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Synergistic Roles of Antibody and Interferon in Noncytolytic Clearance of Sindbis Virus from Different Regions of the Central Nervous System[▽]

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Sindbis virus (SINV) is an alphavirus that causes infection of neurons and encephalomyelitis in adult immunocompetent mice. Recovery can occur without apparent neurological damage. To better define the factors facilitating noncytolytic clearance of SINV in different regions of the central nervous system (CNS) and the roles of innate and adaptive immune responses at different times during infection, we have characterized SINV infection and clearance in the brain, brain stem, and spinal cords of severe combined immunodeficiency (SCID) and C57BL/6 (wild-type [WT]) mice and mice deficient in beta interferon (IFN-β) (BKO), antibody (μMT), IFN-γ (GKO), IFN-γ receptor (GRKO), and both antibody and IFN-γ (μMT/GKO). WT mice cleared infectious virus by day 8, while SCID mice had persistent virus replication at all sites. For 3 days after infection, BKO mice had higher titers at all sites than WT mice, despite similar IFN-α production, but cleared virus similarly. GKO and GRKO mice cleared infectious virus from all sites by days 8 to 10 and, like WT mice, displayed transient reactivation at 12 to 22 days. μMT mice did not clear virus from the brain, and clearance from the brain stem and lumbar spinal cord was delayed, followed by reactivation. Eighty-one days after infection, μMT/GKO mice had not cleared virus from any site, but titers were lower than for SCID mice. These studies show that IFN-β is independently important for early control of CNS virus replication, that antiviral antibody is critical for clearance from the brain, and that both antibody and IFN-γ contribute to prevention of reactivation after initial clearance.

Sindbis virus (SINV) is an arthropod-borne alphavirus of the family *Togaviridae*, related to eastern equine encephalitis and western equine encephalitis viruses. In humans, SINV infection results in diseases ranging from a self-limiting flu-like illness to fever, polyarthritis, and rash. In mice, SINV infects neurons of the central nervous system (CNS) (26, 27), resulting in a well-characterized encephalomyelitis (19). The severity of disease in infected mice is dependent on the viral strain and the age and genetic background of the mouse (26, 27, 51, 55, 58). While neonatal mice die within the first few days of infection, adult immunocompetent mice clear infectious virus within 8 days after infection without neurological sequelae. Although infectious virus has not been detected after clearance, viral RNA is not completely eliminated, and the independent contributions of various components of the immune response to long-term control of virus replication are not known (28, 32, 34, 56). As a result, SINV-induced encephalomyelitis is an excellent model for identification of innate and adaptive immune responses critical for control and clearance of virus from the CNS and for prevention of reactivation.

Because neurons are terminally differentiated cells with very limited capacity for regeneration, recovery from encephalomyelitis requires noncytolytic mechanisms for clearance of intracellular virus. Antiviral antibody is one important mechanism. Passive transfer of antibodies to persistently infected severe combined immunodeficiency (SCID) mice clears infectious SINV from all regions of the CNS, but virus production resumes as antibody levels decline (34, 35). However, mice unable to make antibodies can clear infectious virus from the brain stem and spinal cord, but not the brain, at least in part through the action of gamma interferon (IFN-γ) produced by $CD4^+$ and $CD8^+$ T cells (6). Furthermore, $CD8^+$ T-cell-deficient mice clear SINV from the CNS through the production of antibody, but clearance of viral RNA is delayed, suggesting an independent contribution of cellular immunity to noncytolytic virus clearance (29). In vitro, IFN-γ treatment of differentiated neurons infected with SINV restores cellular protein synthesis and downregulates viral RNA transcription and protein synthesis, facilitating activation of cellular mechanisms that lead to neuron survival and virus clearance (7). These studies suggested that T cells play a synergistic role with the humoral response in virus clearance from the CNS.

However, even in mice with normal antibody and T-cell responses, the innate immune response is essential for initial control of SINV replication and prevention of death. Mice deficient in either the type I IFN-α/β receptor or the IFN-activated transcription factor Stat-1 succumb to alphavirus infection and die before mounting a virus-specific immune response (8, 61). In vitro, treatment of infected cells with antibody to the SINV E2 glycoprotein facilitates suppression of virus replication by IFN-α (12). Collectively, these studies suggest that although antibody responses are critical for noncytolytic clearance of SINV from all types of neurons, IFN-α/β

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is necessary for initial control of virus replication and that IFN-α/β and IFN-γ facilitate antibody-mediated clearance. Furthermore, different populations of neurons may require different mechanisms or combinations of mechanisms for successful virus clearance.

Despite clearance of infectious virus, viral RNA persists in the CNS and there is a need for immune-mediated prevention of reactivation (34, 56). The mechanisms for prevention and control of reactivation are poorly understood. To further define the relative importance of different components of innate and adaptive immune responses for alphavirus control, clearance, and prevention of reactivation in vivo, we have studied SINV infection of mice deficient in the production of IFN-β and antibody; in production of, or response to, IFN-γ; and in production of both antibody and IFN-γ.

