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REVIEW

Interplay between rabies virus and the mammalian immune system

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Abstract

Rabies is a disease caused following infection of the brain by the rabies virus (RABV). The principle mechanism of transmission is through a bite wound. The virus infects peripheral nerves and moves to the central nervous system (CNS). There appears to be little involvement of other organ systems and little detectable immune stimulation prior to infection of the CNS. This failure of the mammalian immune system to respond to rabies virus infection leads, in the overwhelming majority of cases, to death of the host. To some extent, this failure is likely due to the exclusive replication of RABV in neurons and the limited ability to generate, sufficiently rapidly, an anti-viral antibody response *in situ*. This is reflected in the ability of post-exposure vaccination, when given early after infection, to prevent disease. The lack of immune stimulation during RABV infection preceding neural invasion is the Achilles heel of the immune response. Whilst many viruses infect the brain, causing encephalitis and neuronal deficit, none are as consistently fatal to the host as RABV. This is in part due to prior replication of many viruses in peripheral, non-neural tissue by other viruses that allows timely activation of the immune response before the host is overwhelmed. Our current understanding of the correlates of protection for rabies suggests that it is the action of neutralising antibodies that prevent infection and control spread of RABV. Furthermore, it tells us that the induction of immunity can protect and understanding how and why this happens is critical to controlling infection. However, the paradigm of antibody development suggests that antigen presentation overwhelmingly occurs in lymphoid tissue (germinal and non-germinal centres) and these are external to the CNS. In addition, the blood-brain-barrier may provide a block to the delivery of immune effectors (antibodies/plasma B-cells) entering where they are needed. Alternatively, there may be insufficient antigen exposure after natural infection to mount an effective response or the virus actively suppresses immune function. To improve our ability to treat this fatal infection it is imperative to understand how immunity to RABV develops and functions so that parameters of protection

are better defined.

Key words: Rabies virus; Immune stimulation; Central nervous system; Vaccination; Blood-brain-barrier

Core tip: Rabies is a devastating disease in developing countries with a very high case-fatality rate. The delayed immune response to infection with rabies virus could be a defining factor in poor prognosis following infection. Understanding the reasons for this muted response and identifying ways to manipulate immune effectors may lead to new therapeutic approaches to the treatment of rabies. This article reviews the reasons for the apparent failure of the immune response and identifies areas for therapeutic development.

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INTRODUCTION

Rabies virus infection

Rabies has been with humanity since antiquity[1] and is caused by viruses of the *Lyssavirus* genus within the family *Rhabdoviridae*. This genus includes the rabies virus (RABV) and a number of related viruses that consist of an enveloped virus particle with a negative-stranded RNA genome approximately 11000 base pairs in length. The genome of the virus is remarkably simple, coding for only 5 proteins in the order nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G) and polymerase (L)[2]. All viruses within the genus have this basic genomic structure (Figure 1). Each protein is multifunctional and expression of these five proteins from the virus genome is sufficient to enable efficient replication of virus in susceptible cells.

The lyssaviruses are hypothesized to have evolved in bats, with RABV being present in many species of new world bats[3]. However, at some point, the virus made the jump from the order Chiroptera to Carnivora and became established in new reservoir species, and the translocation of the virus to regions where it is now endemic, mainly Africa and Asia[4]. The principal reservoir is the domestic dog. Indeed, virtually all human infections are due to dog bites[5]. Bat bites are also a source of infection but are responsible for a small number of cases. This means that control of rabies is technically simple, control dog rabies. This unfortunately is not the case in parts of the world where public health is under-resourced and uncoordinated. However, due to the relatively long incubation period between exposure to virus following a bite, and development of clinical disease, which is often measured in months, post-exposure vaccination, as pioneered over one hundred years ago by Louis Pasteur, is effective. Therefore, no one needs to die of rabies and yet they continue to do so in their thousands every year[6]. Only mammals appear to be infected naturally. This includes livestock that cause further threat to public health and economic liabilities for developing countries[7].

