Induction of Long-Term Protective Antiviral Endogenous Immune Response by Short Neutralizing Monoclonal Antibody Treatment

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Long-term immune control of viral replication still remains a major challenge in retroviral diseases. Several monoclonal antibodies (MAbs) have already shown antiviral activities in vivo, including in the clinic but their effects on the immune system of treated individuals are essentially unknown. Using the lethal neurodegeneration induced in mice upon infection of neonates by the FrCas\textsuperscript{K} retrovirus as a model, we report here that transient treatment by a neutralizing MAb shortly after infection can, after an immediate antiviral effect, favor the development of a strong protective host immune response containing viral propagation long after the MAb has disappeared. In vitro virus neutralization- and complement-mediated cell lysis assays, as well as in vivo viral challenges and serum transfer experiments, indicate a clear and essential contribution of the humoral response to antiviral protection. Our observations may have important therapeutic consequences as it suggests that short antibody-based therapies early after infection should be considered, at least in the case of maternally infected infants, as adjunctive treatment strategies against human immunodeficiency virus, not only for a direct effect on the viral load but also for favoring the emergence of an endogenous antiviral immune response.

The therapeutic use of monoclonal antibodies (MAbs) has increased spectacularly in recent years (10, 26, 27). It now concerns a wide range of diseases with 13 MAbs approved for human use by the Food and Drug Administration and more than 400 others currently tested in clinical trials (27), including the treatment of chronic viral diseases such as hepatitis B virus (15, 20), hepatitis C virus (15) and human immunodeficiency virus (4, 11, 59) infections. Due to their potential in the treatment of AIDS, several human immunodeficiency virus-neutralizing MAbs have already been obtained and studied (22, 23, 53) and others are being generated by various laboratories worldwide.

Some of the available MAbs have already shown antiviral activity in vivo in a variety of adult and neonatal animal and human settings (see Discussion). Immediate antiviral effects in these experiments were due to direct virus neutralization. However, whether short-term MAb-based immunotherapies could, in addition, favor the emergence of endogenous antiviral immune responses contributing to the protection of infected individuals in the long term has hardly been considered thus far. As the elucidation of fundamental concepts in retroviral immunology is easier to attain in immunocompetent mouse models than in humans or monkeys, we turned to the neonatal period of immunocompetence acquisition in young organisms, a situation which is reminiscent of that of perinatal infant infection by human immunodeficiency virus.

FrCas\textsuperscript{K} is an ecotropic mouse retrovirus (50). Upon inoculation to newborn animals under the age of 5 to 6 days, it first propagates in the periphery and, then, penetrates into the central nervous system, where it causes a rapid noninflammatory spongiform degenerative disease involving primarily the motor centers of the brain and the spinal cord (14, 36). This leads to the death of 100% of the mice within 1 to 2 months. In contrast, mice infected at a later stage do not develop any neurological illness. Instead, the virus replicates only in the periphery, where it induces splenomegalies and leukemias in 80% of the animals within 3 to 6 months postinfection (our unpublished observations). MAb 667 is a neutralizing MAb that binds to the Env of CasBr but not to that of other ecotropic retroviruses (19, 42, 48). We recently showed that its in vitro neutralizing activity results from binding to the VRA domain of Env (19), a motif crucial for attachment to the viral receptor. We also reported that 667 exerts a strong in vivo antiviral activity in passive immunization experiments or when produced in mice upon implantation of encapsulated MAb-producing cells (47). So far, these experiments were performed on short periods of times and in the continuous presence of 667 (47). Moreover, the mechanisms underlying the in vivo effect were not studied. We now report that transient treatment by 667 shortly after infection can, after an immediate antiviral effect, favor the development of a strong protective host immune response, containing viral propagation for more than one year, i.e., long after the MAb has disappeared, via mechanisms involving a strong humoral contribution. This potentially opens new therapeutic perspectives for the immunotherapy of retrovirally induced pathologies such as AIDS.
MATERIALS AND METHODS

Virus stocks and monoclonal antibody production. Culture supernatants of Mus dunni fibroblasts transfected with the FrCasE proviral clone (50) were used as viral stocks (47). The anti-murine leukemia virus Env mouse 667, 709, 672, 678 (42) and rat 83A25 (21) Mabs and the anti-murine leukemia virus p12GagMab (12) were purified from hybridoma cell culture supernatants and assayed as previously described (19).

