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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^E 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 observation 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 infection system by the FrCas^E retrovirus to address this issue. This model constitutes an invaluable tool to address the development of a protective immune response during the critical period of immunocompetence acquisition in young organisms, a situation which is reminiscent of that of perinatal infant infection by human immunodeficiency virus.

FrCas^E 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.

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MATERIALS AND METHODS

Virus stocks and monoclonal antibody production. Culture supernatants of *Mus dunni* fibroblasts transfected with the $FrCas^E$ 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 p12Gag MAb (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. Cell-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 antiserum. For assaying virus neutralization activity of mouse sera, 4×10^2 $FrCas^E$ 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 2×10^4 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 5×10^4 $FrCas^E$ 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 0, 2 and 5 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-$FrCas^E$ serum immunoglobulin concentrations assay. After clotting at room temperature for 15 min, blood samples were centrifuged at 6,000 × *g* for 15 min and serum aliquots were stored at −20°C until use. Fifteen micrograms of 667 per injection was considered an optimal dose in our experiments, as prior work indicated that 667 doses less than 10 μg protected infected mice from the neurodegeneration but not from leukemia, whereas doses higher than 10 μg protected 100% of mice from both diseases in the long term (not shown).

RNA purification, synthesis of cDNA, and reverse transcription-PCR analysis. Total RNAs from brain and splenocytes 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 cDNAs were synthesized 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 $MgCl_2$ and 2.5 units of TacI 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 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 rat MAb, which recognizes an epitope different from that of 667 in Env (21) with no difference in final outcomes. Spleens were dissociated in cell culture medium and washed once by centrifugation and resuspension in PBS. Red blood cells were eliminated by adding the ACK lysing buffer (Biowhittaker). White blood cells were recovered by centrifugation, washed twice in PBS and resuspended in DMEM containing 10% fetal calf serum. Samples of 5×10^5 cells were incubated at room temperature for 1 h in the presence of 5 μg/ml of anti-Env MAb, in PBS-bovine serum albumin (PBS plus 0.2% bovine serum albumin) washed twice in PBS-bovine serum albumin and incubated at room temperature with a secondary fluorescein isothiocyanate-conjugated anti-mouse or anti-rat IgG rabbit antiserum for 1 h. Cells were washed twice in PBS-bovine serum albumin, resuspended in 500 μl of PBS, and analyzed using the FACScalibur flow cytometer from Becton Dickinson.

ELISA of anti-$FrCas^E$ antibodies. The 667 MAb and serum anti-$FrCas^E$ antibodies were assayed as described previously (47). Antibodies used for the standard curve and serum samples were diluted in PBS containing 0.1% Tween 20 (PBST) plus 1% bovine serum albumin. The 667, 709, 672, and 678 MAbs were used as standards for anti-$FrCas^E$ immunoglobulin G2a (IgG2a), IgG2b, IgG1, and IgM detection, respectively. Secondary peroxidase-conjugated anti-mouse IgG2a, IgG2b, IgG1, and IgM rabbit antisera (Serotec) were used as secondary antibodies.

Env binding assay. Assays were performed as previously described (19). Viruses were cleared from culture supernatants by ultracentrifugation and experiments were conducted with soluble Env amounts corresponding to 5×10^4 FrCasE FFU. Env-containing aliquots were mixed with serial dilutions of either purified 667 or of a pool of sera withdrawn from the nine infected-treated animals at the age of 4 months (see Fig. 3A) in a volume of 0.1 ml and let at room temperature for 30 min. Then, mixtures were added to 5×10^5 BALB/c 3T3 cells for another 30 min at room temperature. Cells were washed twice with PBA (PBS plus 2% fetal calf serum plus 0.1% sodium azide) and stained at 4°C for 45 min with the 83A25 rat anti-Env MAb. Following two washes with PBA, cells were incubated at 4°C for 45 min in the dark with a fluorescein isothiocyanate-conjugated anti-rat immunoglobulin rabbit antiserum, then, counterstained for 5 min in the presence of 20 μg/ml propidium iodide and washed twice in PBA. Cell fluorescence was analyzed with the FACScalibur device.

Complement-mediated cell lysis. All experiments were carried out using culture medium devoid of phenol red. $FrCas^E$-infected *Mus dunni* cells (3×10^4) were seeded per well of 96 well culture plates. The following day, the culture medium was removed and cells were incubated in quadruplicate at 4°C for 30 min in the presence of 80 μl of sera samples diluted 50-fold in RPMI medium. As negative and positive controls, 80 μl of RPMI medium alone or containing 5 mg/ml of purified 667 MAb were used in place of sera samples, respectively; 20 μl of rabbit complement (Sera Lab) diluted 6-fold in RPMI medium were, then, added to half of the wells whereas 20 ml RPMI medium alone was added to the other half to quantify nonspecific cell lysis. After 30 min at 37°C, 20 μl of MTS reagent (Promega) were added to each well and incubation was pursued for another 3 h. Spectrophotometric analysis of plates was performed at 490 nm, 1, 2 and 3 h after addition of MTS. Two wells, in which cells were killed by addition of 100 μl of 70% methanol were used as 100% mortality controls.

Virus challenge and serum transfer experiments. The infected/treated and noninfected/treated mice (see text) were injected intravenously with 300 μl of a 5×10^4 FFU/ml $FrCas^E$ suspension 14 months after the first infection. Blood samples were collected every 2 days during the first 2 weeks postchallenge to assay viremia and endogenous anti-$FrCas^E$ IgG concentrations. On day 14, mice were sacrificed and sera were collected, pooled, and diluted in PBS to a concentration of 300 μg/ml anti-$FrCas^E$ IgGs as assayed by ELISA, aliquoted in 50-ml samples, and kept at −20°C until use. For serum transfer experiments, 3-day-old mice were infected with 5×10^4 $FrCas^E$ FFU, and three injections of 50 μl diluted sera were administered intraperitoneally on days 0, 2, and 5 postinfection, as already described for 667.