Once RABV infects peripheral nerves it proceeds rapidly by axonal transport to the spinal cord and then onwards to the brain. This is where virus replication accelerates, symptoms of infection develop and the host is almost certain to die. A number of survivors have been documented but in most cases there was evidence of prior immunization[8]. Survivors have also suffered persistent severe neurological sequelae following acute disease.

The reasons for the high mortality observed following RABV infection are unclear and almost unique for an infectious disease of mammals. It is still uncertain whether there is any replication before RABV infects the nervous system. There is some evidence for low-level replication in muscles, and the nerve-muscle junction is one possible route of entry to the peripheral nervous system[9]. There is also evidence that cells of the monocyte/macrophage lineage can be infected[10]. However, post-mortem investigation of the tissue distribution of RABV inevitably finds the virus exclusively associated with nervous tissue. This may have serious implications for generating protective immunity to RABV infection if replication only occurs in the central nervous system (CNS), with negligible extracellular antigen availability, there may be insufficient antigen to initiate T and B cell responses. If virus replication and amplification occurs exclusively in the CNS, the consequences for the host are profound.

Immune responses to RABV vaccination

What can we learn from the effectiveness of vaccination in controlling RABV infection? The earliest vaccines were crude nervous tissue preparations with virus inactivated by desiccation or subsequently, by chemical means. Current human vaccines are cell-culture expressed virus that is inactivated with beta-propiolactone (extensively reviewed by Wu *et al*[11]). The development of cell-culture vaccines was a critical step in eliminating myelin, present in all neuronal tissue, from human vaccines and reducing the risk of adverse autoimmune reactions from vaccination. A range of vaccination protocols are effective, with induction of neutralizing antibodies (VNAs) being considered the correlate of immunity whether the vaccine is given pre- or post-exposure[12,13]. In both cases, the mechanism of protection is the prevention of virus infecting neuronal cells by neutralising antibodies directed against the principal virus antigen, the glycoprotein. A number of studies have investigated the longevity of the VNA response following anti-rabies vaccination. This appears to be highly variable

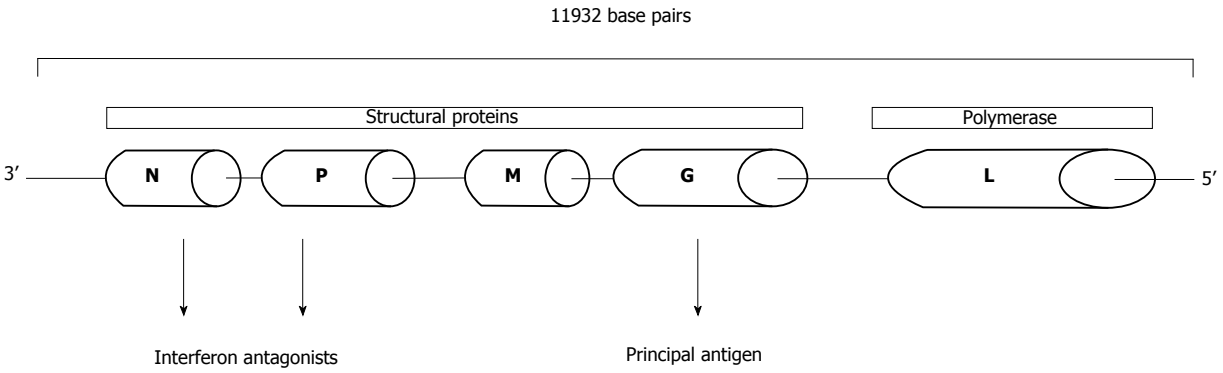

Figure 1 Schematic of the rabies virus genome. Coding regions are shown as cylinders and intergenic regions are shown as single lines. The genome consists of the nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G) and RNA dependent RNA polymerase (L). Attenuation of RABV has been achieved through the addition of point mutations within the G protein, specifically at amino acid position 333 in the SAG2 vaccine strain. Virus attenuation has also been achieved through inclusion of multiple copies of the G protein or by addition of mammalian immunity genes[41]. RABV: Rabies virus.

