The spatial dynamics of prion disease

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An important component of the latency period of the transmissible spongiform encephalopathies (prion diseases) can be attributed to delays during the propagation of the infectious prion isoform, PrP^{Sc}, through peripheral nervous tissues. A growing body of data report that the host prion protein, PrP^C, is required in both peripheral and central nervous tissues for susceptibility to infection. We introduce a mathematical model, which treats the PrP^{Sc} as a mobile infectious pathogen, and show how peripheral delays can be understood in terms of the intercellular dispersal properties of the PrP^{Sc} strain, its decay rate, and its efficiency at transforming the PrP^C. It has been observed that when two pathogenic strains co-infect a host, the presence of the first inoculated strain can slow down, or stop completely, the spread of the second strain. This is thought to result from a reduced concentration of host protein available for conversion by the second strain. Our model can explain the mechanisms of such interstrain competition, and the time-course of the increased delay. The model provides a link between those data suggesting a role for a continuous chain of PrP-expressing tissue linking peripheral sites to the brain, and data on prion strain competition.

Keywords: latency; prion disease; prion kinetics; prion strain competition; spatial model

1. INTRODUCTION

A characteristic feature of the prion diseases—including Creutzfeldt–Jakob disease (CJD), scrapie, and bovine spongiform encephalopathy (BSE)—is an extended latency before the appearance of symptoms. Susceptibility to these diseases is dependent on the presence of the cellular host protein (PrP^C), as demonstrated by genetically engineered mice that do not express PrP^C, and remain resistant to infection (Büeler *et al.* 1993; Weissmann *et al.* 1993). Under intraperitoneal inoculation, latency depends on the time to access the central nervous system (CNS) (Carp *et al.* 1994). When PrP^{Sc} is introduced via the oral route or directly into the peritoneum, disease onset requires the expression of PrP^C in peripheral compartments, such as the spleen, and in the CNS (Brandner *et al.* 1995). In the earliest phase of infection, prions replicate in the lympho-reticular system, and are associated with differentiated B cells (Klein *et al.* 1997). The appearance of prions in the CNS depends on a continuous chain of PrP^C-expressing tissues linking central and peripheral sites (Blättler *et al.* 1997).

After intraperitoneal inoculation, infectivity in the peripheral nervous system (PNS) reaches a plateau long before the onset of clinical symptoms (Kimberlin *et al.* 1983). The same is true after intra-ocular inoculation in mice and hamsters (Scott *et al.* 1992). These observations imply, for that component of latency between appearance in the PNS and appearance in the CNS, that it is not the rate at which PrP^{Sc} replaces PrP^C at a particular locality that is the rate-limiting process, but rather the rate of spread between neighbouring localities. Thus it appears that an important component of delays attending peripheral pathogenesis can be thought of in terms of a

‘travelling wave’ of infection, spreading along a PrP^C-expressing resource towards the CNS.

2. DISEASE AS A TRAVELLING WAVE

To model the active propagation of PrP^{Sc} we need equations that account both for processes of neuronal transport and for processes of PrP^{Sc} replication. Although the actual mechanisms of scrapie replication remain elusive, various hypotheses have been suggested. The most compelling of these are Prusiner’s heterodimer model, some form of nucleation-dependent aggregation (Griffith 1967; Come *et al.* 1993) or the participation of a so far undetected virus. As this question remains unresolved, we model scrapie replication by using a simple linear autocatalysis term (i.e. the replication rate is proportional to the concentration of scrapie, and also proportional to the concentration of available host protein). Physiologically, the spread of peripheral infection is consistent with slow axonal transport. If we assume that the direction of movement of any one PrP^{Sc} molecule is arbitrary, then the trajectory of each molecule is described by a ‘random walk’ process. This means that the spatial movement of the statistical assemblage of particles can be described by means of a mathematical form equivalent to that for a normal diffusion process.