Virus titers and 667 Mab neutralization activity assay. Viral titers were determined using a focal immunofluorescence assay (57). Dilutions of virus-containing samples were added to 25% confluent Mus dunni cell cultures in the presence of 8 µg/ml of Polybrene. Cells-to-cell spread of replication-competent retroviruses was allowed to proceed for 2 days and focus-forming units (FFU) were visualized by indirect immunofluorescence using the 667 Mab and a fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin rabbit antisem. For assaying virus neutralization activity of mouse sera, 4 × 10 FrCasE FFU were diluted in a 1:1 ratio with serum samples previously diluted 100-fold in phosphate-buffered saline (PBS (0.15 M NaCl, 0.01 M Na phosphate, pH 7) and incubated at 37°C for 1 h. Mixes were used to infect 2 × 10 cells cultured in 12 well culture plates overnight. The infection medium was replaced by fresh culture medium and cells were allowed to reach confluence, at which time FFU were scored as above.

Infection experiments. Each experimental group consisted of an individual litter of approximately 10 Swiss mice kept with the mothers until 3 weeks of age. Three day-old mice were injected intraperitoneally with 5 × 10 FrCasE FFU in 100 µl of Dulbecco's modified Eagle's medium. Three doses of 15 µg of purified 667 or of 548 MAB in 50 µl of PBS were administered intraperitoneally on day 2, 2 weeks later, unless indicated otherwise. Mice were examined for clinical signs of neurodegeneration (50) daily until day 30 and weekly afterwards. They were bled at the retroorbital sinus for viremia and anti-FrCasE serum immunoglobulin concentrations. For assaying virus neutralization activity of mouse sera, 4 × 10 FrCasE FFU were diluted in a 1:1 ratio with serum samples previously diluted 100-fold in phosphate-buffered saline (PBS (0.15 M NaCl, 0.01 M Na phosphate, pH 7) and incubated at 37°C for 1 h. Mixes were used to infect 2 × 10 cells cultured in 12 well culture plates overnight. The infection medium was replaced by fresh culture medium and cells were allowed to reach confluence, at which time FFU were scored as above.

RNA purification, synthesis of cDNA, and reverse transcription-PCR analysis. Total RNAs from brain and spleenocytes were prepared using RNAzol as specified by the supplier (Eurobio) and were treated with RNase-free DNase I (Promega) at room temperature for 10 min. DNase I was inactivated by heating the samples at 65°C for 10 min and first strand cDNA syntheses were performed using the Superscript II RNase H reverse transcriptase (Gibco-BRL) as recommended by the supplier. PCR amplification was carried out using a hot start protocol (3 min at 94°C) in a final volume of 50 µl containing 2 ml of each cDNA, 50 pmol of each primer, 1.5 mM MgCl2, and 2.5 units of Taq polymerase (Eurobio, Paris, France); 45 cycles (94°C for 3 min, 65°C for 45 s, 72°C for 45 s) for Env and 25 cycles (45°C for 1 min, 65°C for 45 s, 72°C for 45 s) for β-actin were followed by an elongation period of 10 min at 72°C. Nucleotide sequences of amplification primers are available on request.

Flow cytometry analysis of infected splenocytes. Two set of flow cytometry experiments were always conducted in parallel using either 667 or of the 83A25 murine leukemia virus p12Gag protein (12) were purified from hybridoma cell culture supernatants and assayed as previously reported (47), only the former control group was used in further experiments.

RESULTS

A short 667 Mab treatment is sufficient to protect mice from FrCasE retrovirus-induced diseases on the long term. We first asked whether short MAbs treatments are sufficient to prevent the development of the FrCasE-induced diseases. In a first series of experiments, 3-day-old pups were infected and, one hour later, i.e., a time sufficient for allowing infection to proceed (63), given a first dose of 667 Mab (infected/treated mice). The MAb administration was repeated on days 2 and 5 postinfection. Three control groups were included in the study. One was infected with FrCasE but not treated with 667 (infected/nontreated mice). Another one was infected with FrCasE and treated with the nonneutralizing 548 IgG2a/k MAb directed to the murine leukemia virus p12Gag protein (12) (infected/control MAb-treated mice). The third one was not infected but received the 667 Mab (noninfected/treated animals). All noninfected/treated mice survived and all infected/ nontreated, as well as all infected/control MAb-treated mice (not shown), died within a few weeks. Since infected/treated and infected/control MAb-treated animals behaved similarly, as previously reported (47), only the former control group was used in further experiments.