RESULTS

A short 667 MAb treatment is sufficient to protect mice from $FrCas^E$ retrovirus-induced diseases on the long term. We first asked whether short MAb treatments are sufficient to prevent the development of the $FrCas^E$-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 $FrCas^E$ but not treated with 667 (infected/nontreated mice). Another one was infected with $FrCas^E$ 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

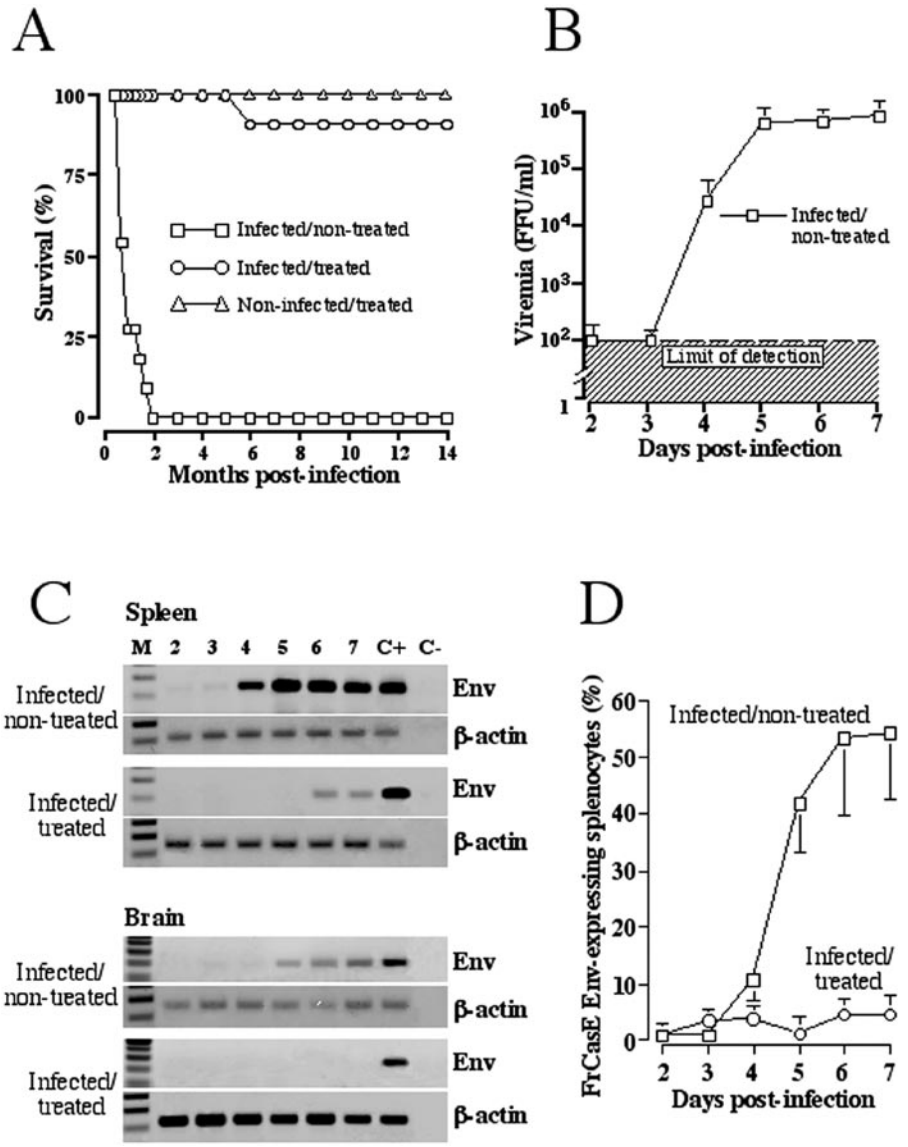

FIG. 1. In vivo antiviral activity of intraperitoneally injected 667 MAb and infection kinetic analysis. (A) Survival of FrCas^E-infected animals. Two groups of ten 3-day-old mice were infected with FrCas^E, one of which was treated with 667 and the other not. As a control group, 10 pups were treated with 667 but not infected. (B) Viremia. Thirty 3-day-old animals were infected with FrCas^E 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 FrCas^E Env mRNA. Spleens and brains were recovered from the same animals as in B. Subgenomic FrCas^E 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 FrCas^E-infected mouse sacrificed 2 weeks postinfection and C- to a negative control with H_2O 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.

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 develop 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 FrCas^E permit mice to survive healthy for more than one year.

Residual peripheral infection but no detectable brain infection in 667 MAb-treated animals. We then asked whether long-term survival of infected/treated mice was associated with a complete elimination of FrCas^E during the first week postinoculation, which corresponds to a critical and acute phase of

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, $FrCas^E$ 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, $FrCas^E$ 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, $FrCas^E$ 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 $FrCas^E$ 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 $FrCas^E$. 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-$FrCas^E$ 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-$FrCas^E$ IgM concentration correlated with an increase in anti-$FrCas^E$ IgG. Maximal levels were reached by week 10 and remained stable for 1 year (Fig. 2A). Neither anti-$FrCas^E$ IgM (not shown) nor IgG (Fig. 2A) were detected in noninfected/treated 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-$FrCas^E$ 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-$FrCas^E$ humoral response with high anti-$FrCas^E$ IgG2a serum concentrations.