MATERIALS AND METHODS

Mice. C57BL6/J wild-type (WT), antibody knockout (μMT) C57BL/6-Igh-6^{tm1Cgn}, IFN-γ knockout (GKO) C57BL/6-*Ifng*^{tm1Ts}, IFN-γ receptor knockout (GRKO) B6.129S7-*Ifngr*^{tm1Agt}, and SCID C57BL/6J-*Prkdc*^{scid}/SzJ mice were purchased from The Jackson Laboratory at 3 to 4 weeks of age. μMT and GKO mice were bred to produce double-knockout (μMT/GKO) mice. IFN-β knockout (BKO) mice on a B6 background (14) were kindly provided by Mary Stenzel-Poore (Oregon Health Science University). For genotyping, tail snips were digested and genomic DNA was isolated with a Fast-DNA kit from Q-Biogene (Carlsbad, CA). The μMT/GKO mice were genotyped by PCR using IFN-γ, neo, and μM primers as suggested by the Jackson Laboratory: IFN-γ, oIMR0126, 5′-AGAAGTAAGTGGAAGGGCCCAGAAG-3′, and oIMR0127, 5′-AGGGA AACTGGGAGAGGAGAA ATAT-3′; neo, oIMR0128, 5′-TCAGCGCAGGG GCGCCCGGTTCTTT-3′, and oIMR0129, 5′-ATCGACAAGACCGGCTTCC ATCCG-3′; and μM, μMF, 5′-CTGTCTTGCTTGCTCTGCTG-3′, and μMR, 5′-CTCAGCCGCACAACCATACT-3′. The BKO mice were genotyped using PCRs assembled with 25 pmol of primers: IFN-βF, 5′-TATCTTCAGGGCTGT CTCCTTTCT-3′; IFN-βR, 5′-ACCTGTTGTTCATGATGGAAGCCA-3′; and λ2a-s, 5′-GGCATAGTTACTAGTTGTAACAGC-3′. The PCR was programmed for 30 cycles with annealing temperatures of 60°C for 30 s. The WT gene gave a band of 373 bp, and the knockout gene gave a band of 535 bp (50).

Virus and animal infections. Stocks of the TE strain of SINV (His at residue 55 of the E2 glycoprotein) (37, 55) were produced by transcribing viral RNA from the TE cDNA plasmid, transfecting the RNA into BHK cells, and collecting supernatant fluids at 24 h after transfection. Virus stocks were assayed by plaque formation on BHK cells and contained 10^9 PFU/ml.

For infection, 1,000 PFU of SINV in 20 μl phosphate-buffered saline (PBS) was injected intracerebrally (i.c.) into 4-week-old mice under isoflurane anesthesia. For tissue collection, mice were anesthetized with isoflurane and then perfused with PBS. The blood, brain, cerebellum, brain stem, and cervical, thoracic, and lumbar spinal cord were collected, snap frozen, and stored at −80°C. The tissues were homogenized in cold PBS to produce 10% (wt/vol) homogenates that were serially diluted and assayed by plaque formation on BHK cells (21). Each time point represented the geometric mean and standard error of the mean (SEM) of three to five mice from multiple experiments. Tissues with no detectable virus were assigned a value midway between zero and the limit of detection for purposes of calculation and graphing.

Anti-SINV antibody measurement and characterization. Serum samples were analyzed for anti-SINV immunoglobulin M (IgM), IgG, IgG1, IgG2a, and IgG2b by enzyme immunoassay (EIA) in triplicate. Plates were coated with 50 μl of 3-mg/ml polyethylene glycol-precipitated SINV overnight at 4°C, blocked, and incubated with serum samples (1:100) at 37°C for 1 h. Bound IgG was detected with biotin-conjugated antibody specific for each of the immunoglobulins (BD Biosciences) and developed with horseradish peroxidase-conjugated streptavidin (Vector Laboratories, Burlingame, CA) and TMB substrate (Sigma). Avidity was determined following the same EIA protocol, with an additional incubation step, after addition of the serum samples, of increasing concentrations of NH_4SCN ranging from 0 to 3 M. The avidity index was the concentration of NH_4SCN resulting in 50% elution of antibody. Neutralizing anti-SINV antibodies were quantitated by incubating serial dilutions of serum with 100 PFU SINV and calculating the dilution that resulted in 80% plaque reduction in BHK cells.

EIAs for cytokine production. Tissue homogenates from three mice at each time point were pooled and centrifuged at 300 × *g* for 5 min. IFN-β, IFN-α, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) were assayed in triplicate according to the manufacturer's instructions (Biosource, Camarillo, CA).

Statistics. Comparisons between groups were performed using Student's *t* test.

