Vaccination for Disease

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Recombinant virus vaccines that express a limited number of epitopes are currently being developed to prevent disease by changing the relative balance between viral spread and the immune response. Some circumstances, however, were found in infections with a noncytopathic virus in which vaccination caused disease; sensitive parameters included the genetic background of the host, the time or dose of infection, and the constituents of the vaccine. Thus, immunopathologic damage by T cells may be an unwanted consequence of vaccination with the new types of peptide or recombinant vaccines that are being investigated for the human immunodeficiency viruses and other pathogens.

EFFECTIVE VACCINATION PROTECTS A host from viral infections by initiating the appropriate cellular or humoral response (*1*). In infections involving cytopathic viruses, immunological protection is equivalent to the early containment of viral spread. In infections by noncytopathogenic viruses, the host cell is not destroyed directly by the virus, but rather by the immune response. Therefore, the damage done by T cells after infection with hepatitis B virus in man (*2*), or lymphocytic choriomeningitis virus (LCMV) in mice (*3*), is determined by the kinetics of both viral spread and the T cell immune response. Accordingly, mice infected intracerebrally (i.c.) with low doses [10^2 plaque-forming units (pfu)] of LCMV usually develop a fatal T cell–mediated choriomeningitis (*3, 4*) that is dependent upon induction of virus-specific cytotoxic T cells (*4*). T cell–deficient mice do not get the disease, which can be adoptively transferred by cloned cytotoxic T cells (*5*). There is no evidence for the involvement of antibodies in this immunopathologically induced LCM. Mice infected with high doses (10^6 pfu) of the LCMV isolate WE (LCMV WE) or low and high doses of LCMV DOCILE (*3, 6*), however, do not develop lethal T cell–mediated disease and survive. This particular virus-host relationship is not unique to LCMV but may also apply to hepatitis B virus and human immunodeficiency viruses (HIVs) (*2, 7*). It is unclear whether LCMV suppresses the immune response directly or via immunopathological mechanisms (*8*), by recruitment of cells to sites other than the meninges (*3, 6*), or by the rapid selection of virus variants (*6, 9*).

We evaluated the capacity of recombinant vaccinia viruses (*10*) that express either LCMV-glycoprotein (GP) (vacc-GP), or LCMV-nucleoprotein (NP) (vacc-NP) to protect high-, low- and non-responder mice from LCM disease. The generation of cytotoxic T lymphocytes specific for LCMV-GP or LCMV-NP is dependent on the mouse H-2 haplotype (*11*). Immunization of various mouse strains with either vacc-GP or vacc-NP usually protected them from low dose i.c. infection in an H-2–dependent manner (Fig. 1, left panel). H-2^d mice (BALB/c) responded to LCMV predominantly with cytotoxic T lymphocytes specific for L^d and NP. Therefore, BALB/c mice immunized with vacc-NP or with LCMV were protected from low doses (10^2 pfu) of LCMV WE, whereas unprimed or vaccinia virus–primed mice and vacc-GP–vaccinated mice died of LCM. BALB/c mice immunized with vacc-GP and then infected i.c. with LCMV WE (10^2 pfu) on the average