Interestingly, 9 out of the 10 infected/treated mice were still alive 14 months later (termination of the experiments) (Fig. 1A). Careful examination of infected/treated mice revealed no neuropathological signs, normal hematocrits, no splenomegaly
and no detectable viremia at any stage of the follow-up. As for the animal which died on month 6, the reason of its death was unclear. However, it presented no sign of retroviral disease. In another series of experiments, the first dose of 667 was administered 1 or 2 days postinfection. None of the infected/treated animals developed any detectable viremia or neurodegeneration (not shown), indicating that administration of 667 immediately after infection is not an absolute requirement for a strong therapeutic effect in the long term. Under the experimental conditions used, the 667 MAb returns to undetectable levels between 14 and 20 days after the first administration, as assayed by enzyme-linked immunosorbent assay (ELISA) (not shown). This indicates that transient neutralizing MAb-based treatments shortly after infection by FrCasE permit mice to survive healthy for more than one year.

FIG. 1. In vivo antiviral activity of intraperitoneally injected 667 MAb and infection kinetic analysis. (A) Survival of FrCasE-infected animals. Two groups of ten 3-day-old mice were infected with FrCasE, one of which was treated with 667 and the other not. As a control group, 10 pups were treated with 667 but not infected. As a control group, 10 pups were treated with 667 but not infected. (B) Viremia. Thirty 3-day-old animals were infected with FrCasE and half of them were treated with 667 as in A. Two animals per group were sacrificed each day and their sera were pooled for viremia assay by immunofluorescence assay. Infected/treated animal viremia was below the detection limit. Data are presented as the mean ± standard error of the mean. (C) Reverse transcription-PCR detection of FrCasE Env mRNA. Spleens and brains were recovered from the same animals as in B. Subgenomic FrCasE Env mRNA accumulation was assayed by reverse transcription-PCR as described in Materials and Methods. β-Actin was used as an internal amplification standard. C+ corresponds to a newborn FrCasE-infected mouse sacrificed 2 weeks postinfection and C− to a negative control with H2O instead of RNA. (D) Expression of cell surface Env in splenocytes. Splenocytes from mice infected in (B) were analyzed by flow cytometry for expression of cell surface-expressed Env. Values are the results of 2 experiments performed in triplicate and are presented as the mean ± standard error of the mean.
virus propagation (50). New groups of mice were infected and treated as described before and sacrificed every day from day 1 to day 7 postinfection for monitoring serum viremia, the expression of viral Env in splenocytes, and the presence of Env subgenomic mRNA in spleen and brain. Spleen and brain were selected because the former constitutes one of the major peripheral organs for viral replication and the latter is the site of the neurodegeneration.

High viremias were already detected on day 4 postinfection in infected/nontreated mice, reached maximal levels (10^6 FFU/ml range) on day 5, and remained stable thereafter. In contrast, they were undetectable (limit of detection, 10^2 FFU/ml) in infected/treated animals for the whole period of the follow-up (Fig. 1B). However, Env protein and RNA analysis showed residual infection in the periphery but suggested absence of central nervous system infection. Thus, FrCasE Env mRNA was detected as early as day 5 in the brain of nontreated animals, whereas it remained undetectable until day 7 in infected/treated animals despite the high number of PCR cycles used (Fig. 1C). As for the spleen, FrCasE mRNA and cell surface-expressed Env became detectable on day 4 and reached maximal and stable levels on day 5 in nontreated animals (Fig. 1C and 1D). In contrast, FrCasE mRNA accumulation was delayed by 2 days and strongly reduced in 667-treated animals (Fig. 1C). Consistently, only low levels of Env-expressing cells were detected in these animals (Fig. 1D). Thus, the treatment of mice by 667 shortly after infection by FrCasE dramatically reduces but does not eliminate systemic viral spread and inhibits infection of the brain, which explains the absence of neurodegeneration.