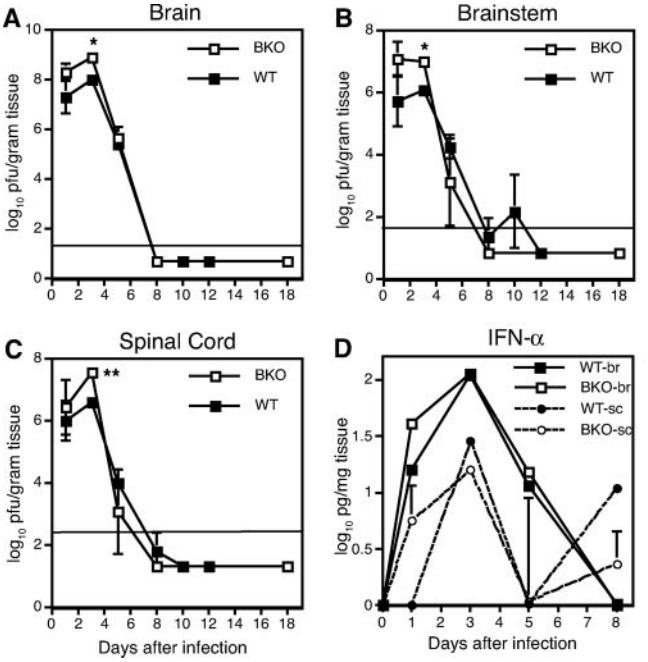

FIG. 1. SINV replication in IFN-β knockout and WT B6 mice. Mice were inoculated i.c. with 1,000 PFU SINV, and amounts of infectious virus were measured in brains (A), brain stems (B), and lumbar spinal cords (C) by plaque formation at various times after infection. The horizontal lines indicate the limits of detection (brain, 1.4; brain stem, 1.7; and spinal cord, 2.7). Samples without detectable infectious virus were assigned a value of half the limit of detection. Each point represents the average and SEM for three mice. *, $P < 0.05$; **, $P < 0.01$; Student's *t* test. (D) Brains (br) and spinal cords (sc) pooled from three BKO or WT mice were analyzed for IFN-α protein by EIA.

RESULTS

To investigate the individual and combined roles of IFN-β, IFN-γ, and antibody in the control of SINV replication, clearance from the CNS, and prevention of reactivation, we studied BKO, GKO, GRKO, μMT, μMT/GKO, and SCID mice in comparison with WT mice. All mice survived infection except for one SCID mouse that died at 61 days.

BKO mice. IFN-β is the earliest IFN produced and amplifies the IFN-α response in vitro (38). To determine the importance of IFN-β for control of SINV replication in the CNS, BKO mice were studied (Fig. 1). SINV replication in the brain (Fig. 1A), brain stem (Fig. 1B), and spinal cord (Fig. 1C) was higher than in B6 mice at days 1 and 3 after infection, with significant differences at all sites on day 3 ($P = 0.036$ for brain, 0.016 for brain stem, and 0.009 for spinal cord). The absence of IFN-β did not compromise the production of IFN-α in the CNS after infection (Fig. 1D) and did not affect SINV clearance or recovery (Fig. 1A to C).

SCID and B6 mice. WT B6 mice cleared infectious virus from all regions of the CNS within 8 days after infection, although small amounts of virus were detected in the brain

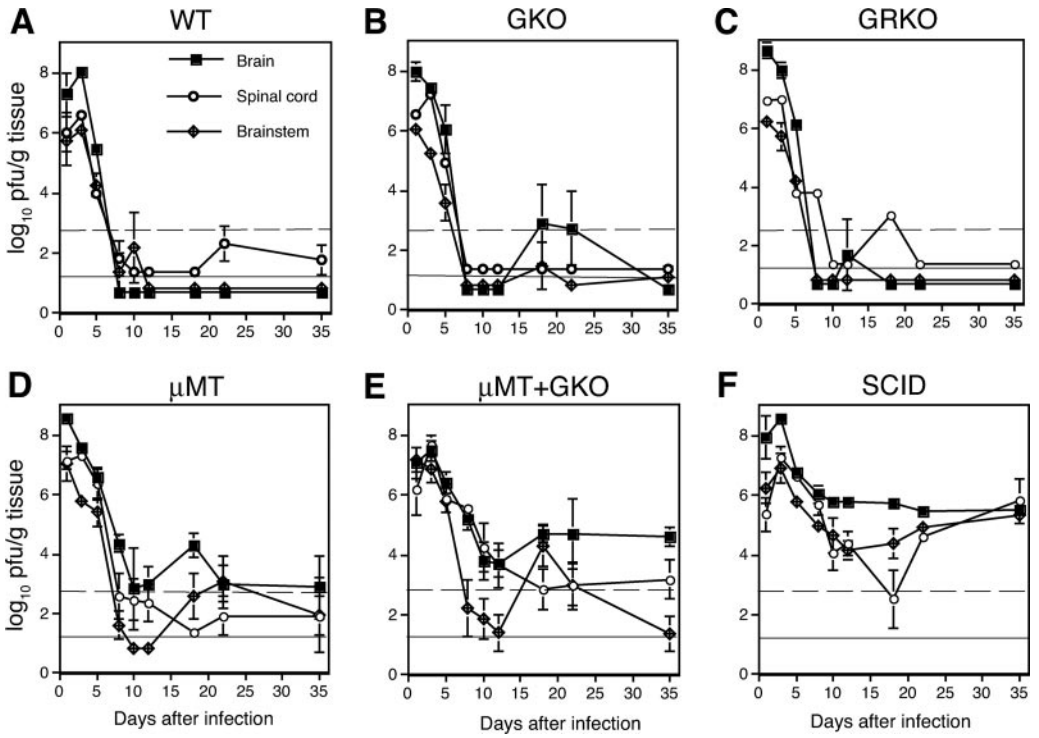

FIG. 2. SINV replication in WT, GKO, GRKO, μMT, μMT/GKO, and SCID mice. Mice were inoculated i.c. with 1,000 PFU SINV, and amounts of infectious virus in the brain, lumbar spinal cord, and brain stem were measured at various times after infection of WT (A), GKO (B), GRKO (C), μMT (D), μMT/GKO (E), and SCID (F) mice. Samples without detectable infectious virus were assigned a value of half the limit of detection. The limit of detection for the brain was 1.4 (solid line), for the brain stem was 1.7, and for the spinal cord was 2.7 (dashed line). Each point represents the geometric mean and SEM for three to six mice, except for spinal cords from GRKO mice on days 1 to 8 and 18 to 35, which were pooled.

stems of two mice at day 12 and in the spinal cords of two mice at day 22 and one mouse at day 35 (Fig. 2A). SCID mice were unable to clear virus from any region of the CNS and maintained virus titers between 2×10^5 and 3×10^6 PFU/gram in the brain and 4×10^3 to 5×10^6 PFU/gram in the brain stem (Fig. 2F) without signs of neurological disease. SINV replication in the lumbar spinal cords of SCID mice slowly declined during the first 2 weeks, reaching geometric mean titers of 3×10^3 PFU/gram at day 18. However, after that time, the mean titers increased to 10^6 PFU/gram on day 35. Virus was readily detected at all sites 81 days after infection (Fig. 3).