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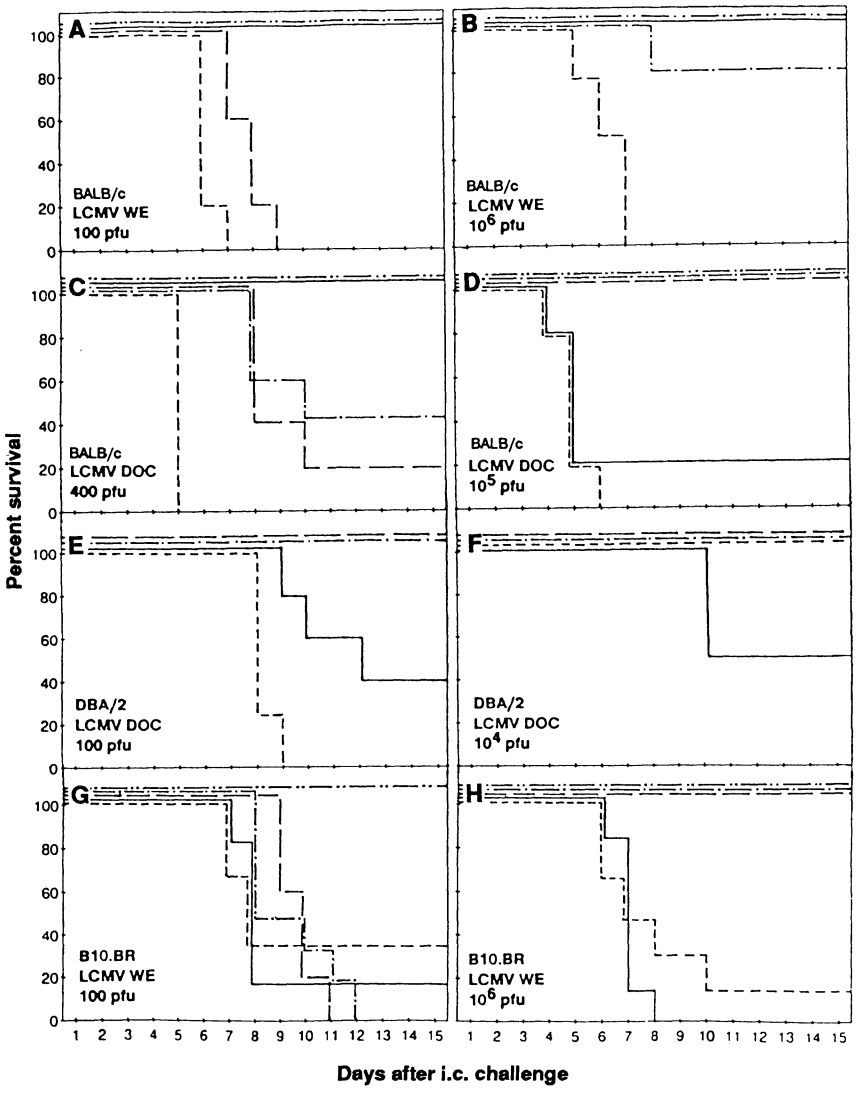

Fig. 1. Parameters influencing degree of infection or increase of susceptibility to LCM by previous vaccination: Dependence upon immunizing LCMV-antigen, H-2 haplotype, and dose and strain of challenging i.c. LCMV. Five to six BALB/c (H-2^d) mice (**A** to **D**), DBA/2 (H-2^d) mice (**E** to **F**), and B10.BR (H-2^k) mice (**G** to **H**) per group were intravenously infected with recombinant vaccinia virus (*10, 11*) (2×10^6 pfu) expressing either LCMV-GP (- - -), LCMV-NP (——) or with wild-type vaccinia virus (negative controls, —— ——); other mice were infected with LCMV WE (100 pfu, —·—·) as positive controls or were left unprimed (—·—). Three weeks later the mice were infected i.c. with the LCMV isolates as indicated (low doses, left; high doses, right). The time to death caused by LCM was registered over a time period of 25 days; survival on day 25 was identical to that shown on day 15. All experimental mice have been kept in accordance to institutional guidelines.

died 1 day earlier than vaccinia–primed control mice; thus, a low level immunization may accelerate development of disease.

Uninfected BALB/c mice or those immunized with vaccinia virus survived a high i.c. dose of LCMV-WE, because of LCMV-induced immune suppression (Fig. 1B). Mice primed with vacc-NP or LCMV were protected and survived, but paradoxically mice primed with vacc-GP died of LCM (Fig. 1B). Comparable results were obtained with B10.BR (H-2^k) mice (Fig. 1, G and H). H-2^k mice respond efficiently to LCMV antigens other than LCMV-GP or LCMV-NP (*11, 12*). Consequently, immunization with vacc-GP and vacc-NP did not protect B10.BR mice from low i.c. doses of LCMV WE (Fig. 1G). Again paradoxical effects of vaccination were observed in H-2^k mice: B10.BR mice immunized with wild-type vaccinia virus or unimmunized survived a high i.c. dose of LCMV; however, if the mice had been immunized with vacc-GP or vacc-NP they died of acute LCM (Fig. 1H). As seen before, LCMV-immunized mice survived because of effective immune protection.