Anti-$FrCas^E$ immunoglobulins produced by infected/treated animals display a strong antiviral activity in vitro. As a first step to link the anti-$FrCas^E$ 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 $FrCas^E$ 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

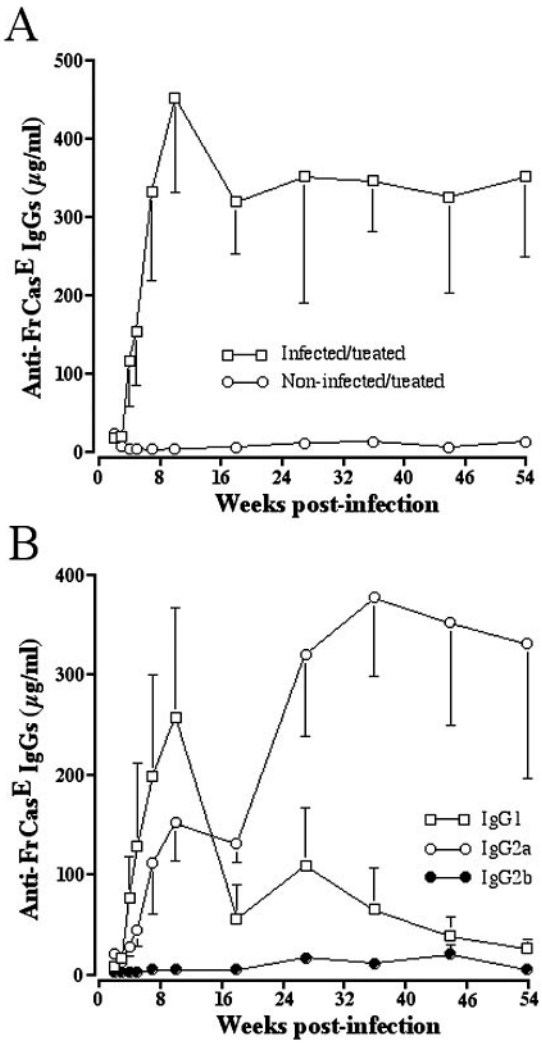

FIG. 2. Endogenous anti-$FrCas^E$ immunoglobulin response in infected/treated mice. Experiments were performed using litters of 9 to 10 animals. (A) Total anti-$FrCas^E$ IgG. Blood samples were collected from the infected/treated and noninfected/treated mice presented in Fig. 1A. Total anti-$FrCas^E$ IgGs were assayed by ELISA. (B) Anti-$FrCas^E$ IgG isotypes. Anti-$FrCas^E$ IgG2a, IgG2b, 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.

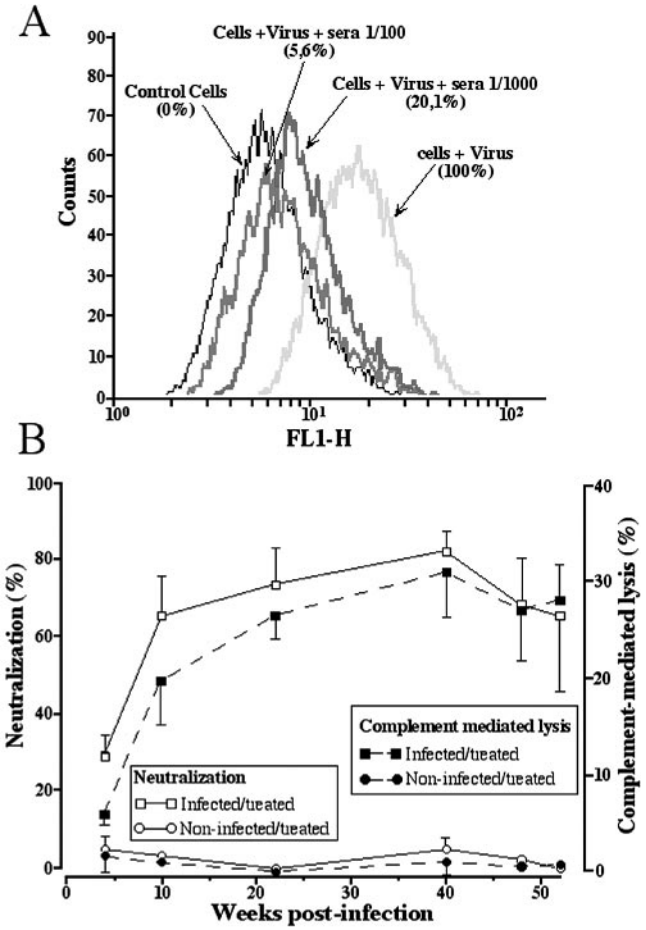

FIG. 3. In vitro antiviral activity of anti-FrCas^E IgGs contained in sera from infected/treated mice. (A) Inhibition of FrCas^E Env binding to BALB/c 3T3 fibroblasts. FrCas^E binding onto BALB/c 3T3 cells was tested by flow cytometry in the presence of 1/100 and 1/1,000 dilutions of sera pooled from the 9 mice infected and treated with 667; 100% of binding corresponds to cells incubated only in the presence of FrCas^E 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 FrCas^E 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. 3B). Complement-mediated lysis activity of FrCas^E-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 paralleling that of the neutralization activity (Fig. 3B). Thus, the anti-FrCas^E humoral immune response induced in infected/treated mice is capable of both virus neutralization activity and complement-mediated lysis of infected cells.