and with clear inter-individual dependence. However, serum levels of VNA generally decline over time and a booster vaccination is recommended to raise the level of VNA[14-16]. Perhaps because of its effectiveness over many decades, there has been little incentive to study the immune stimulation that follows inoculation with inactivated virus. A single study has demonstrated peak levels of blood IgG plasma cells 10 d following primary vaccination with inactivated RABV vaccine[17]. This appears earlier and to higher levels in those individuals who have previously been vaccinated, consistent with a classical T-dependent recall response. However, the long term fate of these plasma cells is not known. Some form of T-cell stimulation would be expected after vaccination although this has not been investigated. Natural killer cell responses have been reported[18] although their long-term persistence or role in preventing infection is unclear.

More recent work has focused on the immune response to the next generation of rabies vaccines. These are based on live-attenuated viruses that either lack one of the virus proteins or have an immune response gene inserted into the genome region between the glycoprotein and polymerase coding sequences (Figure 1). These studies are discussed later in this review.

Immunity in the central nervous system

Until recently the CNS was considered an immune-privileged site within the body[19]. This was primarily due to the absence of immune cells in the normal brain and the presence of the blood-brain-barrier (BBB). The BBB is an impediment to free movement of molecules, proteins and cells between the blood supply to the brain and spinal cord, and the structures of the CNS[20]. A key feature is the endothelial layer on the luminal side of the vasculature. This expresses low levels of adhesion molecules and there are tight junctions that form between endothelial cells to exclude movement of cells across the vessel wall. However, immune privilege does not mean an absence of immune surveillance. There appears to be little antigen presentation within the CNS itself and this appears to be limited to the microglial cell component that can present antigen following stimulation[21]. In addition, there is lymphatic drainage from the brain[22] that enables movement of soluble antigens to the deep cervical lymph nodes[23]. Activated $CD4^+$ T cells also appear to move between the brain and cervical lymph nodes[24]. Despite this limited immune footprint in the brain during the steady-state, diseases such as multiple sclerosis show that immune cells can, and do, invade the brain under certain circumstances.

The alternative focus in recent years has been the stimulation and action of the innate immune response within the infected brain. RABV, principally through a range of functions of the phosphoprotein, can prevent type 1 interferon activation[25-27], a property shared by all members of the genus[28]. More recent work has suggested that the nucleoprotein also inhibits type 1 interferons and that this function has been mapped to a specific region of the protein[29,30]. However, the mechanism through which this defined region of the nucleoprotein inhibits immune responses has not been identified. Interferon inhibition appears to be an early effect as numerous studies have shown substantial innate immune stimulation within the brain of an infected host during the clinical phase of infection. Stimulation of interferons[31], toll-like receptors[32,33] and chemokines[34-36] have all been reported but these may equally cause tissue damage in addition to restricting virus replication[37].

A key model for understanding the immune response to infection with RABV has been infection of mice with attenuated RABVs. Early studies used the attenuated strain CVS-F3 that has an arginine-to-glutamine substitution at position 333 within the glycoprotein gene[38]. Peripheral inoculation of mice with a virulent RABV leads to neuroinvasion and death within 10-20 d. Infection with CVS-F3 leads to transient weight loss and neuroinvasion. However, the virus is rapidly cleared and the mice survive. Mice that are unable to respond to interferon or produce T cells also survive infection with

CVS-F3. However, mice unable to generate antibody develop disease in a similar manner to infection with virulent virus, confirming the central role of antibody in controlling RABV infection[38]. Direct comparison of infection with isolates derived from a silver-haired bat, a representative virulent virus, and an attenuated virus (CVS B2C) demonstrate clear differences in the immune response to both viruses[39]. In the former, immune responses are muted and the outcome of infection is poor, whereas replication of the attenuated strain is extensive but controlled by the immune response. This may be important as the widespread nature of the attenuated virus infection may mean increased antigen availability resulting in an enhanced immune response to the virus.

