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Antibody-mediated clearance of viruses from the mammalian central nervous system

Bernhard Dietzschold

The novel role of antibody in clearing virus from the central nervous system without the help of other immune effectors is an important phenomenon that has only recently been documented. Possible routes for antibodies across the blood–brain barrier and how they work in the CNS are discussed here.

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The clearance of viruses from infected tissues is thought to depend on several nonspecific and specific immune defenses. In the case of virus infections of the central nervous system (CNS), these defense mechanisms are severely restricted by the immunological privilege of the CNS. The existence of the blood–brain barrier and the lack of essential elements in the CNS that are necessary to produce an effective immune response have important consequences for the immune surveillance of the CNS[1]. In general it is believed that T cell responses are more important than antibodies in clearance of viruses from the CNS[2]. In particular, $CD8^+$ cytotoxic T cells have been shown to be effective in reducing virus titers in the brain after experimental infection with coronavirus, Theiler's virus or lymphocytic choriomeningitis virus (LCMV)[1,3,4].

However, antibodies have also been implicated as major effectors in the control of viral infection of the CNS. For example, antibodies play a major role in the recovery from lethal infection with Theiler's virus and it has been suggested that the antibodies limit viral spread within the CNS by neutralizing extracellular virus[1,4]. Recently a novel function for antibodies in protection against viral CNS infection has been discovered. Two studies have reported that antibodies can mediate complete clearance of virus from the CNS by a mechanism distinct from antibody-dependent cell-mediated cytotoxicity or complement-dependent lysis[5,6]. These findings indicate the great potential of antiviral antibodies as effective therapeutics against viral infections of the CNS. Here, I summarize current information on antibody-mediated viral clearance from the CNS, and discuss parameters that might be involved in the clearance process.

Protection of the CNS against viruses

In recent years the lack of a correlation between an antibody's neutralizing activity *in vitro* and its protective activity *in vivo* has been revealed in many viral diseases[5,7,8]. Furthermore, the ability of an antibody to protect *in vivo* cannot be uniformly related to a particular immunoglobulin class[5,6]. For example, the protective activity of a specific antibody in LCMV infection appears to be related to

Table 1. Isotypes of virus-neutralizing mAbs that are implicated in clearing virus from the CNS

Virus infection	mAb	Ig type	Protection	Viral clearance
Rabies[6]	1112-1	G1	Yes	Yes
	523-11	G2b	Yes	Not known
	509-6	G2a	Yes	Not known
Sindbis[5]	50	G2a	Yes	Yes
	R6	G2a	No	Yes
	209	G3	Yes	Yes
LCMV[9]	2.11.10	G2a	Yes	Not known
	67.2	G2a	Yes	Not known
	258.2.11	G2a	Yes	Not known

the IgG2a subclass, whereas in other neurotropic virus infections such as rabies and Sindbis the protective activity is not related to a specific antibody subclass (Table 1).

We have identified an IgG1 antibody (mAb 1112-1) that is protective and able to clear rabies virus from the CNS (Fig. 1). At 24 h after infection, viral RNA was detected in the brain of all five rats tested (Fig. 1a), whereas at 24 h or 96 h after mAb treatment (Fig. 1b and 1c, respectively), the brains of four out of five rats showed no evidence of viral RNA. Moreover, post-exposure treatment with this antibody protects 80–100% of the animals against lethal rabies virus infection, and no viral RNA was detected in the brain of animals that had survived such infection[6].