GKO and GRKO mice. To determine the independent effect of loss of IFN-γ or IFN-γ receptor signaling on clearance from each region of the CNS, GKO (Fig. 2B) and GRKO (Fig. 2C) mice were studied. These mice controlled virus replication similarly and cleared SINV from the brain, brain stem, and lumbar spinal cord by days 8 to 10. However, control of virus replication was not maintained in any region between 12 and 35 days after infection. In the brain, SINV was detected in one GRKO mouse at day 12 and two GKO mice each at day 18 and day 22. In the brain stem, SINV was detected in one GKO mouse at days 18 and 35. At day 18 postinfection (p.i.), all GKO mice had infectious virus detected either in the brain or the brain stem. In the lumbar spinal cord of GRKO (Fig. 2D) mice, SINV was also detected at day 18. However, the percentages of samples positive between days 12 and 35 (GKO, 6/36; GRKO, 2/30) were not different from that observed for WT mice (5/39). These data suggest that IFN-γ is not necessary for clearance of infectious virus from the CNS.

μMT and μMT/GKO mice. SINV was not cleared from the brains of μMT mice (Fig. 2D), but amounts of virus were reduced compared to SCID mice ($P < 0.05$) (Fig. 2F) at most times. Most μMT mice were able to clear virus from the brain stem by days 8 to 10 after infection. However, 2/3 spinal cords were still positive at day 12 compared to 0/3 for WT, GKO, and GRKO mice, and brain stems became positive again in all μMT mice 18 and 22 days after infection. Thus, there was no clearance in the brain, initial clearance with recrudescence in the brain stem, and slow clearance in the lumbar spinal cord of mice without B cells.

To understand the combined roles of antibody and IFN-γ in SINV clearance from different regions of the CNS and to determine whether additional factors might contribute to the clearance of infectious virus, we bred antibody and IFN-γ knockout mice to produce μMT/GKO mice. Control of SINV replication in the brains of μMT/GKO mice (Fig. 2E) was slower ($P < 0.005$ at day 8 p.i.) and reduction in titers was less than for μMT mice (Fig. 2D). Virus was detected in brains at all times, but titers were substantially lower than in SCID mice at the same time points ($P < 0.05$ at days 10, 12, 18, and 35). Likewise, virus remained detectable in the spinal cords of μMT/GKO mice (Fig. 2E), but the titers were intermediate between those of SCID (day 8, $P = 0.03$) and μMT (day 8, $P =$

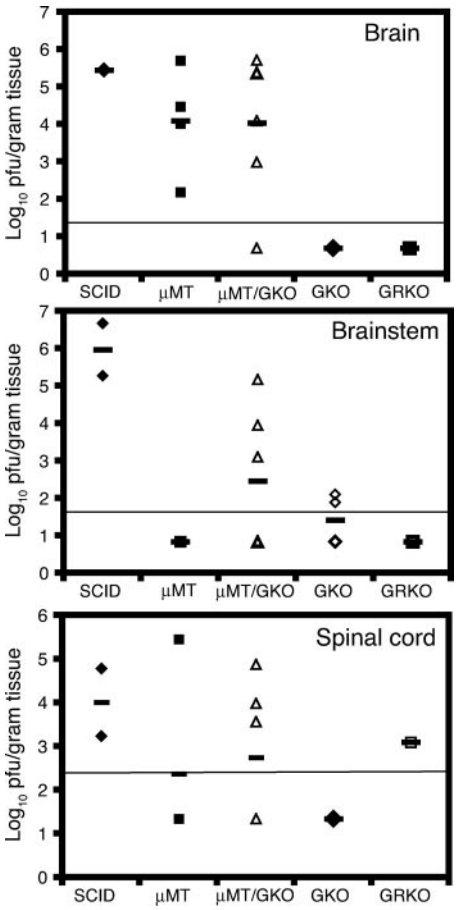

FIG. 3. Detection of infectious SINV 81 days after infection. SCID ($n = 2$), μMT ($n = 4$), μMT/GKO ($n = 6$), GKO ($n = 4$ to 5), and GRKO ($n = 3$) mice were inoculated i.c. with 1,000 PFU SINV. The presence of infectious SINV was assessed in the brain (A), brain stem (B), and lumbar spinal cord (C) collected 81 days after infection. Samples without detectable infectious virus were assigned a value of half the limit of detection (brain, 1.4; brain stem, 1.7; spinal cord, 2.7), indicated by the horizontal line. The bar for each group indicates the geometric mean. GRKO spinal cord homogenates were pooled.

0.005) mice. Unlike μMT mice, SINV was not cleared from the spinal cords of μMT/GKO mice.