Here the host PrP^C is treated as a limited resource covering an extended region of space. The PrP^C can be transformed into PrP^{Sc} by one or more mobile prion strains moving from peripheral to central sites of infection. We let the concentration of PrP^C at a time t and at spatial position x be given by $H(x, t)$ and the concentration of the prion strain by $A(x, t)$. The diffusion coefficient of the host protein is given by D_H and of the

prion strain by D_A . The PrPC is assumed to be produced at a constant rate f and to degrade at a rate g , whereas the PrPSc degrades at a slower rate m_A . Conversion to PrPSc occurs at rate j_A . Thus the equations describing the dynamics of the system are

$$\begin{aligned}\partial H/\partial t &= D_H \partial^2 H/\partial x^2 + f - gH - j_A AH, \\ \partial A/\partial t &= D_A \partial^2 A/\partial x^2 + j_A AH - m_A A.\end{aligned}$$

In the absence of infection, the host protein is present at the equilibrium level $H_0 = f/g$, whereas when strain A is present the equilibrium level of the host protein is $H_A = m_A/j_A$. When the pathogenic strain is inoculated, the dynamics of the wavefront are described by a 'Skellam equation', from which the speed of the wave can be predicted (see Appendix A):

$$C_0^A \approx 2\sqrt{d_A \cdot \Delta H_A}. \quad (1)$$

The speed of the wavefront depends on two critical parameter groupings, as follows: (i) $d_A = D_A j_A$ is a measure of the 'effective dispersal' of strain A, and equals the diffusion coefficient multiplied by the transformation coefficient; and (ii) $\Delta H_A = H_0 - H_A$ is the 'competitiveness' of strain A, and equals the difference in concentration of the host protein before and after invasion by the pathogenic strain. This clarifies the balance between the principal processes at work: the movement of protein from one location to the next, and the accumulation at a certain locality. Strain-dependent factors that will lead to faster speeds are higher diffusion and lower decay rates; a higher transformation rate also leads to faster speeds, but depends on both host and strain properties. The sole host-only factor is the initial concentration of PrPC (at lower concentrations of PrPC there is a slower propagation of the prion along the the PrPC-expressing tissues).

Much of the information required by the model is available in the prion protein literature. The rate of propagation of the prion protein in hamsters has been estimated at around 1 mm d^{-1} and has been related to slow axonal transport, which proceeds at between 0.5 and 2 mm d^{-1} (Scott 1993). It is not known how different prion strains vary in their speed of propagation through peripheral tissues, but it is apparent that their spatial distributions in the CNS vary in a systematic fashion (Bruce 1993). The decay rate of the PrPSc is observed to be much slower than that of PrPC (Borchelt *et al.* 1990), which has a half-life estimated at between three and six hours (Caughey 1994; Harris *et al.* 1996). The slow decay of PrPSc is associated with the accumulation of a degraded form of PrPSc that can form amyloid plaques (Caughey & Chesebro 1997). The rate at which PrPSc converts PrPC is estimated to be of the order of hours, and is thought to occur within endosomes during the transport of PrPC from the cell surface into the cytoplasm (Taraboulos *et al.* 1995). Transgenic experiments have shown that the level of PrPC expression influences the incubation period of disease: halving the level of PrPC gene expression can double the latency period (Prusiner 1993). Our model tells us that there is a critical level of PrPC expression below which PrPSc will be unable to propagate (when $H_0 < H_A$). Thus disease prevention need not require PrPC gene ablation, which can be associated

with a deleterious phenotype (Tobler *et al.* 1996; Sakaguchi *et al.* 1996). A more modest PrPC downregulation would suffice.

3. INTERSTRAIN COMPETITION

The model can be used to explore the latency of peripheral infection when two prion strains are presented in the same host. Experiments on competition between strains of scrapie in mice were carried out by Dickinson *et al.* (1975) and Kimberlin & Walker (1985), who found that inoculation with a 'slow' infectious scrapie agent could block the pathogenesis of a second 'quick' agent. In some cases the blocking was partial (the latency period of the quick strain was prolonged by the presence of the slow strain); in others, full blocking occurred (the symptoms of the second strain never become manifested). The explanation offered for these results was the 'restricted-site hypothesis', in which the replication of an 'unconventional virus' occurs while bound to a protein for which there is restricted access (Dickinson & Meikle 1971). The latency of a pure strain will depend on its transformation ability, which is thought of as the rate-limiting step in pathogenesis. However, when presented together with a second strain, as in the blocking experiments, a higher binding affinity allows one strain to outcompete the other. Thus the strain with the higher binding affinity can block the second strain, even when the high-affinity strain would by itself have a longer incubation period. In this way binding and transformation, and not merely transformation, become the rate-limiting reactions.