The host-virus relationship varied not only with the mouse major histocompatibility complex (MHC; H-2) but also with growth kinetics of the LCMV strain used. LCMV DOCILE is a variant of LCMV WE that replicates more rapidly in mice than LCMV WE (*6, 13*). BALB/c mice infected with a low dose (400 pfu) of LCMV DOCILE were protected when immunized with vacc-NP, but the time to death was accelerated by 3 to 4 days when mice were immunized with vacc-GP (Fig. 1C). All BALB/c mice infected with a high dose of LCMV DOCILE (10^5 pfu) survived (Fig. 1D), except for those vaccinated either with vacc-GP or with vacc-NP; these mice again died relatively early, around day 5. This contrasts with the results in vacc-NP–immunized BALB/c mice that survived infections with high LCMV WE doses (Fig. 1B). Thus a high dose of rapidly replicating LCMV DOCILE virus spreads widely in the brain before the moderately efficient vacc-NP–primed T cell response is fully developed and therefore further viral spread and lethal immunopathology cannot be prevented. The LCMV WE isolate, however, does not spread as quickly, giving the immune response of vacc-NP–immunized BALB/c mice enough time to develop before too

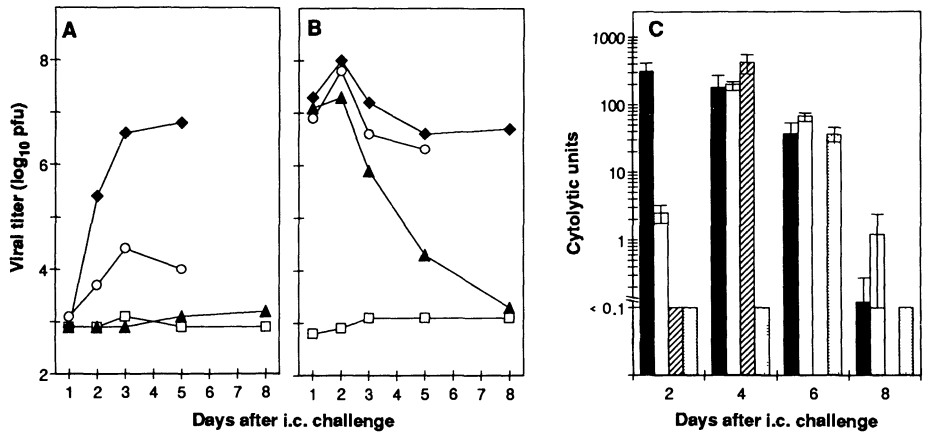

Fig. 2. Antiviral response in spleens and brains of BALB/c mice as measured by virus titers (**A** and **B**) and LCMV-specific cytotoxic T cells (**C**). Results represent viral titers per gram of spleen of four mice per group (means, SEM were below 0.3 log_{10} for all results shown) intravenously immunized with recombinant vaccinia virus (2 × 10^6 pfu) expressing either LCMV-NP (▲), LCMV-GP (○), LCMV WE (100 pfu, □), or wild-type vaccinia virus (◆). Twenty days later all mice were infected i.c. with 10^2 pfu (A) or 10^6 pfu of LCMV WE (B). The spleens were removed on the days indicated, homogenized, aliquoted, and frozen at −70°C. After thawing the homogenates were centrifuged and the supernatants were tested for plaque-forming units on L929 fibroblast cells (*25*). Because of deaths by LCM in some groups, the day 8 virus titers could only be determined in the remaining groups. All values <3.2 log_{10} are maximal estimates, because most of the individual values were below the detection level of 2.8 log_{10} pfu per gram of spleen. (C) LCMV-specific cytotoxic T cell response measured as cytolytic units (*26*) per milliliter of cerebrospinal fluid (CSF) of mice immunized as described above with LCMV-NP (white columns), LCMV-GP (hatched), LCMV WE (black), or wild-type vaccinia (dots) and infected i.c. with a high dose of LCMV WE (10^6 pfu). Four mice per group were anaesthetized and exsanguinated by perfusion with a balanced salt solution containing heparin (1 U/ml), then the CSF was collected. The brains were removed and washed and the harvested lymphocytes were tested in a ^{51}Cr release assay on LCMV WE infected target cells for 4.5 hours (*11, 27*). The spontaneous release was <18%. Lytic units (LU) were calculated as the numbers of lymphocytes causing 30% specific lysis during the standard test of 4.5 hours; values represent means ± SEM of three to four individual determinations.