Treatment of infected mice with the 667 MAb leads to the development of a sustained and long-lasting endogenous humoral immune response against FrCasE. As viral spread is not totally abolished after administration of 667 to infected mice, we assessed whether the absence of neurodegeneration and leukemia in infected/treated animals still alive 14 months postinfection was due to the development of an endogenous immune response. To this aim, anti-FrCasE serum immunoglobulins from infected/treated and noninfected/treated mice were assayed by ELISA. A transient IgM response was observed from weeks 2 to 6 postinfection in infected/treated mice (not shown) and peaked on week 3. The decrease in anti-FrCasE IgM concentration correlated with an increase in anti-FrCasE IgG. Maximal levels were reached by week 10 and remained stable for 1 year (Fig. 2A). Neither anti-FrCasE IgM (not shown) nor IgG (Fig. 2A) were detected in noninfected/nontreated animals, whereas an attenuated IgM response was seen in infected/nontreated mice just before death (not shown). The three main IgG subclasses were also examined in infected/treated mice. Anti-FrCasE IgG2b remained low for the whole follow-up, whereas virus-specific IgG1 and IgG2a were detected from week 4 onwards and increased rapidly until week 10 (Fig. 2B). Then, concentrations of IgG1 decreased dramatically, whereas those of IgG2a increased and remained elevated till the end of the experiments (Fig. 2B). Thus, infected/treated mice developed a sustained and long-lasting anti-FrCasE humoral response with high anti-FrCasE IgG2a serum concentrations.

Anti-FrCasE immunoglobulins produced by infected/treated animals display a strong antiviral activity in vitro. As a first step to link the anti-FrCasE humoral response generated in infected/treated mice to the absence of retroviral pathology, we analyzed its antiviral activity in vitro. We tested whether sera from infected/treated mice taken at week 30 postinfection could inhibit the binding of FrCasE Env to mouse BALB/c 3T3 fibroblasts. A clear dose-dependent inhibition was observed (Fig. 3A), whereas sera from control noninfected/treated mice had no effect on viral binding (not shown). Moreover, a time course analysis indicated that sera from infected/treated mice displayed a strong neutralizing activity in infection assays of Mus dunni fibroblasts. This activity was detectable as early as week 4 postinfection and was stable from week 10 onwards.

FIG. 2. Endogenous anti-FrCasE immunoglobulin response in infected/treated mice. Experiments were performed using litters of 9 to 10 animals. (A) Total anti-FrCasE IgG. Blood samples were collected from the infected/treated and noninfected/treated mice presented in Fig. 1A. Total anti-FrCasE IgGs were assayed by ELISA. (B) Anti-FrCasE IgG isotypes. Anti-FrCasE IgG1, IgG2a, and IgG1 isotypes were assayed in the same serum samples of infected/treated animals as in A. Values are the results of 3 experiments, each one performed in triplicate. Values are presented as the mean ± standard error of the mean. Each point is the average of the different individuals within each experimental group. The errors are calculated from individual variations within each one of these groups.
Complement-mediated lysis activity of FrCasE infected cells was also assessed. It became detectable in sera of infected/treated mice but not in those of control animals 4 weeks postinfection and thereafter reached a plateau parallel-

Viral challenge stimulates the endogenous anti-FrCasE immune response in infected/treated mice. We next tested whether infected/treated mice could respond to a virus challenge 14 months after the first infection. Seven infected/treated and seven noninfected/treated mice were inoculated with FrCasE and serum samples were collected at different times for 2 weeks to assay viremia and endogenous anti-FrCasE antibodies. In noninfected/treated mice, high but transient virus titers were measured, whereas no viremia was detected in challenged infected/treated mice (Fig. 4A). Contrastingly, with a slow and modest increase in anti-