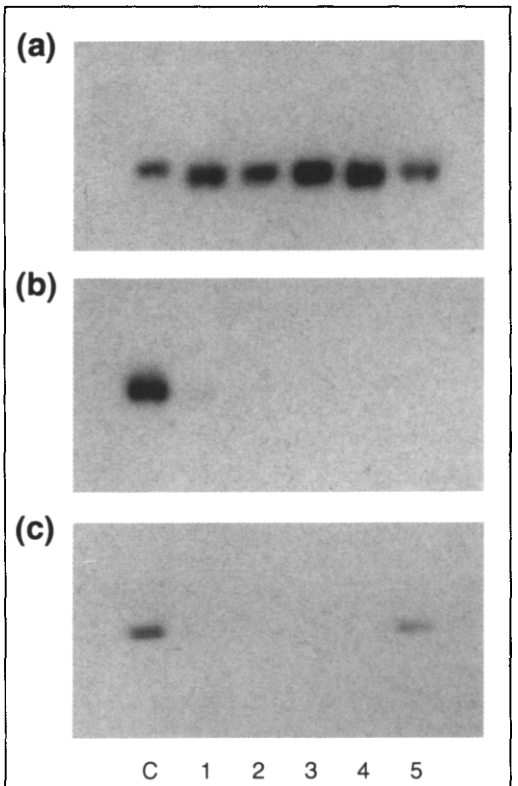

Fig. 1. Clearance of rabies virus RNA from the brain. Three groups of five rats were infected intranasally with rabies virus. After 24 h RNA was isolated from the brains of one group of rats **(a)**, and rats in the other two groups were treated intramuscularly with mAb 1112-1; brain RNA from the latter groups was isolated 24 h **(b)** or 96 h **(c)** after mAb treatment. Rabies N protein mRNA was amplified by RT-PCR (reverse transcriptase polymerase chain reaction). The control lane (C) shows PCR-amplified rabies N cDNA. (Unpublished data of B. Dietzschold.)

The protective role of antibodies has also been demonstrated in a SCID (severe combined immunodeficiency disease) mouse model of persistent Sindbis virus (SV) encephalomyelitis: here, adoptive transfer of a hyperimmune serum or certain mAbs resulted in clearance of the virus from the brain and spinal cord within 48 h after intraperitoneal administration[5]. In addition, the number of cells expressing viral RNA was dramatically reduced within 6 d after antibody transfer. Based on the lack of both a specific role for T cells in virus clearance and evidence for an antibody-dependent cytolytic mechanism in this particular animal model, the authors concluded that anti-SV antibody directly inhibits viral replication[5].

In another study, Wright and Buchmeier[8] showed that the treatment of LCMV-infected mice with mAb at 1 or 2 d after infection resulted in suppression of virus replication and significant protection. These investigators also concluded that antibody can prevent disease by limiting the extent of viral replication in the CNS[8].

The protective effect of antibodies has also been demonstrated *in vitro*. In SV-infected neuron cultures treated for 2 d with mAbs that mediated viral clearance *in vivo*, production of infectious virus ceased, even after the antibody was removed from the culture medium[5]. In contrast, neuroblastoma cells infected with rabies virus and treated with mAbs that were protective *in vivo* did not block viral replication completely, although these mAbs did inhibit virus spread from cell to cell. While most of the virus-neutralizing mAbs inhibited virus spread only at a relatively high virus-neutralizing activity (2 or 10 IU ml^{-1}), inhibition of virus spread by mAb 1112-1 was observed even at a concentration of 0.08 IU ml^{-1}. Thus, the ability of a mAb to inhibit virus spread *in vitro* appears to correlate with its protective activity *in vivo*[6].

Possible mechanisms

It appears that cell-mediated effector mechanisms are not critically involved in antibody-mediated virus clearance from the CNS. Levine *et al.*[5] have demonstrated that suppression of natural killer cell functions or complement depletion in SCID mice persistently infected with SV has no effect on the ability of antibodies to clear virus from the CNS.

The lack of a correlation between the classical function of antibodies – that is, virus-neutralizing activity or complement-dependent lysis – and the ability of antibodies to clear virus from the CNS suggests the involvement of a different effector mechanism in the antibody-mediated clearance process. It has been concluded that antibodies can mediate viral clearance by restricting viral gene expression, since termination of the production of infectious virions in antibody-treated neurons was preceded by a decrease in viral protein synthesis[5]. Similarly, treatment of rabies-virus-infected neuroblastoma cells with antibodies resulted in a marked inhibition of

rabies virus RNA transcription[6]. However, of the five mAbs used, only mAb 1112-1 markedly inhibited rabies virus RNA transcription, suggesting that the high protective activity of this mAb is related to its ability to restrict rabies virus gene expression. In the same context, Schneider-Schaulies *et al.*[10] recently reported that antibody treatment of cells of neuronal origin persistently infected with measles virus leads to a significant reduction of measles-virus-specific transcripts within 24 h.