Further studies have used attenuated viruses that have been derived through the ability to mutate the virus genome and rescue recombinant RABV[40]. Such viruses could form the basis of future vaccines for rabies (reviewed by Hicks *et al*[41]) and one variant has been trialled as an oral vaccine for wildlife[42]. The method of attenuation falls into two categories. The first are those constructs that have a gene deletion, usually the phosphoprotein[43] or matrix[44]. The second are those that have an immunity gene, for example a chemokine such as CCL3[45], inserted into an intergenic region. The investigation of immune responses to these viruses has resulted in important advances in understanding immune stimulation in response to infection[46]. Single-dose vaccination with a matrix-deficient recombinant RABV induces germinal centre-independent B-cell development and antibody secretion more rapidly than a preparation of inactivated RABV[44].

BARRIERS TO THE IMMUNE RESPONSE TO RABV IN THE CNS

A number of barriers need to be overcome in order for the immune response to react effectively to RABV infection in the CNS. This is summarised in Figure 2 and can be grouped into three categories.

Detection

The first is timely detection of infection. All evidence suggests that the first significant replication of RABV occurs in the CNS. Mammalian cells detect infection with negative stranded viruses through detection of non-self-pattern, structures associated with the virus or virus intermediates generated during the replicative process. This is mediated by proteins including retinoic acid-inducible gene Ⅰ (RIG-Ⅰ)-like receptors or melanoma differentiation associated gene 5 proteins[47]. In the case of RABV, RIG-Ⅰ detects the 5'-triphosphate RNA and leads to activation of interferon and interferon-inducible genes[48]. However, as discussed above, negative-stranded virus actively suppresses this response at the cellular level[49], and rabies is no exception. This early suppression of the interferon response could lead to a failure to fully orchestrate the immune response required to limit virus spread. In comparison with attenuated strains, the early chemokine response to virulent RABV appears suppressed, possibly as a result of inhibition of transcriptional activators[39]. This delay could have profound consequences for the host.

Temporal

A known, critical barrier to an effective response is time. This takes two forms: The time from virus detection to the influx of immune effectors into the CNS that can control infection and second, the time from infection to the generation of a high quality antibody response. Pathogens can induce characteristic responses, whereby they vary in the kinetics of induction of antibody responses and the squelae of their induction, such as the persistence of memory for example *Salmonella* infections and helminth infections in mice[50]. Typically, after primary viral infections, immunoglobulin M (IgM) appears in the first days post-infection, followed by a gradual rise in IgG titres in subsequent weeks and months[51]. This parallels the long-term dominance of antibody derived plasma cells selected through the germinal centre, compared to the extrafollicular response alone[52]. A key consideration is that antibody responses typically develop in secondary lymphoid tissues and although some long-term plasma cells can reside in sites such as the spleen, they more typically localise to the bone marrow. From these sites antibody can be readily released into the blood, but this does not necessarily mean the antibody can cross into the cerebrospinal fluid (CSF) or brain parenchyma as antibody is blocked by an intact BBB. Therefore measurement of serum antibody titres may not reflect titres in the CSF and furthermore it is unclear whether the IgG isotypes present in serum correlate with those in CSF. Close examination of the antibody response to neurotropic viruses reveals layers of complexity when making direct comparisons between serological and intrathecal responses[52]. Virus-specific antibody secreting cell (ASC) numbers can peak in peripheral lymphoid tissue prior to their accumulation in the CNS and following inoculation with high doses of RABV, a neutralising antibody response can be detected within 4-5 d (APHA, unpublished data). However, this is unrepresentative of a normal infection where virus appears to enter the CNS by stealth before replication and detection can occur. Such a dichotomy of responses to the same antigen raises a question as to whether this reflects impairment in the development of antibodies to viral antigens. Engagement of the B cell receptor is the initiating event for an antibody response and this requires exposed antigen. If the virus is restricted to an intracellular location during the early stages of infection then antigen presentation will be delayed. There is a clear precedent for a lack of antigen availability resulting in a failure to generate a productive antibody response. This is the observation that many non-vaccinated individuals infected with *Corynebacterium*