SINV clearance rates from the brain stems of μMT/GKO (Fig. 2E) and μMT (Fig. 2D) mice were very similar. Although SINV was not detected in the brain stems of μMT mice 10 and 12 days after infection, it was detected in at least 1 μMT/GKO mouse at all times after day 8, in all mice at day 18, and in 4/5 mice at day 22.

Long-term control of SINV replication. To investigate the roles of antibody and IFN-γ in the long-term control of SINV replication, CNS tissues were collected 81 days after infection (Fig. 3). SCID mice showed persistent virus replication at all sites. All μMT and μMT/GKO mice had virus in the brain, while GKO and GRKO mice did not (Fig. 3A). In the brain stem, virus was detectable in 3/5 μMT/GKO mice, but not in μMT mice (Fig. 3B). SINV was detected in the lumbar spinal cords of four of five μMT/GKO mice but in only one of four μMT mice. GKO and GRKO mice had sporadic virus detection in both brain stem and spinal cord.

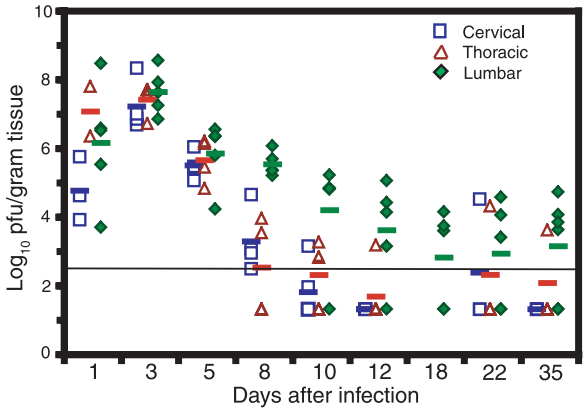

FIG. 4. SINV clearance from the cervical, thoracic, and lumbar spinal cord regions of μMT/GKO mice. μMT/GKO mice were inoculated i.c. with 1,000 PFU of SINV, and the amounts of infectious virus in different spinal cord regions were assessed by plaque assay. The bars indicate the geometric means for each region. The horizontal line indicates the limit of detection.

Persistent infection in μMT/GKO mice is localized primarily to the lumbar spinal cord. SINV is particularly likely to infect motor neurons of the anterior horn of the spinal cord (18, 51), and mechanisms of virus clearance from this site are of particular interest because of their importance for paralysis. To determine if there were differences in clearance from anterior horn neurons in various regions of the spinal cord, cervical, thoracic, and lumbar regions of spinal cords from μMT/GKO mice were analyzed separately (Fig. 4). Peak virus replication (day 3 p.i.) and the initial phase of clearance (day 5) were similar in all regions. However, after day 8, SINV was no longer detected in the cervical and thoracic regions of most μMT/GKO mice, while detectable virus was present in the lumbar spinal cords of the majority through day 81 (Fig. 3 and Table 1). However, levels of virus were lower than those observed in the lumbar spinal cords of SCID mice (day 35, $P = 0.012$). These data indicated that IFN-γ is a particularly important contributor to antibody-mediated control of SINV replication in the lumbar spinal cord.

Antibody production in GKO and wt mice. Because IFN-γ may play a role in the quantity and quality of antibodies produced, the time course of antibody production and the isotypes and avidities of the antibodies produced were assessed (Fig. 5). Production levels of neutralizing anti-SINV antibody were similar (Fig. 5A). Anti-SINV IgM (Fig. 5B) and IgG (Fig. 5C), as

TABLE 1. Amounts of virus in various regions of the spinal cord in μMT/GKO and SCID mice

Spinal cord	Virus titer ($\log_{10}$ PFU/g tissue) on day:		
	22	35	81
μMT/GKO cervical	2.42 (±1.31)	<2.70[a]	1.86 (±0.56)
μMT/GKO thoracic	2.35 (±1.22)	2.12 (±0.94)	<2.70[a]
μMT/GKO lumbar[b]	2.97 (±0.77)	3.18 (±0.66)	2.75 (±0.71)
SCID lumbar[b]	4.61 (±0.11)	5.81 (±0.43)	4.01 (±1.10)

[a] All samples were less than the spinal cord limit of detection.
[b] Comparison of SCID and μMT/GKO lumbar spinal cord titers by Student's *t* test: day 22, $P = 0.12$; day 35, $P < 0.012$; day 81, $P = 0.35$.