Viral challenge stimulates the endogenous anti-FrCas^E immune response in infected/treated mice and protects from infection. 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 FrCas^E and serum samples were collected at different times for 2 weeks to assay viremia and endogenous anti-FrCas^E antibodies. In noninfected/treated mice, high but transient virus titers were measured, whereas no viremia was detected in challenged infected/treated mice (Fig. 4A). Contrasting with a slow and modest increase in anti-

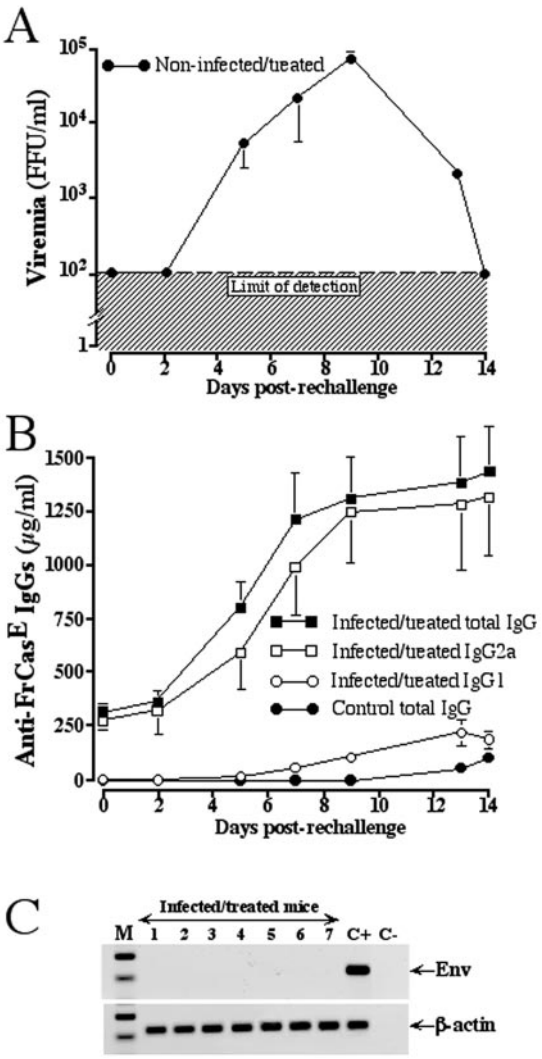

FIG. 4. Viremia and anti-FrCas^E immunoglobulins in challenged infected/treated mice. (A) Plasma viremia. 14 month-old infected/treated and noninfected/treated (control) mice were inoculated with FrCas^E. Serum samples were collected at various time points for viremia assay by immunofluorescence assay. Viremia of infected/treated mice were below the detection limit. (B) Plasma IgGs. Anti-FrCas^E 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) FrCas^E 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 FrCas^E and C- is as in Fig. 1C. β-Actin was used as an internal standard.

FrCas^E 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 FrCas^E 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 FrCas^E due to the efficient existing antiviral immune response, which is restimulated by the viral challenge.

Sera from challenged infected/treated mice protect FrCas^E-infected newborn mice. Finally, we assessed whether the humoral anti-FrCas^E 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 FrCas^E-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-FrCas^E 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-FrCas^E 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-FrCas^E IgGs concentrations increasing with time. This strongly suggests that the humoral anti-FrCas^E response developing in infected/treated animals actually contributes to the antiviral effect more than 1 year after the initial infection and treatment.