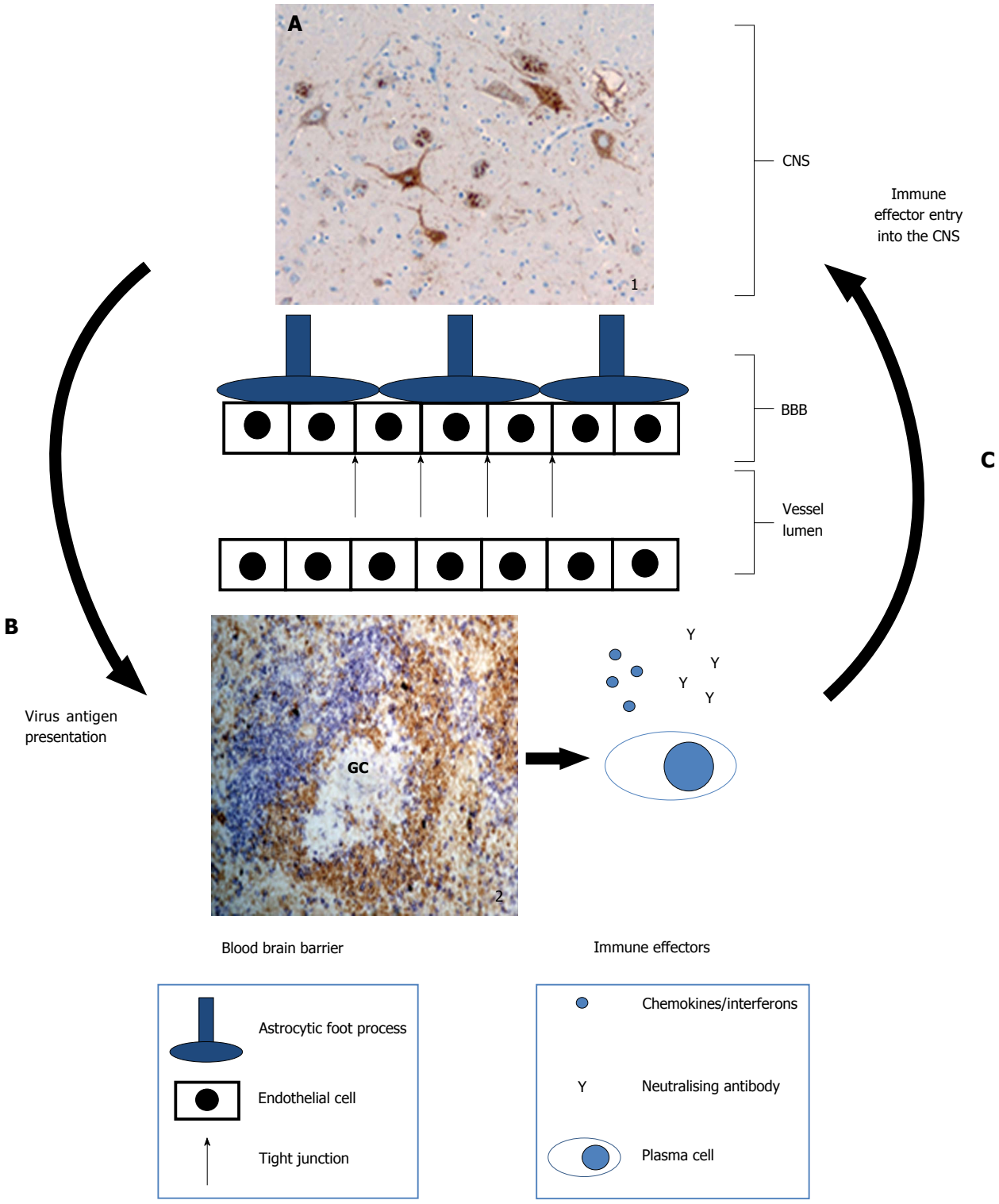

Figure 2 Model showing the movement of rabies virus antigen from the central nervous system to lymphoid tissue and then immune effectors (chemokines, antibodies and plasma cells) return to the central nervous system. Inset 1 shows a section of RABV infected brain stained with anti-nucleoprotein antibodies. Brown staining shows accumulation of nucleoprotein within neuronal cells. Inset 2 shows a murine spleen section stained with anti-CD3 for T cells (blue staining) and anti-IgD for naïve B cells (brown staining). The location of a GC is indicated. The arrows indicate the movement of antigen to lymphoid tissue and immune effectors returning to the CNS. The exact route of these is uncertain in the context of RABV infection. Letters signify areas for future research: A: Identification of therapeutics that restrict the replication and spread of RABV between neurons. Critically, such antiviral strategies must work within the CNS and without toxicity; B: Investigate the mechanisms by which RABV antigens reach antigen presenting cells either in situ or through exit of the CNS. Therapies that accelerate this process should assist the development of immune responses to infection; C: Development of methods that improves access of antivirals and immunes effectors (cytokines, antibodies and cells) into the CNS. RABV: Rabies virus; CNS: Central nervous system; GC: Germinal centre.

diphtheriae, the causative agent of diphtheria, fail to generate protection against reinfection. This is because the level of toxin required to induce disease is so low as to not consistently induce an antibody response. In

Table 1 United Kingdom cases of rabies 2001-2012

Yr	Virus	Source	Country of origin	Findings of tests to detect neutralizing antibody in cerebrospinal fluid	Ref.
2001	RABV	Dog	Nigeria	Not tested	[75]
2001	RABV	Dog	Philippines	None detected on hospital admission	[76]
2002	EBLV-2[1]	Bat	Britain	None detected in any sample submitted	[77]
2005	RABV	Dog	India	Not tested	[78]
