Correspondence

Killing *Yersinia enterocolitica*

In a recent article, Gibb *et al*[1] presented studies dealing with the role of *Yersinia enterocolitica* in transfusion transmitted disease. They proposed that the increased number of reported transfusion complications during recent years, caused by *Y enterocolitica* infected blood components, is related to the use of additive solutions for red cell storage, which brings about a decrease in complement activity. They showed that dilution of plasma with an additive solution to a concentration corresponding to that in red cell units decreases complement killing of *Y enterocolitica* strains at 20°C. These authors assume that the blood of subjectively healthy donors contains *Y enterocolitica* organisms which possess a virulence plasmid rendering them resistant to complement. The organisms are thought to be present free in the donor's plasma. When collected blood is cooled to 20°C, the plasmid is no longer expressed and the organisms become sensitive to complement. The authors suggest that the normal two to six hour delay before separation into components may not be enough for complement killing.

This hypothesis is very interesting and Gibb *et al* presented some evidence in support of it. They do not mention, however, that there is another possible explanation. We have suggested that *Y enterocolitica* organisms are transferred intracellularly in donor leucocytes, where they are obviously protected from the action of plasma complement.[2] After days or weeks of storage, any leucocytes present in red cell units will begin to disintegrate, releasing any organisms they contain. *Y enterocolitica* can grow rapidly at 4°C. Whether the storage medium is undiluted plasma or plasma diluted with an additive solution, no killing by complement will be expected at this temperature, particularly if the bacteria are released after more than a week of storage.

This alternative hypothesis is compatible with the observation that most of the severe complications have been seen with blood units stored for ≥21 days. That no *Y enterocolitica* complications were reported before 1975 may be explained by a combination of insufficient identification of species before the 1970s and that red cells were generally stored for less than 21 days at that time.

The mechanism by which *Y enterocolitica* causes these complications is not just of academic interest. As suggested by Gibb *et al*, the length of time collected blood is held at 20–25°C is important, whether bacteria are present free in donor plasma or whether blood has been contaminated during collection or component separation. Complement killing of *Y enterocolitica* was quick in Gibb *et al*'s study and in a previous study by my group.[3] Removal by phagocytosis is likely to be slower. However, if the major mechanism of transmission of *Y enterocolitica* is via leucocytes infected in vivo, the most logical way of overcoming this problem would be to remove the leucocytes from the red cell preparations, either by removing the buffy coat layer, as is done in many European countries, or by leucocyte filtration.

When discussing possible ways of improving the safety of transfusion, it should be remembered that severe transfusion complications as a result of *Y enterocolitica* infected blood products are extremely rare. The interested reader is referred to a recent review of the subject.[4]

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1 Gibb AP, Poling N, Murphy WG. Failure to kill Yersinia enterocolitica by plasma diluted to the concentration found in red cell units. *J Clin Pathol* 1996;**49**:434–6.
2 Högman CF, Gong J, Hambraeus A, Johansson CS, Eriksson L. The role of white cells in the transmission of Yersinia enterocolitica in blood components. *Transfusion* 1992;**32**:654–7.
3 Gong J, Rawal BD, Högman CF, Vyas GN, Nilsson B, Gustafsson I. Complement killing of Yersinia enterocolitica and retention of the bacteria by leucocyte removal filters. *Vox Sang* 1994;**66**:166–70.
4 Högman CF, Engstrand L. Factors affecting growth of Yersinia enterocolitica in cellular blood products. *Transfus Med Rev* 1996 (in press).

Dr Gibb comments:

Professor Högman's hypothesis was discussed in a previous study by myself and colleagues.[1]

Professor Högman and I agree that those *Y enterocolitica* cells which go on to multiply in donated blood probably survive for some time inside human cells. We disagree about the location of the bacterium at the time of blood collection. As this is such a rare event, we may never know the answer with any certainty, but evidence from animal models suggests that invading *Y enterocolitica* is an extracellular pathogen.[2 3] The plasmid borne, temperature regulated virulence factors of *Y enterocolitica* code for resistance to phagocytosis, as well as complement resistance, at 37°C.[4] These virulence factors are not expressed at low temperature so that complement mediated bacterial killing, or entry into cells, might occur some hours after blood has been collected. If complement mediated killing is impaired by plasma dilution, then this would presumably increase the probability that bacteria could reach a safe intracellular location.