We offer an alternative explanation for the results of the blocking experiments, based on a combination of the protein-only model of prion replication (Griffith 1967; Prusiner 1982, 1991) and our knowledge of the spatial propagation of prion infection (Scott 1993; Blättler *et al.* 1997). The model has parallels with the ecological question of what happens when two consumers (two PrPSc strains) feed on a single resource (the host PrPC). The ecological principle of 'competitive exclusion' tells us that whichever consumer can subsist on a lower level of resource will exclude the other. The principle applies equally well to interstrain competition in prion diseases: if two pathogenic strains (labelled A and B) are introduced into the same host, then the superior competitor is the strain that would, when presented alone, lead to a lower equilibrium level of host protein. In terms of our mathematical model, the superior competitor is the strain with the lower value out of H_A and H_B (hence the decision, above, to term $\Delta H_A = H_0 - H_A$ as the 'competitiveness'). However, under the travelling-wave context introduced above, the roles of H_A and H_B become more subtle. The formula for predicting the speed of a travelling wave of strain A, when the region of space that it is travelling across has previously been invaded by strain B, is (see Appendix A)

$$C_B^A \approx 2\sqrt{d_A \cdot (\Delta H_A - \Delta H_B)},$$

where $\Delta H_B = H_0 - H_B$ is the 'competitiveness' of strain B, and $H_B = m_B/j_B$ is the equilibrium concentration of PrPC if only strain B (but not A) were present. This has

the same functional dependence as the speed described in equation (1), except that the concentration of host protein before invasion by strain A is modified by the presence of strain B. The prior presence of strain B reduces the equilibrium level of PrPC, and thus reduces the speed of invasion of the strain introduced later.

As a consequence of the relative properties of the different proteins, there are four possible categories of resultant behaviour. We label the 'quick' strain as strain A (faster when not in competition), and in each case take this to be the later-injected strain. The conditions and formulae are described in the appendix.

(a) True full-blocking

If strain B is the better competitor, then strain A can never invade.

(b) Effective full-blocking

If strain A is the better competitor, then it can invade the space already occupied by strain B. The travelling wave of A will form, pursuing and displacing strain B as it goes. However, if the wavefront of A is slower than the wavefront of B, then the second strain never catches up with the first. The first strain to be injected is always the first to reach the CNS and to initiate spongiform degeneration.

(c) Apparent full-blocking and (d) partial blocking

As in (b), strain A is the better competitor, with A invading and spreading after B. The difference here is that the wavefront of A, despite being slowed down by the presence of B, is still faster than the strain B wavefront ahead of it. However, if there is not sufficient time for strain A to catch up with strain B then full blocking will appear to have occurred (case (c)). If, alternatively, there is sufficient time, then strain A can catch up and pass strain B (case (d)). In this case the peripheral latency period has two components: the period when the wavefront of A is behind that of B (during which the speed is C_B^A); and the period after the wavefront of A has superseded that of B (during which the speed of A is C_0^A). Here, pre-injection with the 'slow' B strain can only retard, but not stop, the progress of strain A. When 'partial blocking' by B prolongs the latency period of strain A, measurement of the effect can be used to estimate the speed of strain A when it is invading territory already infected by B. The appropriate formula is given in equation (A3).

Dickinson *et al.* (1975) observed a full blocking effect of the 'quick' 22C scrapie strain by the 'slow' 22A strain, in RIII mice expressing the s7 allele of the *sinc* gene (now known to be identical with the host PrPC gene (Moore *et al.* 1998)). In our model this could be explained by category (a), (b) or (c). Kimberlin & Walker (1985) observed various degrees of partial blocking with the same two scrapie strains, in Compton white mice expressing the s7 allele. In our model this would fall under category (d).