FIG. 3. In vitro antiviral activity of anti-FrCasE IgGs contained in sera from infected/treated mice. (A) Inhibition of FrCasE Env binding to BALB/c 3T3 fibroblasts. FrCasE binding onto BALB/c 3T3 cells was tested by flow cytometry in the presence of 1/100 and 1/1,000 dilutions of sera pooled from the 9 mice infected and treated with 667; 100% of binding corresponds to cells incubated only in the presence of FrCasE and 0% to cells incubated with no virus. Virus binding was expressed as the percentage of maximal binding, which was measured using cells incubated in the presence of FrCasE but in the absence of any mouse serum. The data presented are representative one of three experiments performed independently. (B) Neutralizing and complement-dependent cell lysis activities. Neutralization activity (full lines) was assayed by immunofluorescence assay in the presence of 1/100 dilutions of sera from infected/treated and noninfected/treated mice. Complement-mediated cell lysis experiments (dotted lines) were performed using 1/50 dilutions of sera from infected/treated and noninfected/treated mice. Data are presented as the mean ± standard error of the mean of 3 experiments. Each point is the average of the different individuals within each experimental group. The errors are calculated from individual variations within each one of these groups.

FIG. 4. Viremia and anti-FrCasE immunoglobulins in challenged infected/treated mice. (A) Plasma viremia. 14 month-old infected/treated and noninfected/treated (control) mice were inoculated with FrCasE. Serum samples were collected at various time points for viremia assay by immunofluorescence assay. Viremia of infected/treated mice were below the detection limit. (B) Plasma IgGs. Anti-FrCasE immunoglobulins were assayed by ELISA in the same samples as in A. Values are presented as the mean ± standard error of the mean. Each point is the average of the different individuals within each experimental group. The errors are calculated from individual variations within each one of these groups. (C) FrCasE Env mRNA in spleen. The presence of Env mRNA was assayed by reverse transcription-PCR in total RNA from splenocytes of mice sacrificed 14 days postinfection. C corresponds to a noninfected/treated mouse inoculated with FrCasE and C is as in Fig. 1C. β-Actin was used as an internal standard.
FrCasE immunoglobulins in control animals, a rapid and robust response was stimulated in infected/treated mice (Fig. 4B), which is suggestive of a primary immune stimulation in the first group of mice and a secondary response in the second one. Noteworthy, IgG2a was the main antibody subclass contributing to the response in challenged infected/treated animals (Fig. 4B), as during the primary endogenous immune response (Fig. 2B).

All mice were sacrificed on day 14 postinfection for analysis of FrCasE Env mRNA expression. No signal was detected in the spleens of challenged infected/treated mice, whereas control animals were strongly positive (Fig. 4C). This strengthens the idea that challenged infected/treated animals resist reinfection by FrCasE due to the efficient existing antiviral immune response, which is restimulated by the viral challenge.

Sera from challenged infected/treated mice protect FrCasE-infected newborn mice. Finally, we assessed whether the humoral anti-FrCasE response observed in challenged infected/treated animals was protective on its own. To address this, sera from 4 of the challenged infected/treated- and from 4 of the control challenged noninfected/treated mice were collected on the day of sacrifice and administered to litters of 3-day-old FrCasE-infected animals. On weeks 5, 6, and 7 postinfection, 3 animals per group were sacrificed for assay of viremia, expression of env RNA in spleen and brain, and plasma anti-FrCasE IgG levels. One litter of infected mice, not subjected to any serum transfer, was used as a control. Animals not subjected to serum transfer or treated with sera from challenged noninfected/treated mice developed the severe ataxia and paralysis preceding death between weeks 5 and 7. In contrast, the neonates treated with sera from infected/treated mice did not show any pathological signs. Consistently, the two groups of control mice showed high viremia, env RNA in spleen, and low or undetectable anti-FrCasE IgG levels (Fig. 5A to C), whereas animals treated with sera from infected/treated mice showed neither detectable viremia nor env RNA in spleens but high anti-FrCasE IgGs concentrations increasing with time. This strongly suggests that the humoral anti-FrCasE response developing in infected/treated animals actually contributes to the antiviral effect more than 1 year after the initial infection and treatment.