The point in history at which *Y enterocolitica* became a problem is important because of its possible relation to changes in blood transfusion practice. There was an isolated case in 1975, but no further cases were reported until 1982, about the time when additive solutions were introduced into practice.[1] This suggests that growth of *Y enterocolitica* in blood was exceptionally rare before 1982, but that it could be detected if it did occur.

Readers will be aware of the major contribution of Professor Högman to the development of additive solutions for red cell storage.[5 6] It is clear that this work has had important benefits in improving the supply of red cells and plasma products. I have speculated that this process may also have contributed to the growth of *Y enterocolitica* in donated blood. I agree with Professor Högman that this speculation is not proven. Even if there was a proven link, it is likely that the benefit from improved red cell and plasma supply outweighs the possible harm from the rare occurrence of transfusion related *Y enterocolitica* infection.

The harm done by *Y enterocolitica* in blood transfusion is small when compared with other current problems. Nevertheless, we should attempt to understand what has happened and consider strategies to prevent it.

1 Gibb AP, Martin KM, Davidson GA, Walker B, Murphy WG. Modelling the growth of Yersinia enterocolitica in donated blood. *Transfusion* 1994;**34**:304–10.
2 Lian CJ, Hwan WS, Pai CH. Plasmid-mediated resistance to phagocytosis in Yersinia enterocolitica. *Infect Immun* 1989;**55**:1176–83.
3 Hanski C, Kutschka U, Schmoranzer HP, Naumann M, Stallmach A, Hahn H, *et al*. Immunohistochemical and electron microscopic study of interaction of Yersinia enterocolitica serotype 08 with intestinal mucosa during experimental enteritis. *Infect Immun* 1989;**57**:673–8.
4 Poerregard A. Interactions between Yersinia enterocolitica and the host with special reference to virulence plasmid encoded adhesion and humoral immunity. *Danish Med Bull* 1992; **39**:155–72.
5 Högman CF, Hedlund K, Zetterström H. Clinical usefulness of red cells preserved in protein-poor mediums. *N Engl J Med* 1978;**299**:1377–82.
6 Högman CF, Andreen M, Rosén I, Åkerblom O, Hellsing K. Haemotherapy with red-cell concentrates and a new red-cell storage medium. *Lancet* 1983;**i**:269–72.

Book reviews

Laboratory Techniques In Rabies. 4th edn. Meslin F-X, Kaplan MM, Koprowski H, eds. (Pp 476; SW Fr 115.00.) World Health Organisation Publications. 1996. ISBN 92 4 154479 1.

This is a fourth and decidedly fatter edition of a book which is already an indispensable tool for rabies scientists. The editors, Meslin, Kaplan and Koprowski, deserve to be congratulated for a skilful updating, and the WHO for making it available at a reasonable price (SW Fr 115.00 and SW Fr 85.00 in developing countries).

Some of the content is unchanged from the previous edition because there has been no need for change—for instance, the chapters on examination for Negri bodies and the electron microscopy of rabies viruses. Other chapters are right up to date, for instance Tordo's review of the molecular biology of rabies and his discussion with Sacramento and Bourhy of the use of PCR in diagnosis, typing and epidemiological study of rabies.

Different readers will use this book for different purposes. General readers who want to update themselves on rabies will find the first 50 pages very instructive. Specialists in rabies diagnosis, vaccine and standardisation will find their needs equally well served later in the book. At the heart of the book is a series of chapters by expert authors on practical rabies procedures, but in spite of the multi-authored approach, all of the chapters are easy to read. This does credit to the editors and means that the volume is the obvious current source to go to for an exposition of any laboratory technique associated with the study of rabies.

The book is also a salutary reminder to UK readers that throughout most of the world rabies is a serious threat to human and animal health, and that its control is a continuous struggle between what is most desirable and what is possible and affordable. Alongside the newer diagnostic and therapeutic procedures linger classic ones like mouse inoculation and the use of sheep brain vaccines and equine serum for post exposure