring infections with transfusions is multi-faceted and falls into the following categories:

1. Identification and exclusion of potential donors who may be unknowingly infected
2. Measures to reduce the risk of contamination of blood at the time of donation collection
3. Aseptic processing and storage of the components, with filtration to remove white blood cells
4. Monitoring components for bacterial contamination
5. Screening for infections in all donations (e.g. HIV, HBV, HCV, Human T-Cell Leukaemia Virus (HTLV) and Syphilis)
6. Measures instituted to reduce the risk of variant Creutzfeldt-Jakob Disease (vCJD) in donations
7. Pathogen Inactivation of any Fresh Frozen Plasma (currently with Methylene Blue) sourced from USA
8. Monitoring for emerging and re-emerging infectious diseases

Identification of potential donors who may be unknowingly infected

The initial stage in the process of minimising the infectious risks presented by blood transfusions is the questionnaire completed by the donor before any donation takes place. The questionnaire covers many aspects of risk behaviour of potential donors e.g. life style, travel, to decline identified ‘at risk’ donors and prevent them entering the blood donation system. This approach is far from foolproof but does have a great effect on reducing the infectious risk from first time donors (Table 1). Although this risk is greater than for repeat donors the incidence of infection detected in first time donors is less than the incidence of infections in the general population.

Measures to reduce the risk of infection at the time of donation collection

The next stage in the process is the blood donation. These days the collection sets and all subsequent processing of the donated blood occurs in closed systems, i.e. the blood is not exposed to air or the environment throughout this process. Additional measures are now taken during the donation process to minimise the risk of infections in the donations. NBS now uniformly uses Chloraprep, an arm cleansing preparation, containing both Chlorhexidine and alcohol. When correctly applied, including the dwell time, in house studies have shown that there is a 16-fold reduction in the incidence of residual bacterial contamination of the prepared skin compared to previously used cleaners. This donation process is regularly monitored, using contact plates to culture any bacteria present on the arm before and after application of Chloraprep and the failure rate, i.e. bacterial growth on the post-treatment contact plate has reduced from >14% to <2%.

The next risk reduction measure is the use of a side-arm pouch on each of the collection kits. This side arm pouch collects the first 25 ml of the donation, including any bacteria introduced as the needle passes through the skin. This step reduces the risk of bacterial contamination by 50%.

Processing and storage of the components

The donation is then processed into plasma, red blood cells and platelet concentrates. Some plasma is used as fresh frozen plasma, following inactivation with a solvent-detergent process, which inactivates enveloped viruses e.g. HIV, HCV, HBV (to a lesser extent). The red blood cell (RBC) components are stored at 4°C and have a shelf life of 38 days. As a result of the storage temperature there is an extremely low risk of bacterial growth in these components and data from Serious Hazards of Transfusion (SHOT) reports, containing the annual review of transfusion transmitted infections, indicates that these components are very rarely involved in bacterial transmissions to recipient patients. A greater risk is presented by platelet concentrates, which are stored at 20°C for a shelf life of 5 days. At this temperature a range

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Table 1. Estimated prevalence of infections in blood donors 2004 – 2005 (per 100,000 donations)

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<thead>
<tr>
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<th>New Donation</th>
<th>Repeat Donation</th>
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<tbody>
<tr>
<td>HBV</td>
<td>34.46</td>
<td>0.41</td>
</tr>
<tr>
<td>HCV</td>
<td>23.26</td>
<td>0.49</td>
</tr>
<tr>
<td>HIV</td>
<td>5.44</td>
<td>0.65</td>
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of bacteria will grow in this highly nutrient medium and annual reports are produced in SHOT of transfusion-transmitted bacterial infection. It should be noted that the influence of all the previously mentioned measures has contributed to the reduction of post transfusion infections (PTI) (Table 2).

**Monitoring components for bacterial contamination**

Specific measures can be taken to monitor the bacterial contamination rate in platelet components. The two screening processes most widely used are BacTAlert and eBDS. The former uses samples of the component in continuous culture at 37°C. Bacterial growth in the cultured, inoculated bottle alters the pH of the medium and this is detected as a colour change when scanning the bottom of the bottle. The second system measures changes in oxygen concentration of a platelet sample introduced into a pouch containing nutrients and incubated overnight. A current study in the NBS with both these systems has identified around 1 in 6,000 platelet units as confirmed positive for bacteria. However, depending on the transfusion recipients, their immune status and current antibiotic regimens for recipients, most of these transfused bacteria cause no clinical signs. Current SHOT reports indicate a falling incidence of the post transfusion reports to NBS. From SHOT notifications it has been clear that there is a shift in the causal bacteria towards those present in donor with bacteraemia at the time of donation rather than the expected skin commensals e.g. *Strep. bovis; E. coli* etc. It is likely that the implicated organisms in these bacteraemias are present at a higher concentration in blood and therefore in the transfused component, than the skin commensals.