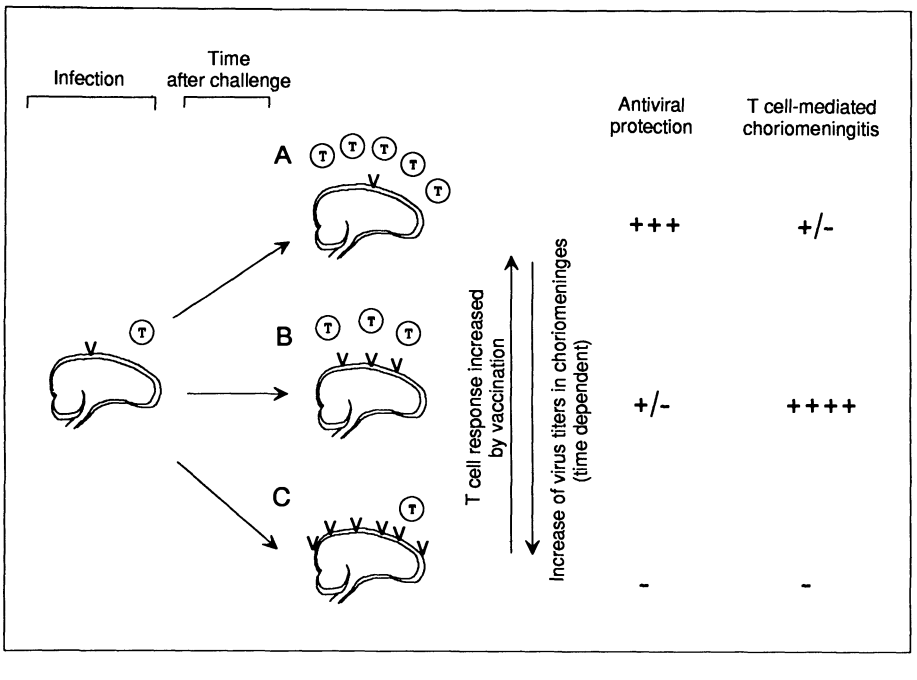

Fig. 3. Model to explain experimental results. After i.c. infection, virus (V) spread and T cell (T) immune response may end up in three possible states, A to C (with all intermediate states possible). (**A**) Relatively few choriomeningeal cells are infected and great numbers of early recruitable effector T cells can stop further virus spread early. Rapidly proliferating immune protection wins over immunopathological damage. (**B**) Many choriomeningeal cells are infected by the time the immune T cells are numerous enough to control virus spread. Cytotoxic T cells attempt to provide antiviral immune protection by trying to stop viral expansion, but instead cause lethal immunopathological damage. (**C**) Virus has overwhelmingly infected many choriomeningeal cells and cells of the immune system, causing immune suppression. The suppressed antiviral T cell response can neither control virus spread nor lead to immunopathological damage. Vaccination before challenge increases T cell frequencies and tends to change the equilibrium from (C) towards (A); more rapidly spreading LCMV or higher infectious doses shift it from (A) towards (C).

many cells are infected; this results in immune protection.