2008	RABV	Dog	South Africa	None detected on hospital admission. Evidence for low levels after 5 d of hospitalization	[73]
2012	RABV	Dog	India	None detected in any sample submitted	[79]

[1]European bat lyssavirus 2. RABV: Rabies virus.

this instance, the extracellular toxin is produced by an extracellular organism and acts through binding of the host cell surface and yet even under such circumstances it is still not sufficient to elicit protective B cell responses. The limited antibody response observed early after RABV contrast with events at later times after infection. Then, a combination of increased antigen availability due to a higher viral load, host cell death and pathology resulting in BBB perturbation can result in measurable antibody responses. This is observed in the late stages of infection in experimental models where evidence for lymphocyte infiltration and antibody in the CSF is clear. The proportion of infiltrating cells that are B-cells is low, often approximately 1% of labelled lymphocytes[53], and these may have effector functions other than antibody secretion. It is not clear where at these later time points B cells and antibody is derived from and this may be important in understanding how best to accelerate the induction of protective responses after infection with RABV.

Physical

Finally there is the barrier presented by the BBB itself. The BBB is the interface between the CNS and the blood supply. It is composed of both physical and physiological barriers that control entry of cells, proteins and large molecular weight molecules into the CNS (described in more detail below). Studies by Hooper *et al*[54] identified opening of the BBB as a critical factor in enabling access of ASCs that might contribute to control of RABV infection. They further deduced that opening of the BBB could lead to successful resolution of infection with a virulent RABV[55]. This provides a new avenue in therapy for RABV for both immune and antiviral compounds that could lead to successful treatment for infection.