Interstrain blocking also occurs when both strains are inoculated intracerebrally (Dickinson *et al.* 1972; Hecker *et al.* 1992; Manuelidis 1998). The general principles we discuss can apply, but the physiological context is more complex and less clearly delimited: the structure of the CNS is three-dimensional, different types of neurons are

Figure 1. Schematic of the travelling wave interpretation of strain competition in prion disease. The graphs show the concentration of host PrPc protein (solid line), the concentration of pathogenic strain B (dotted line), and the concentration of pathogenic strain A (broken line). Inoculation occurs at the left-hand side, after which disease spreads rightwards towards the CNS. (a) Strain B is injected first, and propagates with speed C_0^B . Behind the wavefront the concentration of the host protein is reduced relative to the uninfected level. (b) Strain A (presumed here to be the better competitor) is subsequently injected and propagates with speed C_B^A , displacing strain B as it goes. (c) Strain A may catch up and pass strain B. Once this occurs the reservoir of host protein available for conversion by A is larger than when the wavefront of A was behind that of B, and so the propagation of strain A increases to a speed C_0^A (faster than C_B^A). The prior inoculation with strain B causes the overall time taken for strain A to reach the right-hand side (i.e. to reach the CNS) to be slower, in a predictable way, than if solely strain A were present.

present, different scrapie strains are known to have region-specific accumulation affinities, and PrPC is not a limiting resource in the CNS (Manuelidis 1998). These factors suggest that one would not see the same blocking properties for interstrain competition in the CNS as in the PNS. A different model formulation, and a more sophisticated understanding of prion pathogenesis will be required before intracerebral blocking can be understood.

4. DISCUSSION

We have explored the consequences on disease latency of treating prions as mobile pathogens that propagate along a continuous path of PrP-expressing tissues from peripheral sites to the CNS. Our mathematical model clarifies the factors that influence the propagation of PrPSc and identifies those parameter groupings that are rate determining in the peripheral component of disease

latency. The important parameter groupings (ΔH_A and d_A) are in principle directly obtainable for any particular strain–host pair, given measurements of PrPC concentrations *in vivo*. With this information the model would allow us to specify the extent of PrPC downregulation required to prevent the peripheral phase of infection. Indirect deductions of wave speeds during strain competition are also possible by using equation (A3) of Appendix A. Moreover, if experiments were to be performed so as to produce detailed delay–latency curves for the ‘partial blocking’ scenario of strain competition, then other important information could become available, such as any spatial heterogeneity in PrPC expression, or the presence of an establishment phase for the travelling wave (both of which would be reflected by nonlinearity in the delay–latency curve).

Although the model is able to identify rate-limiting parameters in peripheral propagation, we are not suggesting that spatial bottlenecks are the sole rate-limiting step in prion pathogenesis, but merely that they are one important component thereof. Nowak *et al.* (1998) show how a nucleation-dependent polymerization mechanism (Bessen *et al.* 1995) might delay disease onset. Payne & Krakauer (1998) provide a mechanism for prion latency in a non-spatial model by examining bottlenecks in the intracellular processing of PrPC and PrPSc, and conclude that clinical onset could be therapeutically impeded, without risking loss of PrPC function, by preferential cleaving of PrPSc from the cell surface. The recent confirmation of an essential role for B cells in the initial phase of infection (Klein *et al.* 1997) suggests that a comprehensive model of latency will also need to consider this earliest of steps. It would appear that latency is a composite of many processes, each with different rate-limiting parameters, and hence that a complete description will require a careful consideration of all phases of disease.

Eigen (1996) has pointed out that, under the heterodimer model, individuals will either fail to manifest disease, or exponentially accumulate PrPSc with very little or no disease latency. The choice of outcome will depend on the relative magnitude of PrPSc decay and the conversion of PrPC into PrPSc. By introducing a spatial dimension into the model, we overcome the all-or-nothing dilemma identified by Eigen.

Thinking of the host protein as a limited spatial resource provides a simple explanation for prion strain competition. When two prion strains co-infect a host, the strain that can survive on a lower density of PrPC will outcompete the other; yet, if a slower-propagating strain is inoculated early enough, it can retard or block completely the progress of a more aggressive strain. This has parallels in the delayed onset seen in heterozygous +/– PrPC–null chimeras (Bueler *et al.* 1994), in which the ablation confers a naturally lower level of PrPC expression. Our model suggests that administration of any compound that specifically targeted reduction of PrPC concentration in the PNS, where it may have minimal natural functions, would be a more sophisticated way of preventing the propagation of a prion strain with known clinical effects.

This work was funded by the NERC (RJHP) and the Wellcome Trust (DCK).