Other genes besides the MHC influence the immunological balance (Fig. 1, E to F). LCMV DOCILE, even when injected into immunized mice at a low dose (100 pfu), did not induce lethal LCM in DBA/2 mice (Fig. 1E), in contrast to BALB/c mice (Fig. 1C). This result and high virus titers ($>10^7$ pfu/g of spleen), even on days 8 to 12 after infection, correlate with the relatively low percentage of CD8 cells in DBA/2 mice and their slow T cell response (*14*), particularly against this rapidly replicating virus (*6, 13*). These conditions enabled even a low dose of LCMV DOCILE to induce immune suppression in DBA/2 mice. Therefore the same explanations as given for BALB/c mice infected with high doses of LCMV DOCILE may account for the findings with DBA/2 mice that were not fully protected when immunized with vacc-NP and infected with a low dose of LCMV DOCILE (100 pfu, Fig. 1E). Correspondingly, DBA/2 mice survived i.c. injection of a high dose of LCMV DOCILE (10^4 pfu, Fig. 1F) because of their low frequency of CD8 cells even when immunized with vacc-GP, whereas exactly the same treatments caused early death in vacc-GP–immunized BALB/c (Fig. 1D). These experiments also showed that vaccination with vacc-NP and subsequent i.c. injection of a high dose of LCMV DOCILE caused LCM only in about 50% of the DBA/2 mice but in 80 to 100% of the BALB/c mice (Fig. 1D); again, rapid virus spread can overwhelm immune protection and lead to immunopathology, as in high-responder BALB/c mice, or, alternatively, can induce high dose immune paralysis even in immunized low-responder DBA/2 mice.

These data suggest that immunizing mice with an antigen that induces low cytotoxic T cell (CTL) responses in a given haplotype shifts the relative kinetics between virus spread and immune response. Immune suppression by LCMV did not develop, and establishment of high dose-induced immune paralysis was prevented; therefore lethal LCM disease developed. Thus an intermediate CTL response induced by vaccination was not vigorous enough to eliminate the virus quickly from the brain before too many cells had been infected so that widespread immunopathological damage resulted.

This interpretation was supported by an analysis of viral titers in infected mice and of the LCMV-specific cytotoxic T cells that were generated (Fig. 2). BALB/c mice immunized with vaccinia virus and then infected with LCMV WE (10^2 pfu) showed time-dependent high LCMV titers in spleen. Those mice immunized with LCMV WE or vacc-NP rapidly cleared LCMV, and mice immunized with vacc-GP had intermediate titers of virus in their spleens (Fig. 2A). BALB/c mice infected with a high virus dose (10^6 pfu) controlled LCMV titers completely if they had been immunized with LCMV WE, cleared LCMV rapidly if immunized with vacc-NP, but controlled virus only marginally if they had been previously immunized with vacc-GP (Fig. 2B). Vaccinia-primed mice showed consistent high virus titers similar to unprimed controls. The kinetics of the CTL response in the meninges of BALB/c mice that were infected with 10^6 pfu of LCMV WE (Fig. 2C) were also examined. The response in cerebrospinal fluid was most rapidly established in LCMV and vacc-NP–immunized mice and slower in vacc-GP–immunized mice. The CTL response of vaccinia-immunized control mice was typical of the immunosuppressed response to a high LCMV WE infection (10^6 pfu) (*3, 6, 9, 13*). Therefore the kinetics of virus spread and T cell function correlated with the outcome of disease (Fig. 3): rapid induction of a potent LCMV-specific CTL response resulted in rapid virus clearance and protection from LCM disease. An intermediate CTL response prevented the induction of immune paralysis, but instead lead to fatal LCM. Slow or nonresponsive hosts had quickly spreading virus that established an immunosuppressed state, with no LCM.

This enhancement of susceptibility to LCM by immunization was dependent on $CD8^+$ T cells (Table 1). In vivo depletion of these effector cells by monoclonal antibodies (MAbs) prevented death by LCM in B10.BR mice immunized with vacc-NP and then infected i.c. with a high dose of LCMV WE. Efficiency of anti-CD8 treatment was shown by survival of B10.BR mice infected with low dose LCMV and treated with MAbs to CD8 (*15*).