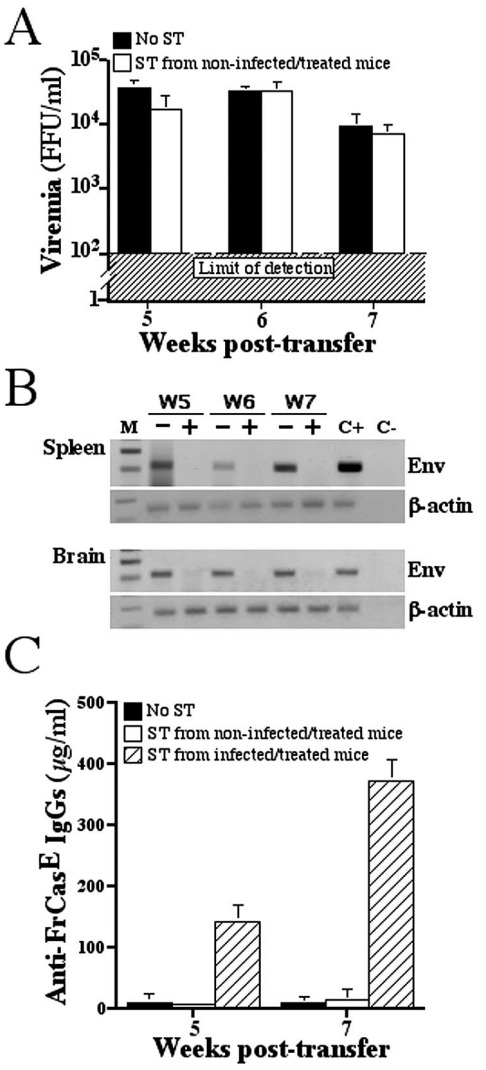

FIG. 5. Serum transfer experiments in FrCas^E-infected newborn mice. Three groups of FrCas^E-infected 3-day-old mice were subjected to no treatment or to transfer of sera (ST) from infected/treated and noninfected/treated mice (see text). Groups of 3 mice each were sacrificed on weeks 5, 6, and 7 for viremia and the anti-FrCasE immunoglobulin assay as well as for testing for the presence of Env mRNA in spleen and brain. (A) Viremia. Viremia were assayed by immunofluorescence assay on individual animals. Infected neonates treated with sera from infected/treated mice were below the detection limit. Values are the mean ± standard error of the mean. Each bar represents the average among different individual within one group of animals and the error is calculated from individual variations within each experimental group. (B) Env mRNA in spleen and brain. Brain and spleens from mice of each group of 3 animals were pooled for total RNA preparation and FrCas^E Env mRNA presence was tested by reverse transcription-PCR. β-Actin transcripts were analyzed in the same samples as internal amplification controls. (+) corresponds to neonates having received sera from infected/treated mice and (−) to neonates having received sera from noninfected/treated mice. C+ corresponds to infected animals having received no treatment and C- to negative amplification controls as in Fig. 1C. (C) Anti-FrCas^E immunoglobulins. Total anti-FrCas^E IgG were assayed by ELISA in individual animals. Values are the mean ± standard error of the mean. Each bar represents the average among different individual within one group of animals and the error is calculated from individual variations within each experimental group.

DISCUSSION

Two-step mechanism for long-term protection of FrCas^E-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 FrCas^E 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 (36, 37, 50), this prevents induction of the lethal neurodegeneration. Then, a strong long-lasting protective anti-FrCas^E 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-FrCas^E IgGs observed from week 10 onwards are suggestive of a continuous stimulation of the immune system by residual, albeit undetectable infection and, most probably, protects 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. Interestingly, 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 histocompatibility complex context.

Crucial contribution of the endogenous humoral immune response to the protective anti-FrCas^E 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 FrCas^E with the Friend retroviral complex may be informative in this respect. The latter induces erythroleukemia when inoculated into adult mice and is the most extensively studied model for analysis of antiretroviral immune responses during adulthood, including in the context of passive immunotherapy (30) and vaccination (16, 17). Efficient protection in this system requires both B and T cells (18, 31). The B-cell contribution is accounted for by neutralizing antibodies (43), whereas that of T cells involves not only helper T but also cytotoxic T lymphocytes (CTL) (18, 31). The reason for this is that, even though B cells and CTLs are both stimulated by Th lymphocytes, they do not have overlapping functions; CTLs kill infected cells, whereas antibodies control virus spread. It will therefore be important to evaluate the contributions of each one of these cell types to the long term protection of FrCas^E-infected/667-treated animals at various times postinfection. Monitoring viral rebounds and/or performing viral challenges in mice depleted of B cells, Th lymphocytes, or CTLs via the administration of cytotoxic antibodies will be useful in this respect.

Whatever the role of CTLs in limiting the spread of viral infection, our observations that (i) in vitro neutralizing and complement-mediated cell lysis activities were found in infected/treated mice sera, (ii) no viremia was detected in challenged mice, and (iii) serum transfer experiments resulted in efficient protection of infected neonates strongly indicate a clear and crucial contribution of the humoral response in the protective anti-FrCas^E effect. Characterizing the precise viral epitopes targeted by this response and determining whether it continues to mature during adulthood, due to the presence of residual infection, constitute important issues deserving future investigations.

Interestingly, Haigwood et al. (28) recently reported that intensive treatment of juvenile simian immunodeficiency virus-infected macaques with anti-simian immunodeficiency virus hyperimmune serum immunoglobulins accelerated the development of neutralizing antibodies, as assayed in vitro, in a fraction of the animals. Moreover, this response was associated with a delay in the onset of the disease in certain monkeys. The experimental setting used was significantly different from ours, as animals were fully immunocompetent at the time of infection and treatment, polyclonal antibodies were used instead of an MAb, and no viral challenge was conducted to test whether the endogenous anti-simian immunodeficiency virus immune response was protective. Nevertheless, these experiments raise the possibility that induction of an endogenous protective immune response upon passive neutralizing immunotherapy may not just concern neonatally infected/treated individuals.

The anti-FrCas^E antibodies we observed in infected/treated mice were predominantly IgG2a, i.e., of the immunoglobulin isotype known as the most potent for complement-mediated cell lysis, binding to Fc receptors and antibody-dependent cell-mediated cytotoxicity in the mouse. Taken with the observations that (i) several viruses, including polio and dengue viruses, 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-FrCas^E protective response was mostly accounted for by this isotype.

Initiation of the anti-FrCas^E 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, accumulating evidence that tolerance is not an intrinsic property of the newborn immune system, and factors like the type of the antigen-presenting cells, the dose of the antigen, and the presence of adjuvant or of cytokines determine whether the outcome is neonatal tolerance or immunization (1, 2, 8, 39). Further supporting this view, our work strongly suggests that treatment by 667 prevents tolerance of FrCas^E as a rapid anti-FrCas^E antibody response is detected with a kinetics corresponding 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 FrCas^E 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 favored by the Th1 rather than by the Th2 response (31): Th1 cells are central for development and maintenance of protective 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. Interestingly, Sarzotti et al. (54) have shown that inoculation of high doses of CasBr-M, a murine retrovirus close to FrCas^E, 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, suggesting that the amount of antigen is critical for regulating the Th1/Th2 balance. Our data showing a rapid anti-FrCas^E IgG2a class switch in infected/treated animals are strongly suggestive of a predominantly Th1-type protection.

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 $FrCas^E$ 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. Tolerization 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 $FrCas^E$ 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 various 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 peri- and postpartum-infected macaques challenged with simian immunodeficiency virus and simian-HIV (22, 33, 34, 44–46, 61).