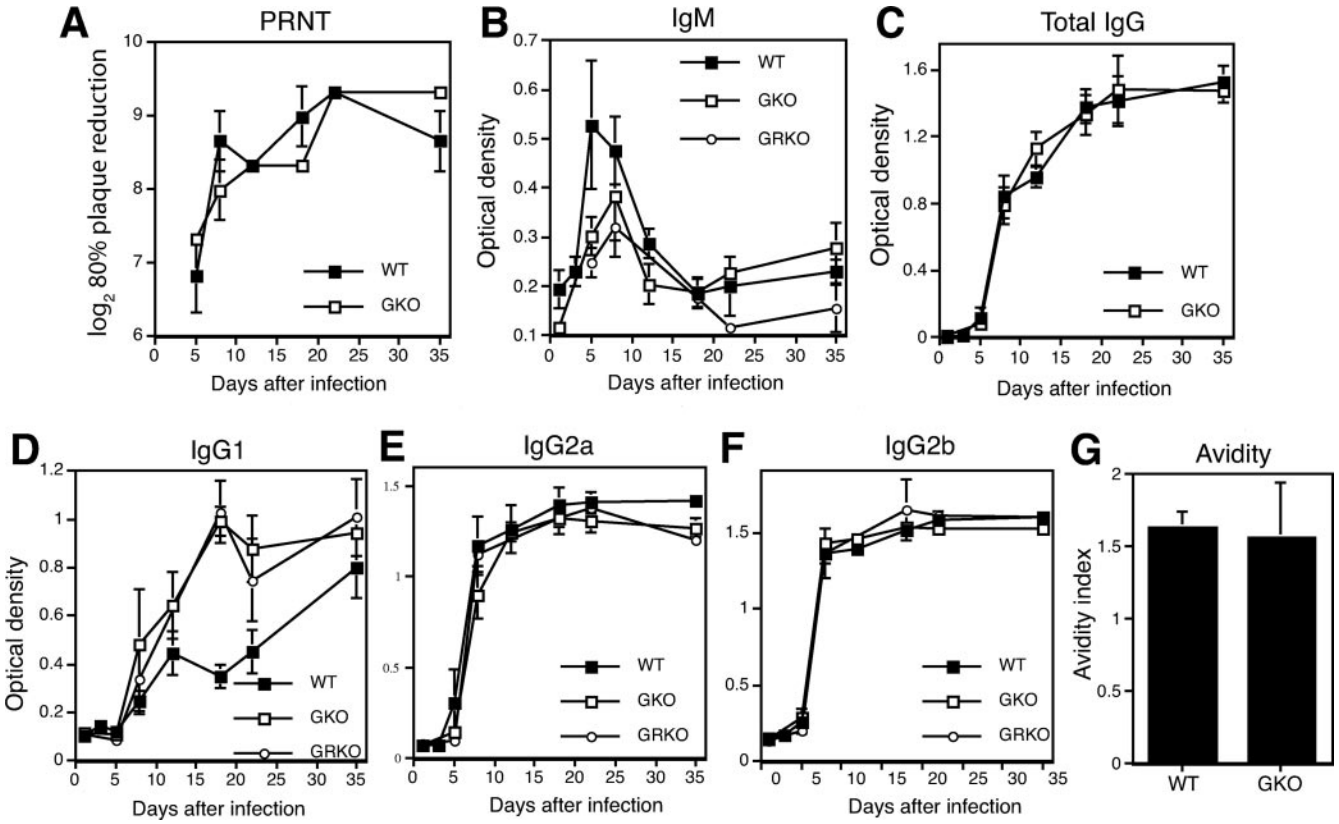

FIG. 5. Production of SINV-specific antibody by GKO, GRKO, and WT mice. Mice were inoculated i.c. with 1,000 PFU SINV, and sera were collected at different times after infection. Neutralizing antibody was measured by an 80% plaque reduction test in BHK cells (A). SINV-specific IgM (B), total IgG (C), IgG1 (D), IgG2a (E), and IgG2b (F) were measured by EIA. The avidity of SINV-specific IgG was measured in sera collected 18 days after infection (G). Each point represents the mean and SEM of sera from three mice analyzed in triplicate.

measured by EIA, were produced with the same kinetics in both GKO and WT mice. However, GKO and GRKO mice produced more IgG1 than WT mice (Fig. 5D), while levels of IgG2a (Fig. 5E) and IgG2b (Fig. 5F) were similar. Eighteen days after infection, the antibodies produced by GKO and WT mice were of similar avidity (Fig. 5G).

Cytokine responses in the brain and the spinal cord. To determine whether mice deficient in IFN-γ and/or antibody differed in innate antiviral responses in the CNS during SINV infection, we analyzed the levels of IFN-β, IFN-α, IL-6, and TNF-α in the brain and spinal cord after infection (Fig. 6). Virus infection triggered rapid production of IFN-β (Fig. 6A and B) and IFN-α (Fig. 6C and D) in all mice, with the highest levels observed in μMT/GKO mice on day 3. SCID, μMT, and μMT/GKO mice, all of which failed to clear virus from the brain (Fig. 2), maintained elevated IFN-β and IFN-α synthesis in the brain, while levels in WT, GKO, and GRKO mice decreased after day 5, when virus clearance was occurring. In the spinal cord, SCID, μMT, and μMT/GKO mice maintained high levels of IFN-β, but this was less apparent for IFN-α.

Patterns of IL-6 synthesis in brains (Fig. 6G) were similar between the strains of mice examined, with the highest levels during acute infection. Production of TNF-α in both brain and spinal cord (Fig. 6E and F) showed less temporal change than IFN-β, IFN-α, or IL-6, and differences between the strains were not identified.

DISCUSSION

Successful recovery from alphavirus infection of neurons is a process involving both innate and adaptive immune responses and has at least three phases: early control of replication and inhibition of spread, clearance of infectious virus, and prevention and control of reactivation (18). This study shows that IFN-β, antibody, and IFN-γ played synergistic roles in SINV clearance that varied in relative importance at different times during the course of infection and for different neuronal populations. IFN-β had an independent role in early control of virus replication in all regions of the CNS, but not for clearance. Production of antiviral antibody was essential for initial clearance from the brain but did not prevent transient reactivation 4 to 10 days after clearance appeared complete. IFN-γ contributed to clearance from the brain and spinal cord and to long-term control of virus replication, and this contribution was most apparent in the absence of antibody. In addition, comparison of virus clearance by μMT/GKO and SCID mice indicated that additional unidentified factors contribute to clearance.