APPENDIX A

The equations describing the dynamics of two pathogenic strains, $A(x,t)$ and $B(x,t)$, both able to transform the same host protein, $H(x,t)$, are

$$\begin{aligned}\partial H/\partial t &= D_H \partial^2 H/\partial x^2 + f - gH - (j_A A + j_B B)H, \\ \partial A/\partial t &= D_A \partial^2 A/\partial x^2 + j_A A H - m_A A, \\ \partial B/\partial t &= D_B \partial^2 B/\partial x^2 + j_B B H - m_B B.\end{aligned}$$

The domain is assumed to be homogeneous.

(a) Wave speeds

If strain A is inoculated first, the dynamics of the wavefront (where A is small) are described by the linear perturbation equation

$$\partial A/\partial t = D_A \partial^2 A/\partial x^2 + (j_A H_0 - m_A)A. \quad (\text{A1})$$

This is a ‘Skellam equation’: i.e. it has the form

$$\partial U/\partial t = D \partial^2 U/\partial x^2 + nU.$$

The solution of this equation converges asymptotically to a travelling wave with speed

$$C_U \geq 2\sqrt{nD}.$$

This inequality delimits the minimum wave speed. Both analytic and numerical studies of a number of scenarios for which the wavefront is well approximated by the Skellam equation have consistently shown the minimum speed, $2\sqrt{nD}$, to be the speed that becomes manifest. An up-to-date discussion of several aspects and applications of the Skellam equation in biological contexts, together with a proof of the wave speed formula, can be found in Shigesada & Kawasaki (1997). In the case of equation (A1) the wavefront is predicted to move with speed

$$C_0^A \approx 2\sqrt{d_A(H_0 - H_A)},$$

where $H_A = m_A/j_A$ is the equilibrium level of PrPC in the presence of strain A only (in absence of B) and $d_A = D_A j_A$.

Now suppose that strain A is only added after strain B has already been inoculated. Moreover, suppose that sufficient time has elapsed that the concentration of host protein behind the wavefront of B (but in front of A) has achieved equilibrium $H_B = m_B/j_B$. Thus when we examine the wavefront of A, moving into the region already occupied by B, the relevant perturbation equation is now

$$\partial A/\partial t = D_A \partial^2 A/\partial x^2 + (j_A H_B - m_A)A.$$

As before, this is a Skellam equation, and we can again write down the wave speed. In this case the predicted speed of the wavefront is

$$C_B^A \approx 2\sqrt{d_A(\Delta H_A - \Delta H_B)}.$$

(b) Latency times

Here we consider only the peripheral component of latency, that is, the time taken for a travelling wave of infection to spread from the site of inoculation to the CNS.

Let L be the distance from site of inoculation to the CNS, and s be the time-delay between injection of strain B and of strain A. Let T_0^A and T_B^A be the latency period of strain A alone, and in presence of strain B, respectively. If only strain A is present, the average wave speed is estimated by

$$C_0^A = L/T_0^A. \quad (A2)$$

Here the establishment phase of the waves is assumed to be brief relative to the overall incubation period, and the length of the wavefront to be short relative to the domain length.

When two strains are in competition, there are four categories of behaviour, as follows:

(a) $H_B < H_A$: strain B is a better competitor, so A can never invade.

(b) $H_A < H_B$; $C_B^A < C_0^B$: strain A invades, but is slower than strain B.

(c) $H_A < H_B$; $C_0^B < C_B^A$: strain A invades and is faster than strain B, but there is insufficient time for A to catch up B ($s + T_B^A < T_0^B$).

(d) As for (c), except with sufficient time for A to catch up B ($s + T_B^A > T_0^B$). In this final case, the total peripheral latency is the time taken for strain A to catch up strain B (speed C_B^A) plus the time to traverse the remainder of the domain (speed C_0^A). Suppose A passes B at time t^* and position l^* . Then the total incubation time is $T_B^A = t^* + (L - l^*)/C_0^A$, from which one can calculate

$$T_B^A = T_0^A + s \frac{(1 - C_B^A/C_0^A)}{(C_B^A/C_0^B - 1)}.$$

Substituting from (A2) and rearranging gives

$$C_B^A = L(s + T_B^A - T_0^A)/\{sT_0^A + T_0^B(T_B^A - T_0^A)\}. \quad (A3)$$

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