**Table 3. Markers of infection used to screen blood donors**

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<th>Detection of mandatory infectious markers in donations</th>
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| The other major area of infectious risk in blood components is associated with viral infection in donors and particularly the blood-borne viruses. Great efforts are made by blood services to detect infections with these viruses. The markers used for screening the donations are listed in Table 3. Currently HBV detection depends on the use of HBsAg assays, with a sensitivity of 0.05 ng/ml HBsAg. In England and Wales these sensitive tests reduced the cases of transmission by transfusion to zero in 2004. Reported cases of HBV transmission have all been from early acute infections rather than episodes of persistent low level carriage of HBsAg in the presence of anti-HBc. HCV and HIV are screened using combined antigen and antibody assays. Use of these assays has reduced the window period (the time in days between infection and first detection of a virus-associated marker) highly significantly and this can be illustrated by the data on residual risk (RR). RR is the incidence of infected donations for each marker remaining after the use of the screening assays (Table 4). It should be noted from Table 4 that the difference in residual risk between pooled donation nucleic acid testing (NAT) testing and the combined assays is very small. When compared with the background lifetime risks (Figure 1) the risk from being infected by a transfusion containing one of these blood-borne viruses is very low. The exception to this is HBV associated with a RR of ~ 1 in 800,000 donations. Use of NAT assays for reducing this risk even further would entail the use of pools of samples of less than 8 donations, rather than the 48 donation pools used at present for HCV and HIV and it would offer only a minute yield.

Screening for evidence of syphilis infection in donors is performed using Treponema Pallidum Haemagglutination Test (TPHA) assays. Most of the positives detected are determined to be past infections but the individuals involved are removed from the donor panels. Currently there
is a rise in acute infections, which follows the national outbreak of acute syphilis which has been identified in recent years. There is a second set of screening undertaken if a donor, at the time of the pre-donation interview, is considered to be at risk, usually resulting from travel or other history. This is termed ‘conditional’ testing and comprises:

- Antibody to Malaria – travel/history
- Antibody to Trypanosoma cruzi (T. cruzi) – travel/history
- Anti-HBc – tattoos, skin piercing

Donors found to be positive for antibodies to malaria and/or Chagas’ disease are excluded from donation. Donors found to be anti-HBc reactive are screened for other markers of HBV infection to determine their current status of infection and, if they are anti-HBc positive, anti-HBs at least 100 iu/ml and HBsAg negative, these donors can remain on the donor panel.

It can clearly be understood that repeat donors, who have previously been screened for these markers, form a group with a much reduced risk of infection with the mandatory markers, than the new donors (Table 4).

### Measures instituted to reduce the risk of vCJD in donations

There are two further areas of infectious risks from blood components. The first of these is from vCJD. There is now evidence that transfusion-transmission of the causative agent PrPres occurs. Three cases have been reported to date and there is evidence from animal models of transfusion transmission in sheep to a level of 40%. Currently this presents NBS with a huge problem. A number of precautionary steps have been taken to reduce the risks of transmission. However, currently there are no blood screening assays available for the detection of PrPres. It is hoped that some CE marked assays will be available next year and the Blood Services are planning for the introduction of this screening. One aspect of this screening, which is being studied, is the impact on willingness to donate that testing for vCJD may have on donors. Rather as in the introduction and early days of HCV blood screening, the sensitivity and specificity of these assays may not be ideal but will improve with later versions of the tests.

An alternative approach to reducing this risk is the use of an additional filtration step to remove vCJD. Several manufacturers are developing such filters, indeed one has a CE mark and should be used. The NBS is examining the performance of such filters in removal of the abnormal prion and any other effects on the filtered RBC components.

### Monitoring for emerging and re-emerging infectious diseases

The final consideration is the threat from emerging and re-emerging infections, as they become apparent throughout the world. Once such a risk becomes apparent, e.g. West Nile Virus (WNV) in North America since 2000, a
committee of experts assesses the risk to the UK blood supply. If there is considered to be a significant risk of that infection being introduced into the UK blood donors, risk reduction measures are introduced to reduce that risk. In the case of WNV the committee assessed that, from 2003 during the mosquito season in the North American continent, blood donors who had visited that area should be screened for WNV using NAT assays. This risk has now reduced and the screening ceased in 2006. Other infections, which have recently been considered, include SARS – no longer a continuing threat; Chikungunya virus – deferral extended to visitors to the risk areas; Malaria cases – the area of risk was extended to include that area in the malaria deferral risk. International monitoring of emerging risks by a range of agencies (WHO, CDC, CFI) who share information with the blood services.

The risks of transmission of infections by transfusion clearly pose continuing problems and Blood Services throughout the world share methods of best practice to reduce these risks. It is also important that manufacturers of assays, to enable sensitive and specific detection of the markers of infection, are kept aware of the range of emerging infections and extending the range of targeted assays as required.

References