Type I IFNs are responsible for early control of replication and inhibition of the spread of many viruses in vivo. In the absence of the IFN-α/β receptor, mice succumb to alphavirus infection before mounting an adaptive immune response (8, 36, 61). Both IFN-β and IFN-α were produced early after

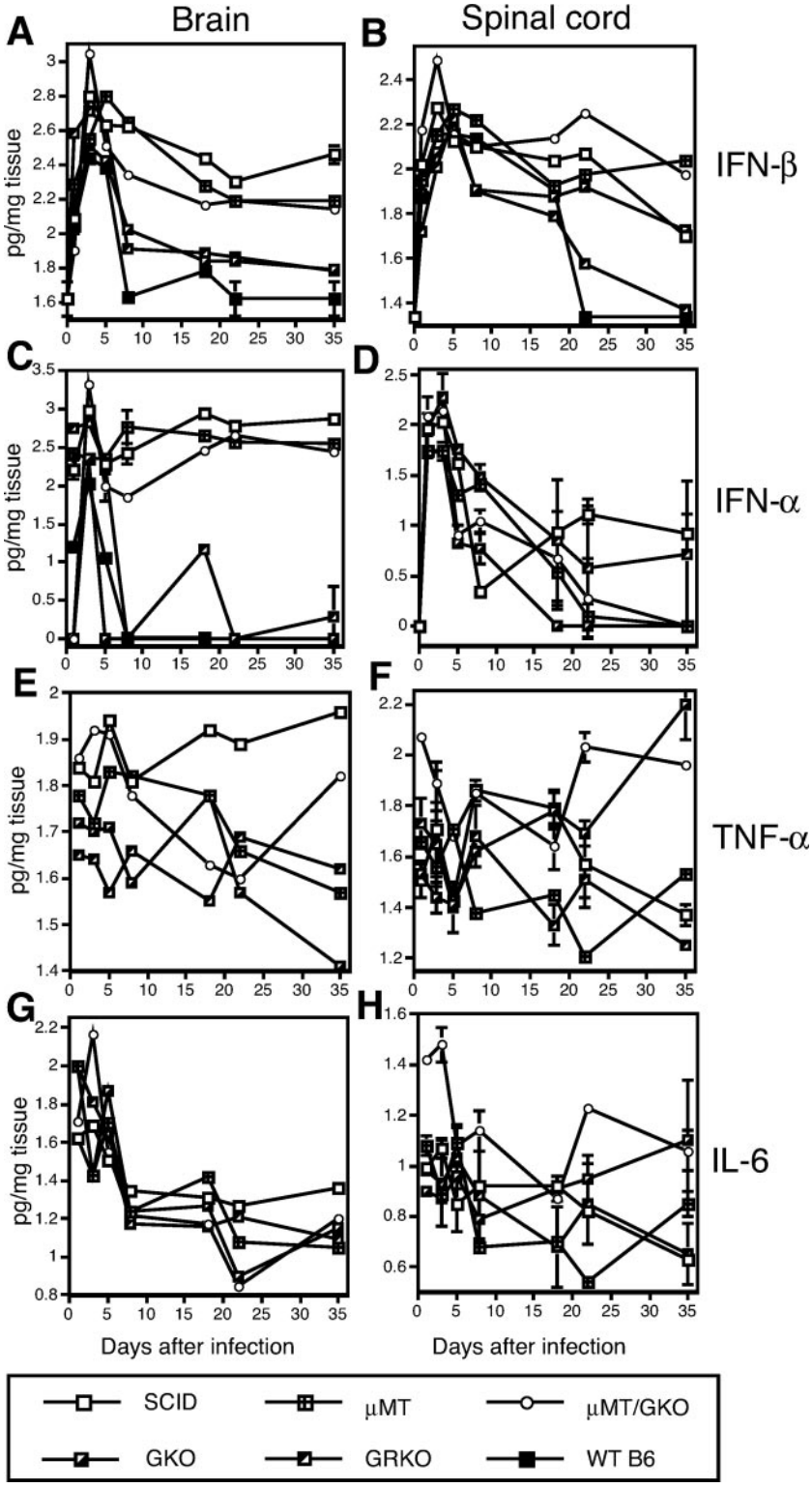

FIG. 6. IFN-β, IFN-α, TNF-α, and IL-6 production in brains and spinal cords of WT, SCID, μMT, μMT/GKO, GKO, and GRKO mice during SINV infection. IFN-β (A and B), IFN-α (C and D), TNF-α (E and F), and IL-6 (G and H) were measured in the brain and spinal cord. Tissues collected in the same experiment from three mice at each time point were analyzed for protein levels by EIA. Spinal cord measurements were standardized by sample protein concentration. Each point represents the average and standard deviation of samples assayed in triplicate.

infection, and production was sustained in mice with persistent SINV replication. Initial SINV replication was higher in BKO mice than in WT mice, indicating an independent role for IFN-β in early control of SINV CNS replication. This may be due to a need for rapid IFN-β production after neuronal infection (43) or for constitutive production of low levels to increase resistance to virus replication in the mature CNS.