INTERVENTION IN HUMAN RABIES

Treatment of human rabies is confounded by a range of factors. Firstly, the majority of cases occur in developing countries that have a poor health infrastructure, low ratios of doctors to the population, little access to pharmacological agents or the ability to deliver intensive medical care[8,56]. This means that prevention of rabies in reservoir populations, particularly the domestic dog, is both a cost-efficient and effective approach to reducing human rabies[57]. A second problem, even encountered in developed countries, is that once a case of rabies has been confirmed, the patient is in an advanced stage of disease. If contact with a rabid animal is not recognised or appropriate post-exposure prophylaxis is not sought, the first indications of neurological disease are usually a sign that the virus has reached the brain. This makes treatment of rabies extremely challenging and identifying potential therapies impossible. Although the Milwaukee protocol or therapeutic coma[58] has been hailed as a possible breakthrough, its successes have been sporadic and has been criticised as it lacks a firm mechanistic basis[8]. Here again, the lack of an experimental model prevents progress in treatment development. Notwithstanding these problems, based on what is known it is possible to speculate on what a potential treatment regimen might include. It will not be simple or based on a single drug.

The role of anti-rabies antibodies in cerebrospinal fluid

The above points highlight the complexity of understanding and interpreting the immune response to rabies as there are multiple variables such as the involvement of multiple anatomical sites and differing levels of viral burden at distinct times during infection. One potential measure of immune response within the CNS is the presence of antibody in CSF. Here the picture is mixed. Some authors report that the presence of immune complexes in the CSF is a valid ante-mortem diagnostic marker of infection[59]. The experience of patients admitted in the United Kingdom with rabies, whilst modest, suggests that CSF antibody is detected at a relatively late stage in the course of infection (Table 1) and all of these patients died as a result of infection. One striking feature of the first survivor to receive therapeutic coma as a treatment for rabies infection was the high levels of neutralising antibody, both IgM and IgG, in both serum and CSF[60].

More recent evidence from animal models of infection suggest that the early appearance of antibody in the CSF is a good indicator of survival[61,62] although these experiments are problematic as intermediate events such as neuroinvasion are not tightly controlled nor is the point at which antibody is detected in the CSF. Also the source of this antibody is not known. Is it produced *in situ* or in the periphery and if this is the case, is it accessing the parenchyma of the CNS where it is needed

to neutralise virus or is it restricted to the CSF? However, the presumption is that controlled opening of the BBB combined with an early antibody response are critical steps required to control infection with RABV. When this does not happen, as in the case of the vast majority of infections, the outcome is death of the host.

Perturbations of the BBB and access of immune mediators and antiviral drugs to the site of infection

The BBB forms the interface between the circulation and the brain parenchyma and is critical to maintaining stable homeostatic control within the CNS. It is formed by endothelial cells on the vasculature of the brain, which are in turn surrounded by astrocyte end-feet[63]. Neuronal cells are also an integral component of the structure. Tight junctions form between endothelial cells that exclude large molecular weight molecules, proteins and cells. In addition, transporter proteins actively reduce molecule concentrations in the brain interstitial spaces. Attempts to overcome the BBB have been extensively studied for the delivery of anti-tumour therapies[64,65]. A range of methods have been developed to open tight junctions ranging from drug mediated effectors to osmotic disruption[66]. Many of these approaches have been used in human medicine and so could be adapted to therapy of acute viral infection.

An antiviral that would stop virus replication and spread within the CNS is the Holy Grail for the effective treatment of clinical rabies. Numerous candidates have been proposed as potential anti-rabies therapeutics (extensively reviewed by Dacheux *et al*[67]). In virtually all cases, promising results in tissue culture systems are not matched by findings in animal models. Partly, this may be due to the difficulties of accessing the CNS and would be alleviated by administration in a coordinated way with BBB opening compounds. However, successful demonstration of this within an appropriate model is challenging.