Table 1. Effect of anti-CD8 treatment on development of lethal LCM in B10.BR mice immunized with vacc-NP and then infected with a low (200 pfu) or high (3×10^5 pfu) dose of LCMV WE. Mortalities and mean days to death (±SEM) of B10.BR mice (five mice per group) that were immunized as in Fig. 1. Five mice were treated intraperitoneally 1 day before and 1 day after i.c. infection with MAb to CD8 (*27*). Mortality rates were registered over a time period of 25 days.

Immunizing agent	Anti-CD8	Mortality (%)	Days to death
	Low dose LCMV		
Vacc-NP	−	100	9.4 ± 0.2
	+	0	−
Vaccinia	−	100	10.3 ± 0.7
	+	0	−
	High dose LCMV		
Vacc-NP	−	100	9.0 ± 0.4
	+	0	−
Vaccinia	−	0	−
	+	0	−

Vaccination usually induces protective antibodies (*1, 16*). However, rare examples exist like Dengue virus (*17*), respiratory syncythial virus (*18*), probably measles (*19*), and possibly HIV (*20*) in which induction of antibodies by vaccination may enhance subsequent infection and disease; in view of the presented results, T cells may be involved as well. Our results document that in noncytopathic virus infections an antiviral T cell response may be induced by vaccination that may aggravate disease caused by a subsequent infection, dependent upon genetic factors of the host (including the MHC) (*21*), the virus isolate (*3, 6, 9, 13*), the infectious dose (*3, 13*), the time after vaccination (that is, the kinetics of immunological memory), the immunocompetence of the host (*3*), and many other factors. In such infections vaccination may shift the balance from low (that is, late) to high (early) responder status and therefore may prevent immunopathologically mediated disease (from C to A in Fig. 3), or it may shift the balance only slightly from a nonresponsive asymptomatic carrier to a low or intermediate responder status causing immunopathology (from C to B in Fig. 3). This happens usually not with whole virus vaccines exhibiting multiple protective T cell epitopes but may be induced when only one or few of the virus epitopes are used for vaccination, as is the case in the newer types of peptide or recombinant vaccines (*10, 22, 23*) that are being investigated for HIV (*22*), leprosy (*23*), and many other infections. Such strategies should be reevaluated carefully because in some cases vaccination may cause, rather than prevent, T cell–mediated immunopathological disease.

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12. Besides the defined major epitopes, there are minor T cell epitopes that represent less than 2 to 5% of primary responses. These minor epitopes specificities have been demonstrated in antiviral protection assays (*11*). Accordingly, H-2^d mice generate H-2–restricted cytotoxic T cells specific for LCMV-GP and H-2^k mice against LCMV-GP and NP, which can be boosted preferentially during secondary or tertiary responses in vitro. The effect of vaccination with recombinant viruses on a subsequent LCMV infection i.c. was studied also on days 10 and 60 to 70 after vaccination with slightly shifted kinetics, but with overall comparable results to the ones shown here for days 20 and 21.
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Altered Synaptic Plasticity in *Drosophila* Memory Mutants with a Defective Cyclic AMP Cascade

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Synaptic transmission was examined in *Drosophila* mutants deficient in memory function. These mutants, *dunce* and *rutabaga*, are defective in different steps of the cyclic adenosine 3′,5′-monophosphate (cAMP) cascade. In both *dunce* and *rutabaga* larvae, voltage-clamp analysis of neuromuscular transmission revealed impaired synaptic facilitation and post-tetanic potentiation as well as abnormal responses to direct application of dibutyryl cAMP. In addition, the calcium dependence of transmitter release was shifted in *dunce*. The results suggest that the cAMP cascade plays a role in synaptic facilitation and potentiation and indicate that synaptic plasticity is altered in *Drosophila* memory mutants.