The $FrCas^E$ 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 $FrCas^E$ 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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REFERENCES

1. **Adkins, B.** 1999. T-cell function in newborn mice and humans. Immunol. Today. **20:**330–335.
2. **Adkins, B., C. Leclerc, and S. Marshall-Clarke.** 2004. Neonatal adaptive immunity comes of age. Nat. Rev. Immunol. **4:**553–564.
3. **Akiyama, K., S. Ebihara, A. Yada, K. Matsumura, S. Aiba, T. Nukiwa, and T. Takai.** 2003. Targeting apoptotic tumor cells to Fc gamma R provides efficient and versatile vaccination against tumors by dendritic cells. J. Immunol. **170:**1641–1648.
4. **Armbruster, C., G. M. Stiegler, B. A. Vcelar, W. Jager, N. L. Michael, N. Vetter, and H. W. Katinger.** 2002. A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1. AIDS **16:**227–233.
5. **Baba, T. W., V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Ayehunie, L. A. Cavacini, M. R. Posner, H. Katinger, G. Stiegler, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, Y. Lu, J. E. Wright, T. C. Chou, and R. M. Ruprecht.** 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat. Med. **6:**200–206.
6. **Baldridge, J. R., and M. J. Buchmeier.** 1992. Mechanisms of antibody-mediated protection against lymphocytic choriomeningitis virus infection: mother-to-baby transfer of humoral protection. J. Virol. **66:**4252–4257.
7. **Baldridge, J. R., T. S. McGraw, A. Paoletti, and M. J. Buchmeier.** 1997. Antibody prevents the establishment of persistent arenavirus infection in synergy with endogenous T cells. J. Virol. **71:**755–758.
8. **Bot, A., and C. Bona.** 2002. Genetic immunization of neonates. Microbes Infect. **4:**511–520.
9. **Britt, W. J., and B. Chesebro.** 1983. Use of monoclonal anti-gp70 antibodies to mimic the effects of the Rfv-3 gene in mice with Friend virus-induced leukemia. J. Immunol. **130:**2363–2367.
10. **Carter, P.** 2001. Improving the efficacy of antibody-based cancer therapies. Nat. Rev. Cancer. **1:**118–129.
11. **Cavacini, L. A., M. H. Samore, J. Gambertoglio, B. Jackson, M. Duval, A. Wisnewski, S. Hammer, C. Koziel, C. Trapnell, and M. R. Posner.** 1998. Phase I study of a human monoclonal antibody directed against the CD4-binding site of HIV type 1 glycoprotein 120. AIDS Res. Hum. Retroviruses. **14:**545–550.
12. **Chesebro, B., W. Britt, L. Evans, K. Wehrly, J. Nishio, and M. Cloyd.** 1983. Characterization of monoclonal antibodies reactive with murine leukemia viruses: use in analysis of strains of friend MCF and Friend ecotropic murine leukemia virus. Virology **127:**134–148.
13. **Coutelier, J. P., J. T. van der Logt, F. W. Heessen, A. Vink, and J. van Snick.** 1988. Virally induced modulation of murine IgG antibody subclasses. J. Exp. Med. **168:**2373–2378.
14. **Czub, S., W. P. Lynch, M. Czub, and J. L. Portis.** 1994. Kinetic analysis of spongiform neurodegenerative disease induced by a highly virulent murine retrovirus. Lab. Invest. **70:**711–723.
15. **Dagan, S., and R. Eren.** 2003. Therapeutic antibodies against viral hepatitis. Curr. Opin. Mol. Ther. **5:**148–155.
16. **Dittmer, U., D. M. Brooks, and K. J. Hasenkrug.** 1999. Protection against establishment of retroviral persistence by vaccination with a live attenuated virus. J. Virol. **73:**3753–3757.
17. **Dittmer, U., D. M. Brooks, and K. J. Hasenkrug.** 1999. Requirement for multiple lymphocyte subsets in protection by a live attenuated vaccine against retroviral infection. Nat. Med. **5:**189–193.
18. **Dittmer, U., and K. J. Hasenkrug.** 2001. Cellular and molecular mechanisms of vaccine-induced protection against retroviral infections. Curr. Mol. Med. **1:**431–436.
19. **Dreja, H., L. Gros, S. Villard, E. Bachrach, A. Oates, C. Granier, T. Chardes, J. C. Mani, M. Piechaczyk, and M. Pelegrin.** 2003. Monoclonal antibody 667 recognizes the variable region A motif of the ecotropic retrovirus CasBrE envelope glycoprotein and inhibits Env binding to the viral receptor. J. Virol. **77:**10984–10993.
20. **Eren, R., E. Ilan, O. Nussbaum, I. Lubin, D. Terkieltaub, Y. Arazi, O. Ben-Moshe, A. Kitchinzky, S. Berr, J. Gopher, A. Zauberman, E. Galun, D. Shouval, N. Daudi, A. Eid, O. Jurim, L. O. Magnius, B. Hammas, Y. Reisner, and S. Dagan.** 2000. Preclinical evaluation of two human anti-hepatitis B virus (HBV monoclonal antibodies in the HBV-trimera mouse model and in HBV chronic carrier chimpanzees. Hepatology **32:**588–596.