**DISCUSSION**

**Two-step mechanism for long-term protection of FrCasE-infected mice by 667.** Passive antibody-based immunotherapies of newborn mice infected by murine leukemia viruses and other viruses, such as lymphocytic choriomeningitis virus, have already been reported (6, 7, 24, 48). However, none of these studies addressed the possible development of protective endogenous immune responses. Using the neonate FrCasE infection model, we show here that transient treatment with the neutralizing 667 MAb shortly after infection induces long-term protection, as infected/treated mice survived and showed no sign of retrovirally induced neurodegeneration, splenomegaly, or leukemia for the 14 months of the follow-up.

Healthy survival of animals most likely involves two complementary and sequential mechanisms. In the first step, 667 exerts an immediate and direct antiviral effect, limiting but not eliminating viral propagation in the periphery. As high peripheral viremia is necessary for viral entry into the central nervous system, neutralization of viremia by FrCasE immunoglobulins is necessary for protection. By the second step, immune responses are restimulated by the viral challenge and contribute to long-term protection. This also suggests that FrCasE immunoglobulins may still be protective in neonatal animals and that the protective effect of the sera could be due to neutralizing antibodies or to immune effectors. A detailed analysis of this aspect is ongoing in our laboratory.
therefore be important to evaluate the contributions of each infected cells, whereas antibodies control virus spread. It will lymphocytes, they do not have overlapping functions; CTLs kill that, even though B cells and CTLs are both stimulated by Th (43), whereas that of T cells involves not only helper T but also tion in this system requires both B and T cells (18, 31). The responses during adulthood, including in the context of passive infection, our observations that (i) several viruses, including polio and dengue vi- ruses, induce mostly strong protective IgG2a responses in the mouse (13, 35, 58), (ii) neurological diseases induced by lactate dehydrogenase-elevating virus and NS1 yellow fever virus are better prevented by IgG2a than by other isotypes (38, 55) and (iii) IgG2a better protect mice from Ebola virus (64) and Friend virus-induced leukemia (9), it is reasonable to assume that the humoral anti-FrCasE protective response was mostly accounted for by this isotype.

Initiation of the anti-FrCasE immune response in infected/treated neonates. The neonatal period corresponds to a window of ontogeny during which the immune system is particularly susceptible to tolerization. There is, however, accumulat- ing evidence that tolerance is not an intrinsic property of the newborn immune system, and factors like the type of the an- tigen-presenting cells, the dose of the antigen, and the pres- ence of adjuvant or of cytokines determine whether the out- come is neonatal tolerance or immunization (1, 2, 8, 39). Further supporting this view, our work strongly suggests that treatment by 667 prevents tolerance of FrCasE as a rapid anti- FrCasE antibody response is detected with a kinetics corre- sponding to that of a classical antiretroviral response in the mouse (31).

How a short passive immunotherapy can orient the immune response towards a protective outcome against FrCasE in neonates constitutes an important issue. CD4+ Th cells are divided into two major functional types, Th1 and Th2. It is commonly assumed that protection against retroviruses is fa- vored by the Th1 rather than by the Th2 response (31): Th1 cells are central for development and maintenance of protec- tive CTLs and humoral immunity predominantly of the IgG2a isotype. At variance, Th2 responses, even though they also provide help for the generation of antiviral antibodies, can suppress the stimulation of antiviral Th1 responses. Interest- ingly, Sarzotti et al. (54) have shown that inoculation of high doses of CasBr-M, a murine retrovirus close to FrCasE, into neonates does not result in immunological nonresponsiveness but to induction of a nonprotective Th2 response. In contrast, low doses triggered Th1-dependent immune protection, sug- gesting that the amount of antigen is critical for regulating the Th1/Th2 balance. Our data showing a rapid anti-FrCasE IgG2a class switch in infected/treated animals are strongly suggestive of a predominantly Th1-type protection.

system (36, 37, 50), this prevents induction of the lethal neu- rodegeneration. Then, a strong long-lasting protective anti- FrCasE immune response, as demonstrated by challenge and serum transfer experiments, is mounted and persists long after 667 MAb has disappeared. These high and constant levels of anti-FrCasE IgGs observed from week 10 onwards are suggestive of a continuous stimulation of the immune system by residual, albeit undetectable infection and, most probably, protec- tects mice from viral rebound and/or viral escape. However, other explanations, such as persistence of long-lived plasma cells, cannot be ruled out and are under investigation. Inter- estingly, administration of 667 1 h, 1 day, or 2 days postinfection is equally effective at preventing the neurodegeneration and result in similar evolutions of viremia over time (not shown), indicating a certain flexibility for successful application of the treatment. Finally, it is important to underline that Swiss mice are outbred, suggesting that induction of an antiviral protective immune response by passive MAb-based immunotherapy is not dependent on a narrow genetic or major histo- compatibility complex context.