The mechanism by which antibodies affect virus transcription remains largely unresolved. It has been suggested that viral proteins expressed on the infected cell surface might function as signal-transducing receptors for an external signal provided by the antibody[10]. This hypothesis is supported by the demonstration that measles-virus-specific antibodies specifically induce an increase in inositoltriphosphate, which can act as a second messenger in the activation of protein kinase C in cells infected with subacute sclerosing panencephalitis virus (SSPE)[11]. Alternatively, it is possible that the antibody exerts its inhibitory activity on virus transcription after it is taken up by the infected cell. We have recently demonstrated the efficient internalization and localization in cellular vesicles, and in the cytoplasm, of a mAb that markedly inhibits rabies virus RNA transcription[6]. However, it remains unclear how the antibody acts to inhibit virus transcription within the cell.

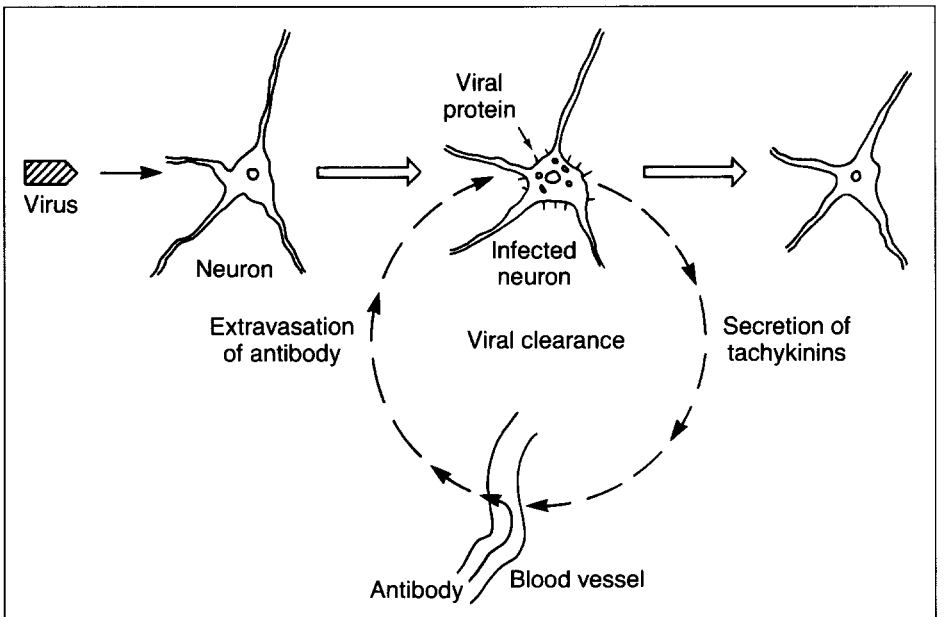

Fig. 2. Model of tachykinin involvement in the antibody-mediated clearance of virus from the CNS.

Crossing the blood–brain barrier

Exactly how antibody can pass the blood–brain barrier is still a puzzling question. Although a specific saturable transfer mechanism for IgG at this barrier has been demonstrated[12], such a transport system is saturated at normal physiological IgG levels. Thus it seems very unlikely that sufficient quantities of administered antibody can infiltrate the brain parenchyma. However, in the virus-infected brain, the endothelial barriers that normally restrict extravasation of blood-borne proteins may become susceptible to endothelial cellular passage[13]. During inflammatory processes in the CNS, soluble factors such as histamine and cytokines are produced that can cause 'leakiness' of the capillary endothelium, thus providing the route for antibody passage. Indeed, breakdown of the blood–brain barrier occurs in the course of several viral CNS infections[14], although predominantly in the terminal stages of the disease when neuronal damage is extensive and antibody treatment is not effective.