SYNAPTIC PLASTICITY OCCURS THROUGHOUT the nervous system from neuromuscular junctions to central synapses (*1, 2*). It has been hypothesized that the cellular processes underlying learning and memory involve modulation of synaptic efficacy by second messenger systems (*3*). Genetic dissection in *Drosophila* provides a powerful tool for identifying and manipulating molecules that control synaptic efficacy (*4*). Among *Drosophila* mutants deficient in learning and memory (*5*), alleles of the *dunce (dnc)* and *rutabaga (rut)* loci have been subjected to extensive behavioral (*5, 6*) and biochemical (*7–10*) studies. Two *dnc* alleles, dnc^{M11} and dnc^{M14}, eliminate cAMP-specific phosphodiesterase II, leading to higher levels of cAMP-dependent phosphorylation in nervous tissues (*8, 10*). The rut^1 mutation abolishes Ca^{2+}-calmodulin (Ca^{2+}-CaM)–dependent activity of adenylate cyclase (*7*), disrupting Ca^{2+}-activated, cAMP-dependent phosphorylation of certain proteins (*10*). In spite of these behavioral and biochemical studies, it remains unknown if and how neural transmission is affected in these mutants. It is technically difficult to study synapses in the circuits for specific learning tasks in *Drosophila*. However, certain fundamental effects of an altered cAMP cascade on synaptic plasticity may be determined in synapses that are more accessible to physiological studies.

The two-microelectrode voltage-clamp method was used to study excitatory junctional currents (EJCs) at the *Drosophila* larval neuromuscular junction (*11*), which has been well characterized physiologically (*4, 12*) and anatomically (*13*). The muscle fiber was clamped at −80 mV (Fig. 1A) to prevent activation of voltage-gated ion channels (*14*). Therefore, transmitter release evoked at different external Ca^{2+} concentrations by nerve stimulation was accurately reflected by the EJC amplitude. The Ca^{2+} dependence of transmitter release in normal larvae thus determined (Fig. 1B) was consistent with that indicated by excitatory junctional potential (EJP) recordings (*12*).

The amplitude of nerve-evoked EJCs was enhanced in both dnc^{M11} and dnc^{M14} (Fig. 1). However, miniature EJCs (MEJCs) caused by spontaneous quantal release (*1, 15, 16*) remained unaltered in the mutants (Fig. 1A), suggesting a presynaptic defect. Instead of the fourth-power relation seen in normal larvae, the Ca^{2+} dependence of transmitter release in both *dnc* alleles (Fig. 1B), as reflected by either EJC amplitude or quantal content (*1*), is closer to a second- or third-power relation (power $n = 2.4$). The power (slope n in logarithmic plots) is usually interpreted to indicate the number of Ca^{2+} ions required in cooperative action to trigger transmitter release (*1, 16*). Thus, the change in *dnc* suggested a decline in Ca^{2+} cooperativity, which led to an increase in release probability (*16*). At a low Ca^{2+} concentration (0.1 mM), a large fraction of nerve stimuli led to failure in release in normal (0.59 ± 0.11, mean ± SD, four fibers) and rut^1 (0.55 ± 0.03, six fibers) larvae, but failure was rare in both *dnc* alleles (0.06 ± 0.06, three dnc^{M11} and three dnc^{M14} fibers, significantly different from normal, $P < 0.02$, and from rut^1, $P < 0.01$, by t test). In rut^1 larvae, EJCs were not significantly altered, displaying a Ca^{2+} dependence parallel to that of normal larvae (Fig. 1).

Facilitation was elicited with repetitive stimuli (2 to 20 Hz) at low external Ca^{2+} concentrations, resulting in a gradual increase in transmitter release. EJCs were recorded at 0.2 mM Ca^{2+} with 4-Hz stimulation (Fig. 2A). To characterize the frequency dependence of this process, we delivered 20 pulses at different frequencies, and averaged the last 10 EJCs from each fiber to determine their facilitated amplitude (Fig. 2B). In normal fibers, facilitation was

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