Immune stimulation

As discussed above, the adaptive immune response to infection appears delayed. Stimulation of immune responses may assist the patient, particularly in clearing the virus from the CNS where antibodies are a key component[38]. Here, live attenuated vaccines may have a role to play in the stimulation of antibody responses. A matrix gene-deleted RABV construct inoculated into mice efficiently stimulated antibody production within 7 d[68]. If this can be accelerated further it may support the patient's ongoing adaptive responses. The major difficulty in achieving this is that RABV-specific B cells, as found for other antigens, will only be present in naïve subjects at a low frequency. This suggests that to achieve an accelerated enhancement of antibody levels a range of methods might be needed including vaccination, passive transfer of antibody or by immunizing with high copy number protective epitopes to engage as many specific B cells as rapidly as possible. An additional unknown is the contribution CD8 T cells make to protection. With the prolonged nature of the infection and its near 100% fatality rate it suggests that they contribute little, and of course adaptive immunity can always exacerbate infection. It is possible that CD8 T cells cannot access the brain parenchyma because of the BBB, which is a key stumbling block to all interventions. Both T and B cells destined for entry into the CNS need to express a range of surface molecules that allow adhesion to the brain vascular endothelium and promote transit across the physical structures of the BBB[69,70]. It is also clear that CD8 T cell responses generated after vaccination in the absence of B cells are not protective in experimental models of infection[38]. However, T cells are the major lymphocyte population forming perivascular cuffs and accessing the brain parenchyma during the late stages of infection with RABV[53]. The role of these cells, either positive or negative, in response to infection should be defined.

Neuroprotection

When patients come to the attention of clinicians, the disease is already well advanced in the CNS. Some neuronal deficit would be expected even if treatment was applied early and was effective. One of the unexpected benefits of the therapeutic coma approach has been an increase in understanding of the metabolic effects of rabies virus infection on the human brain[71]. Using this knowledge it may be possible to develop neurosupportive therapeutic strategies to assist in recovery from infection. Here again, recombinant rabies viruses could play a role in delivering neurotrophins, proteins that act as survival and growth factors for nerves, into the CNS[72].

A major practical consideration is how to monitor patient progress through the treatment period. In past studies where therapeutic coma has been used there have been few indicators of patient progress. Serum levels of neutralising antibodies have been used although this can be a slow, crude measure of the patient's response to infection[73]. Potentially, development of virus-specific antibody in the CSF may provide a more appropriate measure although this would be a harder sample to take at regular intervals. Such a measure might also be an indicator of when treatment has failed.

CONCLUSION

Neutralising antibodies are the main correlate of protection for vaccination against rabies[38] and it is likely that they could play a role in limiting the spread of RABV within the CNS. The absence of antibody in patient serum or CSF coincident with disease onset suggests that there is a delay in development of B cell responses as a result of poor antigen availability. This in turn implies that RABV replicates exclusively in the CNS and that seropositive animals without disease occur either because there has been exposure to antigen in the absence of replication, for example aerosol exposure in bats[74], or RABV replication in the periphery that has

stimulated antibody and then controlled infection. In the latter case such individuals, either animal or human, do not come to the attention of veterinarians or public health professionals. Even when immune effectors are stimulated, limited accessibility of antibody and lymphocytes to the CNS appears to nullify the ability of the adaptive immune response to control rabies. Further investigation is needed to identify ways of accelerating patient immune response and enabling its entry into the CNS. This first requires a suitable model. Most studies use rodents, particularly mice, as they are convenient, but infection with RABV in this model can be unpredictable, result in a short incubation period (< 5 d) and follow a rapid disease course (1-2 d). Rodents are also unsuitable for use in assessing human therapies. Alternative models for rabies pathogenesis and vaccine response include ferrets[80] and non-human primates[81,82]. These are also problematic for similar reasons to those seen in rodents and are considerably more costly. Until a suitable model can be resolved, progress in understanding the complex processes associated with disease pathogenesis and immune response will be slow.

It is well over one hundred years since Louis Pasteur pioneered therapeutic vaccination for rabies. Despite this success, or possibly because of it, it is still not clear why rabies is overwhelmingly fatal to all mammals and continues to affect thousands of people every year[83]. To date no antiviral or therapeutic approach has been identified that improves outcome[8]. This suggests that a range of measures will be needed to treat infected patients and that a single mode of intervention will be insufficient. Advances in the development of research tools to investigate the disease process such as recombinant rabies viruses and other viruses expressing RABV proteins[84] should identify ways to rationally target inhibition of virus replication, stimulate the immune response and provide supportive treatment that will enhance delivery of such inhibitors.

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