In the case of persistently SV-infected SCID mice or in the early stages of rabies, inflammatory processes in the CNS are either absent or minimal. Since antibody-mediated clearance can take place in the absence of inflammation, a different mechanism for extravasation of antibody into the brain parenchyma must exist. It is possible that neurons and probably also microglial cells respond to virus infection in a unique fashion. Virus infection may trigger a complex cascade of events in these cells, such as activation of immediate early gene expression followed by stimulation of late genes, which might result in the production of a variety of neurotransmitters and neuropeptides. The release of neurotransmitters from neurons can induce vasodilation and plasma extravasation (Fig. 2). In particular, the tachykinins substance P (SP) and neurokinin A are potent inducers of plasma extravasation at physiological concentrations[15]. SP-induced extravasation has not yet been demonstrated for the CNS, although this mechanism operates in the periphery[16]. The observation that SP gene expression is increased in the brain during the early phases of rabies virus infection (B. Dietzschold, unpublished) supports the hypothesis that activation of neuronal function in early stages of virus infection is an essential factor in the antibody-mediated virus clearance.

Conclusion

The data reviewed here demonstrate that certain virus-neutralizing antibodies can completely clear virus from the infected CNS without the help of other immune effectors. This antibody-mediated viral clearance appears to be facilitated by a novel mechanism that is distinct from the classical viral clearance processes. However, the molecular events in this clearance process remain to be elucidated. In addition, there is still no explanation for the observation that only a restricted number of antibodies are capable of clearing virus. Future research efforts focused on resolving these questions and

determining how virus infection influences neuronal functions should lead to more rational development and selection of antibodies for the treatment of viral CNS infections.

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Iron acquisition in microbial pathogenesis

Shelley M. Payne

Successful competition for iron by potential pathogens is essential to establish infection. The roles of the various types of microbial iron acquisition systems in host–pathogen interactions depend on the nature of the infection and the location of the pathogen within the host. Microbes infecting the extracellular spaces of the host employ different strategies for iron acquisition than those that invade and multiply within host cells.

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Iron is an essential element, serving as a transporter of oxygen or as a catalyst in electron transport processes, and most microorganisms have specific transport systems for acquisition of this element. Because pathogens typically encounter an extremely iron-limiting environment when attempting to colonize or invade mammalian hosts, the role of iron in microbial infections has been the subject of numerous investigations[1]. Although there is a substantial amount of this element present in the host, most of it is complexed and there is little or no free iron to support microbial growth. The iron is predominantly intracellular, occurring as heme, iron–sulfur proteins or ferritin, with smaller quantities in other iron proteins. The limited amounts of extracellular iron are tightly held by proteins such as transferrin in the serum and lactoferrin on body surfaces; the pools of these proteins are normally only partially saturated with iron.

There is considerable evidence that modulation of the levels of iron in the host influences the establishment and extent of microbial infection. It has been observed in experimental models and in humans with abnormalities of iron metabolism that the incidence of fungal and bacterial infections increases under conditions of iron overload. Similarly, reduced iron levels are associated with enhanced resistance to infection.

Microbial iron transport systems

The ability of microorganisms to compete successfully with the host for iron is an important determinant of their virulence. A variety of iron acquisition systems that may function within the host have been identified, and there is now evidence to show that some of the components are virulence factors. One mechanism of microbial iron acquisition *in vivo* is the direct utilization of host iron sources. Although transferrin and lactoferrin withhold iron from many microbes and inhibit their growth, some pathogens have been found to bind transferrin and/or lactoferrin and to remove and utilize the iron associated with these proteins. They include the pathogenic *Neisseria*[2], *Hemophilus influenzae*[3], and the flagellated protozoan *Trichomonas vaginalis*[4]. The *Neisseria gonorrhoeae* transferrin receptor has been cloned and characterized[2]. Its amino acid sequence is similar to that of other bacterial iron transport receptors.

Another potential iron source for pathogens in the host is heme. The ability to use heme, even complexed to hemoglobin, is characteristic of many pathogens such as *Hemophilus*[5] and *Vibrio cholerae*[6]. When starved for iron, *V.*