IFN-β induces initial synthesis of antiviral genes and of IRF-7, a transcription factor necessary for the production of most IFN-αs, and can prime cells for resistance to infection (24, 30). Alphaviruses are sensitive to the inhibitory effects of IFN (1, 2, 17), and two IFN-induced proteins, ZAP and ISG-15, may play roles in restricting alphavirus replication (5, 33). IFN-β-deficient mice are highly susceptible to coxsackievirus and vaccinia virus infections and exhibit decreased induction of IFN-α and IFN-responsive antiviral genes after infection of peripheral organs (10, 11, 53). However, IFN-α production levels after SINV infection were similar in BKO and WT mice, indicating that IFN-β deficiency did not lead to a deficiency of IFN-α production in the CNS. In fact, the levels of IFN-β and IFN-α in the CNS roughly reflected the amounts of infectious SINV, suggesting that these IFNs were readily induced by virus replication in the CNS.

Previous studies have shown that passive transfer of SINV-specific antibodies clears virus from the brains and spinal cords of persistently infected SCID mice, and the present studies further indicated that antibody is a primary mediator of clearance (6, 35). The mechanism is unclear, but in vitro studies have shown that antibody-mediated clearance requires cross-linking of antibody to viral glycoproteins on the cell surface (57) and involves inhibition of virus budding and restoration of cellular processes, such as protein synthesis and sodium pump function (13). Treatment with antibody results in an improved antiviral response to IFN-α, suggesting a synergistic effect of these two effectors in vivo (12).

Although mice deficient in mature B cells (μMT) were unable to clear virus from the brain and to prevent persistent infection, amounts of virus were lower than in μMT/GKO or SCID mice, indicating that antibody-independent antiviral responses partially control SINV replication. μMT mice do not produce mature B cells or antibody (31) but are also deficient in $CD4^+$ and $CD8^+$ T-cell function. Studies of clearance of other viruses from the CNS suggest that B cells contribute more to control of virus replication than just the production of antibody (4, 23). Lack of complete clearance in μMT mice may be due in part to impaired T-cell responses that are exacerbated in the absence of IFN-γ, thereby underestimating the importance of antibody-independent mechanisms of clearance.

TNF-α and IFN-γ provide noncytolytic control of hepatitis B virus replication in the liver (20, 62) and are candidates for control of virus replication in the CNS. A recombinant rabies virus expressing TNF-α reduces virus replication, increases T-cell infiltration into the CNS, and protects animals (15). However, studies using a recombinant SINV showed that TNF-α enhanced replication in SCID mice (6). Recombinant SINV expressing IFN-γ eliminated infectious SINV from the spinal cord and partially controlled replication in the brain at rates similar to those observed in μMT mice, indicating the importance of this cytokine for clearance, particularly from spinal cord motor neurons (6).

IFN-γ production protects from CNS infection and contributes to clearance of a number of neurotropic viruses (9, 22, 25, 40, 41, 44, 49, 52). The mechanisms by which IFN-γ acts may include direct antiviral effects, as well as regulatory functions important for other immune effector mechanisms (45), such as increasing expression of major histocompatibility complex class I and class II (3). Neurons can respond directly to IFN-γ by signaling through the Jak/Stat-1 pathway to induce expression of a number of antiviral genes (9). Treatment of SINV-infected neurons with IFN-γ restores cellular protein and RNA synthesis and suppresses viral protein and RNA synthesis (7). The IFN-γ-responsive nitric oxide synthase gene is important for suppression of vesicular stomatitis virus replication in neurons, and suppression of nitric oxide synthesis exacerbates SINV-induced disease and may play a role in clearance (9, 54).

μMT/GKO mice deficient in the production of both antibody and IFN-γ developed a persistent infection in the brain and spinal cord that was intermediate in titer between those of μMT and SCID mice, suggesting that nonantibody antiviral factors, in addition to IFN-γ, contribute to clearance. Control of lymphocytic choriomeningitis virus infection in the CNS is defective in granzyme B-deficient mice (63), and T-cell-mediated clearance of West Nile virus, resolution of persistent infection, and enhanced survival of mice are dependent on perforin activity (48). Further studies are needed to examine additional factors that function to control virus replication in different regions of the CNS in the absence of IFN-γ and antibody.

Effective clearance differed between neuronal populations. Virus was least likely to be cleared from brain neurons, where even prior immunization does not always prevent alphavirus replication (39), and most likely to be cleared from motor neurons in the cervical and thoracic regions of the spinal cord. The mechanisms determining differential responses of neuronal populations to immune mediators are poorly understood. IFN-γ production by T cells effects clearance of Borna disease virus from the CNS (22), but IFN-γ protection from neuronal infection is complete in cerebellar cultures, while neurons in hippocampal cultures are only partially protected (16). It is likely that neurons differ either in expression of IFN-γ receptor subunits, in intracellular signaling pathways necessary for an antiviral response, or in the ability to synergize with type I IFN, important for the inhibition of replication of other viruses (42, 46, 47, 59, 60).

Although SINV clearance appeared complete by days 8 to 10 in WT, GKO, and GRKO mice, careful examination of the CNS for the presence of infectious virus revealed a period of recrudescent replication beginning approximately 12 days after infection. These data suggest that persistence of viral RNA in the CNS serves as a source of SINV reactivation even in the presence of competent antibody and T-cell responses. This has also been observed in other RNA virus infections of the CNS. μMT mice clear neurotropic coronavirus infection at rates similar to those of B6 mice in a T-cell-dependent manner but fail to prevent virus reactivation (4). These data suggest that additional immune mechanisms are necessary for complete suppression of virus replication, particularly in motor neurons.

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