Evolutionary genetics

Ambiguous role of CCR5 in *Y. pestis* infection

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Mecsas and colleagues suggest that a deficiency in the chemokine receptor CCR5 in humans is unlikely to confer protection against plague, based on their study of *Yersinia pestis* infection in Ccr5-deficient mice[1]. They were testing the hypothesis that a mutation in the CCR5 gene, frequently found in Caucasians, may have been selected for in the past because it provided protection against (bubonic) plague[2–7]; the mutation, called *CCR5Δ32*, is characterized by a 32-base-pair deletion. We have also tested this hypothesis by using *Y. pestis* infection in mice and, in addition, we have done phagocytosis experiments with macrophages from wild-type and Ccr5-deficient mice. Although, like Mecsas *et al.*, we did not see any difference in the survival of the two groups of mice, we did find that there was a significantly reduced uptake of *Y. pestis* by Ccr5-deficient macrophages *in vitro*. Our results indicate that the role of Ccr5 in *Y. pestis* infection may therefore be more complex than previously thought.

In humans, macrophages are targeted by *Y. pestis*, the causative agent of plague, and are therefore important for successful infection. We tested whether Ccr5 affects the uptake of *Y. pestis* by macrophages *in vitro* by using peritoneal macrophages from $Ccr5^{+/+}$ and $Ccr5^{-/-}$ mice in phagocytosis assays. The uptake by $Ccr5^{-/-}$ macrophages was about 30-fold lower than that by $Ccr5^{+/+}$ macrophages (Fig. 1a; six independent experiments). Our preliminary results indicate that the uptake of *Yersinia pseudotuberculosis* by macrophages from $Ccr5^{-/-}$ mice is much less inhibited in similar experiments (Fig. 1b), suggesting that the inhibition may be specific to *Y. pestis*.

To test the effect of Ccr5 on survival after *Y. pestis* infection, groups of specific pathogen-free $Ccr5^{+/+}$ and $Ccr5^{-/-}$ mice were challenged with lethal inocula of *Y. pestis* GB, a highly virulent strain isolated from a fatal human case of plague. However, there was no significant difference in survival between the groups, even after infection with a low dose of two colony-forming units (CFU) (Fig. 1c).

Our survival data are in agreement with those of Mecsas *et al.*[1], although we used a strain of *Y. pestis* with a different degree of virulence (GB rather than KIM), mice with a different genetic background (C57BL/6 rather than BALB/c) and a different route of infection (subcutaneous rather than intravenous). Our results show that $Ccr5^{-/-}$ mice are not protected against infection with a fatal human isolate of *Y. pestis* and succumb at the same rate as $Ccr5^{+/+}$ mice.

Although these results seem to disprove the 'plague hypothesis', some doubts remain. We consistently observed a marked reduction in the uptake of *Y. pestis* by $Ccr5^{-/-}$ macrophages *in vitro* that appears to be specific to this species of *Yersinia*. The *Y. pestis* strain that caused the great plague pandemic in the fourteenth century was probably quite different from the twentieth-century isolate used for the infection experiments discussed here. Genome analysis indicates that *Y. pestis* evolved rapidly from an enteric organism, which was spread by the faecal–oral route, to a flea-transmitted pathogen of rodents and humans, with acquisition of novel virulence mechanisms along the way[8,9].

In addition, the pathogenesis of *Y. pestis* infection may not be comparable when delivered by injection of mice in the laboratory rather than by flea-borne transmission to humans[10], because infection may be more rapid and acute. The dose of plague bacteria delivered by flea-borne transmission is likely to be more variable and the outcome of infection to depend on an interaction between the pathogen, vector and mammalian host. A previous infection leading to preactivation of the host's immune system would change the course of a subsequent *Y. pestis* infection — as would be expected in people living in the Middle Ages, who were constantly encountering all kinds of infection and in whom a resistance to plague could have developed in association with the *CCR5Δ32* mutation.

Under these circumstances, firm conclusions cannot be drawn from the negative results obtained in Ccr5-deficient mice. Taking all these arguments into consideration, the data on the role of CCR5 in *Y. pestis* infection are still inconclusive because the situation seems to be more complex than previously anticipated.

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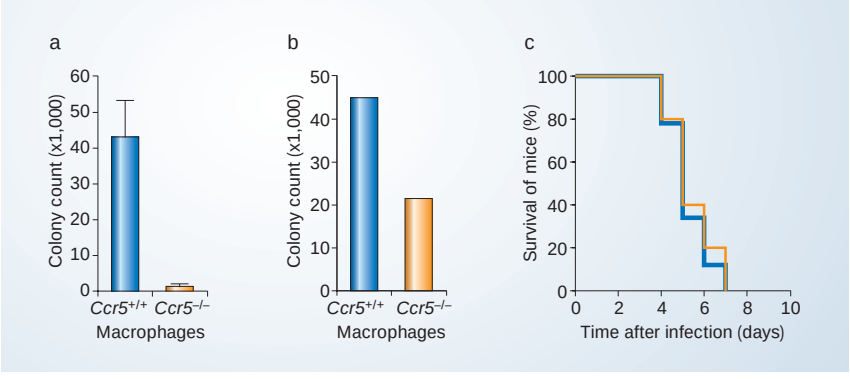

Figure 1 Ccr5 influence on the uptake of bacteria by macrophages *in vitro* and on the survival of mice infected with *Yersinia pestis*. **a, b,** The intracellular bacteria recovered from peritoneal macrophages isolated from C57BL/6 $Ccr5^{+/+}$ and $Ccr5^{-/-}$ mice and incubated ($1x10^6$ cells for 1 h at 37 °C) with **a,** *Y. pestis* GB (multiplicity of infection, 10 colony-forming units (CFU); mean ± s.e.m.) or **b,** *Y. pseudotuberculosis* strain IP32953. Gentamycin was used to kill extracellular bacteria. **c,** Survival of C57BL/6 $Ccr5^{+/+}$ mice (blue; $n = 9$) and $Ccr5^{-/-}$ mice (orange; $n = 10$) after challenge with 2 CFU *Y. pestis* GB (*Biovar orientalis*, Pgm^+, $LcrV^+$; median lethal dose is 1 CFU) subcutaneously.

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