21. **Evans, L. H., R. P. Morrison, F. G. Malik, J. Portis, and W. J. Britt.** 1990. A neutralizable epitope common to the envelope glycoproteins of ecotropic, polytropic, xenotropic, and amphotropic murine leukemia viruses. J. Virol. **64:**6176–6183.
22. **Ferrantelli, F., R. A. Rasmussen, K. A. Buckley, P. L. Li, T. Wang, D. C. Montefiori, H. Katinger, G. Stiegler, D. C. Anderson, H. M. McClure, and R. M. Ruprecht.** 2004. Complete protection of neonatal rhesus macaques against oral exposure to pathogenic simian-human immunodeficiency virus by human anti-hiv monoclonal antibodies. J. Infect. Dis. **189:**2167–2173.
23. **Ferrantelli, F., and R. Ruprecht.** 2002. Neutralizing antibodies against HIV-back in the major leagues? Curr. Opin. Immunol. **14:**495–502.
24. **Fish, D. C., D. B. Djurickovic, and R. J. Huebner.** 1979. Prevention of transplantable tumors by adoptive transfer of spleen cells from immunized rats. J. Immunol. **123:**2658–2663.
25. **Gauduin, M. C., P. W. Parren, R. Weir, C. F. Barbas, D. R. Burton, and R. A. Koup.** 1997. Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. Nat. Med. **3:**1389–1393.
26. **Glennie, M. J., and P. W. Johnson.** 2000. Clinical trials of antibody therapy. Immunol. Today. **21:**403–410.
27. **Gura, T.** 2002. Therapeutic antibodies: magic bullets hit the target. Nature **417:**584–586.
28. **Haigwood, N. L., D. C. Montefiori, W. F. Sutton, J. McClure, A. J. Watson, G. Voss, V. M. Hirsch, B. A. Richardson, N. L. Letvin, S. L. Hu, and P. R. Johnson.** 2004. Passive immunotherapy in simian immunodeficiency virus-infected macaques accelerates the development of neutralizing antibodies. J. Virol. **78:**5983–5995.
29. **Hamano, Y., H. Arase, H. Saisho, and T. Saito.** 2000. Immune complex and Fc receptor-mediated augmentation of antigen presentation for in vivo Th cell responses. J. Immunol. **164:**6113–6119.
30. **Hasenkrug, K. J., D. M. Brooks, and B. Chesebro.** 1995. Passive immunotherapy for retroviral disease: influence of major histocompatibility complex type and T-cell responsiveness. Proc. Natl. Acad. Sci. USA **92:**10492–10495.
31. **Hasenkrug, K. J., and U. Dittmer.** 2000. The role of CD4 and CD8 T cells in recovery and protection from retroviral infection: lessons from the Friend virus model. Virology **272:**244–249.
32. **Heyman, B.** 2000. Regulation of antibody responses via antibodies, complement, and Fc receptors. Annu. Rev. Immunol. **18:**709–737.
33. **Hofmann-Lehmann, R., R. A. Rasmussen, J. Vlasak, B. A. Smith, T. W. Baba, V. Liska, D. C. Montefiori, H. M. McClure, D. C. Anderson, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, H. Katinger, G. Stiegler, M. R. Posner, L. A. Cavacini, T. C. Chou, and R. M. Ruprecht.** 2001. Passive immunization against oral AIDS virus transmission: an approach to prevent mother-to-infant HIV-1 transmission? J. Med. Primatol. **30:**190–196.
34. **Hofmann-Lehmann, R., J. Vlasak, R. A. Rasmussen, B. A. Smith, T. W. Baba, V. Liska, F. Ferrantelli, D. C. Montefiori, H. M. McClure, D. C. Anderson, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, H. Katinger, G. Stiegler, L. A. Cavacini, M. R. Posner, T. C. Chou, J. Andersen, and R. M. Ruprecht.** 2001. Postnatal passive immunization of neonatal macaques with a triple combination of human monoclonal antibodies against oral simian-human immunodeficiency virus challenge. J. Virol. **75:**7470–7480.
35. **LeClerc, C., P. Martineau, S. Van der Werf, E. Deriaud, P. Duplay, and M. Hofnung.** 1990. Induction of virus-neutralizing antibodies by bacteria expressing the C3 poliovirus epitope in the periplasm. The route of immunization influences the isotypic distribution and the biologic activity of the antipoliovirus antibodies. J. Immunol. **144:**3174–3182.
36. **Lynch, W. P., S. Czub, F. J. McAtee, S. F. Hayes, and J. L. Portis.** 1991. Murine retrovirus-induced spongiform encephalopathy: productive infection of microglia and cerebellar neurons in accelerated CNS disease. Neuron **7:**365–379.
37. **Lynch, W. P., S. J. Robertson, and J. L. Portis.** 1995. Induction of focal spongiform neurodegeneration in developmentally resistant mice by implantation of murine retrovirus-infected microglia. J. Virol. **69:**1408–1419.
38. **Markine-Goriaynoff, D., and J. P. Coutelier.** 2002. Increased efficacy of the immunoglobulin G2a subclass in antibody-mediated protection against lactate dehydrogenase-elevating virus-induced polioencephalomyelitis revealed with switch mutants. J. Virol. **76:**432–435.
39. **Marshall-Clarke, S., D. Reen, L. Tasker, and J. Hassan.** 2000. Neonatal immunity: how well has it grown up? Immunol. Today. **21:**35–41.
40. **Mascola, J. R., M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. Louder, C. R. Brown, C. V. Sapan, S. S. Frankel, Y. Lu, M. L. Robb, H. Katinger, and D. L. Birx.** 1999. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. J. Virol. **73:**4009–4018.
41. **Mascola, J. R., G. Stiegler, T. C. VanCott, H. Katinger, C. B. Carpenter, C. E. Hanson, H. Beary, D. Hayes, S. S. Frankel, D. L. Birx, and M. G. Lewis.** 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat. Med. **6:**207–210.