Crucial contribution of the endogenous humoral immune response to the protective anti-FrCasE effect? A first question relates to the components of the immune system responsible for protection of infected/treated animals in the long term. Comparison of FrCasE with the Friend retroviral complex may be informative in this respect. The latter induces erythroleu- kemia when inoculated into adult mice and is the most exten- sively studied model for analysis of antiretroviral immune re- sponses. In adulthood, including in the context of passive therapy is not dependent on a narrow genetic or major histo- compatibility context.

Comparison of FrCasE with the Friend retroviral complex may be informative in this respect. The latter induces erythroleukaemia when inoculated into adult mice and is the most exten- sively studied model for analysis of antiretroviral immune re- sponses. In adulthood, including in the context of passive therapy is not dependent on a narrow genetic or major histo- compatibility context.
Several nonexclusive mechanisms might contribute to the emergence of such a response. First, although high doses of virus were inoculated into mice, the administration of 667 rapidly blunted FrCasE propagation. This, most probably, generated a situation very similar to that of the low-dose infection experiments of Sarzotti et al. (54), i.e., a situation giving time for the whole immune system to mature and to develop a predominantly Th1 response. In addition, the generation of the protective response may have been accelerated and strengthened by the specific use of a MAb as an antiviral agent early in the life of infected mice. Telerization is, at least in part, explained by the lower responsiveness (and the lower number) of professional antigen-presenting cells compared to their adult counterparts (39). It is, therefore, possible that immune complexes formed by FrCasE and 667 were more efficient at activating antigen-presenting cells, via binding to signal-inducing Fc receptors, than free viruses internalized by other routes. Supporting this hypothesis, several groups have already reported that cross-linked immune complexes dramatically improve antigen presentation by dendritic cells (3, 29, 32, 51, 56). Future work will examine formally whether viral immune complexes help to induce a protective endogenous immune response, as this may have important therapeutic consequences in the use of MAbs as antiviral agents.

Potential interest of MAb-based immunotherapies in the case of human immunodeficiency virus neonatal infection. The World Health Organization estimates that approximately 800,000 children were infected by human immunodeficiency virus in 2002, most of them through mother-to-child transmission. The current treatments of infection consist of antiretroviral chemotherapies (60). However, those are not always efficient due to the increasing frequency of spontaneous resistances to drugs (49, 52), and their long-term side effects in individuals treated in infancy and childhood are unfortunately still largely unknown. Therefore, passive immunotherapies based on the administration of highly neutralizing anti-human immunodeficiency virus MAbs represent new attractive therapeutic tools in particular because antibodies show no intrinsic toxicity (reviewed in reference 53). Indeed, their in vivo antiviral potential has begun to receive experimental validation in several preclinical (5, 25, 40, 41, 62) and clinical adult settings (4, 11, 59), as well as in the specific context of perinatal lentiviral infection as antibody administrations could protect perinatally-infected macaques challenged with simian immunodeficiency virus and simian-HIV (22, 33, 34, 44–46, 61).

The FrCasE experimental system is reminiscent of infant contamination by human immunodeficiency virus-infected mothers upon delivery since, in both cases, initial virus propagation in the organism occurs during the period of immunocompetence acquisition. Our work may therefore have important therapeutic consequences since it suggests that short antibody-based immunotherapies of maternally infected infants should be considered, not only for a direct effect on the viral load but also for stimulating a protective endogenous anti-human immunodeficiency virus immune response lasting for a long period of time. The 667 MAb plus FrCasE constitutes one of the rare neonatal immunotherapy models of a retroviral infection that is amenable to in-depth immunological study of large numbers of animals under defined, standardized, and reproducible conditions. It should, thus, reveal an invaluable tool for characterizing parameters permitting us to orient and strengthen antiviral endogenous immune responses with the aim of human applications.

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