References

- ¹ Vel WAC, Namavar F, Verweij-van Vught AMJJ, Pubben ANB, MacLaren DM. Killing of *Escherichia coli* by human polymorphonuclear leukocytes in the presence of *Bacteroides fragilis*. *J Clin Pathol* 1985;38:86-91.
- ² Reznikov M, Finlay-Jones JJ, McDonald PJ. Effect of *Bacteroides fragilis* on the peritoneal clearance of *Escherichia coli* in mice. *Infect Immun* 1981;32:398-9.
- ³ Finlay-Jones JJ, Hill NL, Nulsen MF, Pruul H, McDonald PJ. Opsonins in normal mouse serum for the phagocytic killing of *Proteus mirabilis* by murine neutrophils. *Br J Exp Pathol* 1984;65:711-8.
- ⁴ Verweij-van Vught AMJJ, Namavar F, Sparrius M, Vel WAC, MacLaren DM. Pathogenic synergy between *Escherichia coli* and *Bacteroides fragilis*: studies in an experimental mouse model. *J Med Microbiol* (in press).

Dr Vel and others reply as follows:

Inhibition of phagocytosis and killing of facultative bacteria by polymorphonuclear leukocytes in the presence of anaerobic bacteria is regarded as a possible mechanism by which mixed infections with these bacteria can develop.¹ As many other authors have done, we studied the inhibition of killing in vitro and the effects of anaerobes on the clearance of facultative bacteria in vivo.¹⁻¹⁰ In vitro we found inhibition of the killing of facultative bacteria by human polymorphonuclear leukocytes, provided this killing was dependent on complement.¹⁰ This inhibition could be achieved not only with anaerobes but also with other complement depleting organisms such as *Staphylococcus aureus* or *Zymosan*.¹⁰ In vivo, in a mouse skin model, we found reduction of the clearance of *Escherichia coli* when it was inoculated simultaneously with *Bacteroides fragilis*.⁸ We suggested that the failure to find such an effect by Reznikov *et al*² might have been caused by their *E coli* strain not requiring complement for its killing. Their proof that the *E coli* strain which failed to maintain itself in the presence of *B fragilis* in an experimental infection does need complement to be killed in vitro rules that explanation out.

In our further experiments, the results of which are shown in the Table, *B fragilis* and *B vulgatus* behaved similarly in inhibiting the in vitro killing of *E coli* by polymorphonuclear leukocytes; however, in vivo *B fragilis* had an inhibiting effect on the clearance of *E coli* and *B vulgatus* did not. The presence or absence of synergy seems to be related to the virulence of

Comparison of *B fragilis* BE1 and *B vulgatus* BE16

	<i>B fragilis</i> BE1	<i>B vulgatus</i> BE16
<i>In vitro</i>		
Killing of <i>bacteroides</i> by human polymorphonuclear leukocytes: survival of an inoculum of 7.0 after 90 min*	5.4	6.6
Inhibition of the killing of <i>E coli</i> EB1 in the presence of <i>bacteroides</i> after 90 min†	32	50
Complement depletion by <i>bacteroides</i> ‡	93.3	90.2
<i>In vivo</i>		
Survival of <i>bacteroides</i> in monoinfections after three days (inoculum 8.3)*	6.4	2.0
Survival of <i>E coli</i> in the presence of <i>bacteroides</i> after six days†	2200	4.9

*log viable count

†Inhibitory index: viable count of *E coli* in the presence of *bacteroides* divided by the viable count of *E coli* alone

‡CH₅₀ in serum after absorption with *bacteroides* cells: percentage of control serum, treated with phosphate buffered saline.

Methods of *in vitro* experiments are described by Vel *et al*¹⁰ and those of *in vivo* experiments by Verweij-van Vught⁴

these strains in monoinfections and not to their in vitro killing, complement consumption, or inhibition of killing of *E coli*. We therefore conclude that the synergistic effect in vivo is dependent on the virulence of the "helper organism." Furthermore, the virulence of the *E coli* strain is also of critical importance, since in our hands only with doses within certain narrow ranges can the synergistic effect be seen, not only in our mouse skin model but even more so in an intraperitoneal mouse model (unpublished data).

We therefore believe that our recent results can explain those of Reznikov *et al*²: the ability to consume complement and to inhibit phagocytosis and killing in vitro is no guarantee of synergy in vivo. In our opinion, however, inhibitory effects on phagocytosis and killing may still play a crucial part in the pathogenesis of mixed infections, provided the individual bacteria are virulent enough.

WAC VEL
AMJJ VERWEIJ-VAN VUGHT
F NAMAVAR
DM MACLAREN
Vrije Universiteit,
Faculty of Medicine,
Department of Medical Microbiology and
Parasitology,
1007 MC Amsterdam
The Netherlands

References

- ¹ Ingham HR, Sisson PR, Tharagounet D, Selkon JB, Codd AA. Inhibition of

phagocytosis in vitro by obligate anaerobes. *Lancet* 1977;ii:1252-4.

- ² Namavar F, van Vught AMJJ, MacLaren DM. Bacterial synergy in mixed aerobic and anaerobic infections. *Lancet* 1980;ii:659.

- ³ Jones GR, Gemmel CG. Impairment by *Bacteroides* species of opsonisation and phagocytosis of enterobacteria. *J Med Microbiol* 1982;15:351-61.

- ⁴ Namavar F, Verweij AMJJ, Bal M, van Steenberg TJM, de Graaff J, MacLaren DM. Effect of anaerobic bacteria on the killing of *Proteus mirabilis* by human polymorphonuclear leukocytes. *Infect Immun* 1983;40:930-5.

- ⁵ Wade BH, Kasper DL, Mandell GL. Interactions of *Bacteroides fragilis* and phagocytes: studies with whole organisms, purified capsular polysaccharide and clindamycin-treated bacteria. *J Antimicrob Chemother* 1983;11(suppl C):51-62.

- ⁶ Onderdonk AB, Bartlett JG, Louie T, Sullivan-Seigler N, Gorbach SL. Microbial synergy in experimental intra-abdominal abscess. *Infect Immun* 1976;13:22-6.

- ⁷ Kelly MJ. The quantitative and histological demonstration of pathogenic synergy between *Escherichia coli* and *Bacteroides fragilis* in guinea pig wound. *J Med Microbiol* 1978;11:513-25.

- ⁸ Verweij-van Vught AMJJ, Namavar F, Sparrius M, Vel WAC, MacLaren DM. Pathogenic synergy between *E coli* and *Bacteroides fragilis*: studies in an experimental mouse model. *J Med Microbiol*.

- ⁹ Reznikov M, Finlay-Jones JJ, MacDonald PJ. Effect of *Bacteroides fragilis* on the peritoneal clearance of *Escherichia coli* in mice. *Infect Immun* 1981;32:398-9.

- ¹⁰ Vel WAC, Namavar F, Verweij-van Vught AMJJ, Pubben ANB, MacLaren DM. Killing of *Escherichia coli* by human polymorphonuclear leukocytes in the presence of *Bacteroides fragilis*. *J Clin Pathol* 1985;38:86-91.