42. **McAtee, F. J., and J. L. Portis.** 1985. Monoclonal antibodies specific for wild mouse neurotropic retrovirus: detection of comparable levels of virus replication in mouse strains susceptible and resistant to paralytic disease. J. Virol. **56:**1018–1022.
43. **Messer, R. J., U. Dittmer, K. E. Peterson, and K. J. Hasenkrug.** 2004. Essential role for virus-neutralizing antibodies in sterilizing immunity against Friend retrovirus infection. Proc. Natl. Acad. Sci. USA **101:**12260–12265.
44. **Nishimura, Y., T. Igarashi, N. Haigwood, R. Sadjadpour, R. J. Plishka, A. Buckler-White, R. Shibata, and M. A. Martin.** 2002. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J. Virol. **76:**2123–2130.
45. **Nishimura, Y., T. Igarashi, N. L. Haigwood, R. Sadjadpour, O. K. Donau, C. Buckler, R. J. Plishka, A. Buckler-White, and M. A. Martin.** 2003. Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. Proc. Natl. Acad. Sci. USA **100:**15131–15136.
46. **Parren, P. W., P. A. Marx, A. J. Hessell, A. Luckay, J. Harouse, C. Cheng-Mayer, J. P. Moore, and D. R. Burton.** 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J. Virol. **75:**8340–8347.
47. **Pelegrin, M., M. Marin, A. Oates, D. Noel, R. Saller, B. Salmons, and M. Piechaczyk.** 2000. Immunotherapy of a viral disease by in vivo production of therapeutic monoclonal antibodies. Hum. Gene Ther. **11:**1407–1415.
48. **Pincus, S. H., R. Cole, R. Ireland, F. McAtee, R. Fujisawa, and J. Portis.** 1995. Protective efficacy of nonneutralizing monoclonal antibodies in acute infection with murine leukemia virus. J. Virol. **69:**7152–7158.
49. **Pomerantz, R. J., and D. L. Horn.** 2003. Twenty years of therapy for HIV-1 infection. Nat. Med. **9:**867–873.
50. **Portis, J. L., S. Czub, C. F. Garon, and F. J. McAtee.** 1990. Neurodegenerative disease induced by the wild mouse ecotropic retrovirus is markedly accelerated by long terminal repeat and gag-pol sequences from nondefective Friend murine leukemia virus. J. Virol. **64:**1648–1656.
51. **Rafiq, K., A. Bergtold, and R. Clynes.** 2002. Immune complex-mediated antigen presentation induces tumor immunity. J. Clin. Investig. **110:**71–79.
52. **Richman, D. D.** 2001. HIV chemotherapy. Nature **410:**995–1001.
53. **Safrit, J. T., R. Ruprecht, F. Ferrantelli, W. Xu, M. Kitabwalla, K. Van Rompay, M. Marthas, N. Haigwood, J. R. Mascola, K. Luzuriaga, S. A. Jones, B. J. Mathieson, and M. L. Newell.** 2004. Immunoprophylaxis to prevent mother-to-child transmission of HIV-1. J. Acquir. Immune Defic. Syndr. **35:**169–177.
54. **Sarzotti, M., D. S. Robbins, and P. M. Hoffman.** 1996. Induction of protective CTL responses in newborn mice by a murine retrovirus. Science **271:**1726–1728.
55. **Schlesinger, J. J., M. Foltzer, and S. Chapman.** 1993. The Fc portion of antibody to yellow fever virus NS1 is a determinant of protection against YF encephalitis in mice. Virology **192:**132–141.
56. **Schuurhuis, D. H., A. Ioan-Facsinay, B. Nagelkerken, J. J. van Schip, C. Sedlik, C. J. Melief, J. S. Verbeek, and F. Ossendorp.** 2002. Antigen-antibody immune complexes empower dendritic cells to efficiently prime specific CD8+ CTL responses in vivo. J. Immunol. **168:**2240–2246.
57. **Sitbon, M., J. Nishio, K. Wehrly, D. Lodmell, and B. Chesebro.** 1985. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: distinction of host range classes in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. Virology **141:**110–118.
58. **Smucny, J. J., E. P. Kelly, P. O. Macarthy, and A. D. King.** 1995. Murine immunoglobulin G subclass responses following immunization with live dengue virus or a recombinant dengue envelope protein. Am. J. Trop. Med. Hyg. **53:**432–437.
59. **Stiegler, G., C. Armbruster, B. Vcelar, H. Stoiber, R. Kunert, N. L. Michael, L. L. Jagodzinski, C. Ammann, W. Jager, J. Jacobson, N. Vetter, and H. Katinger.** 2002. Antiviral activity of the neutralizing antibodies 2F5 and 2G12 in asymptomatic HIV-1-infected humans: a phase I evaluation. AIDS **16:**2019–2025.
60. **Thorne, C., and M. L. Newell.** 2004. Prevention of mother-to-child transmission of HIV infection. Curr Opin Infect. Dis. **17:**247–252.
61. **Van Rompay, K. K., C. J. Berardi, S. Dillard-Telm, R. P. Tarara, D. R. Canfield, C. R. Valverde, D. C. Montefiori, K. S. Cole, R. C. Montelaro, C. J. Miller, and M. L. Marthas.** 1998. Passive immunization of newborn rhesus macaques prevents oral simian immunodeficiency virus infection. J. Infect. Dis. **177:**1247–1259.
62. **Veazey, R. S., R. J. Shattock, M. Pope, J. C. Kirijan, J. Jones, Q. Hu, T. Ketas, P. A. Marx, P. J. Klasse, D. R. Burton, and J. P. Moore.** 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat. Med. **9:**343–346.
63. **Weiss, R., N. Teich, H. Varmus, and J. Coffin.** 1985. RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
64. **Wilson, J. A., M. Hevey, R. Bakken, S. Guest, M. Bray, A. L. Schmaljohn, and M. K. Hart.** 2000. Epitopes involved in antibody-mediated protection from Ebola virus. Science **287:**1664–1666.