Brief Definitive Report

Induction of Protective Cytotoxic T Cell Responses in the Presence of High Titers of Virus-neutralizing Antibodies: Implications for Passive and Active Immunization

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Summary

The effect of preexistent virus-neutralizing antibodies on the active induction of antiviral T cell responses was studied in two model infections in mice. Against the noncytopathic lymphocytic choriomeningitis virus (LCMV), pretreatment with neutralizing antibodies conferred immediate protection against systemic virus spread and controlled the virus below detectable levels. However, presence of protective antibody serum titers did not impair induction of antiviral cytotoxic T lymphocyte (CTL) responses after infection with $10^2$ PFU of LCMV. These CTLs efficiently protected mice independent of antibodies against challenge with LCMV-glycoprotein recombinant vaccinia virus; they also protected against otherwise lethal lymphocytic choriomeningitis caused by intracerebral challenge with LCMV-WE, whereas transfused antibodies alone did not protect, and in some cases even enhanced, lethal lymphocytic choriomeningitis. Against the cytopathic vesicular stomatitis virus (VSV), specific CTLs and Th cells were induced in the presence of high titers of VSV-neutralizing antibodies after infection with $10^6$ PFU of VSV, but not at lower virus doses. Taken together, preexistent protective antibody titers controlled infection but did not impair induction of protective T cell immunity. This is particularly relevant for noncytopathic virus infections since both virus-neutralizing antibodies and CTLs are essential for continuous virus control. Therefore, to vaccinate against such viruses parallel or sequential passive and active immunization may be a suitable vaccination strategy to combine advantages of both virus-neutralizing antibodies and CTLs.

Effective control of acute pathogens is usually mediated by the combination of humoral and cellular immune responses. Vaccines used presently against human pathogens primarily induce protective humoral immune responses. However, an isolated humoral immune response is not sufficient for control, particularly against persistent infections with non- or low cytopathic viruses (1-3). Subprotective levels of neutralizing antibodies may even risk an antibody-dependent enhancement of disease (4, 5), which may be caused by antibodies influencing the balance between virus spread and CTL response-mediating immunopathology.

Here we studied whether neutralizing antibodies influenced induction of a CTL response in the well-studied model infections of mice with the noncytopathic lymphocytic choriomeningitis virus (LCMV) and the cytopathic vesicular stomatitis virus (VSV). The results indicate that active vaccination of hosts exhibiting preexistent neutralizing antibodies permits efficient induction of protective T cell immune responses without dangerous enhancement of immunopathology. Therefore, infection accompanied by passive antibody transfer may be a valid approach particularly for vaccination against noncytopathic viruses with a tendency to persist, which are controlled by combined antibody and T cell responses.

Materials and Methods

Viruses. The LCMV isolate WE (LCMV-WE) was obtained from F. Lehmann-Grube (FASEB, Hamburg, Germany). The VSV serotype Indiana (VSV-IND, Mudd-Sommer isolate) was obtained from B. Kolakowsky (FASEB, Geneva, Switzerland). The following recombinant vaccinia viruses were used: Vacc-G2, ex-

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pressing the full-length LCMV-glycoprotein precursor molecule (gift from D.H.L. Bishop, Oxford University, Oxford, U.K.; reference 6); Vacci-N-D-GP, expressing the glycoprotein of VSV-IN-D; and Vacci-N-D-N-P, expressing the nucleoprotein of VSV-IN-D (both gifts from B. Moss, FASEB, Bethesda, MD; reference 7).

Mice. Inbred C57BL/6 and BALB/c mice were purchased from the Institut für Versuchstierkunde, Universität of Zürich. CD8-deficient mice were provided by Tak W. Mak, FASEB, Toronto, Canada (8).

Generation and Characterization of LCMV-Neutralizing mAbs.

The LCMV-neutralizing mAb KL25 has been previously described (9, 10). The LCMV-neutralizing mAbs WEN3 and WEN4 were generated as follows: CD8-deficient (H-2b) mice and CD8-depleted (11) BALB/c (H-2b) mice were immunized intravenously with 106 PFU LCMV-WE. After 40-60 d, mice were boosted with 5 μg purified LCMV or with two intravenous injections of 106 PFU LCMV-WE. 4 d later, spleen cells were fused with P3x63Ag.8.6 mouse plasmacytoma cells. mAb WEN3 originated from a CD8-deficient mouse, and WEN4 from an anti-CD8-treated BALB/c mouse. mAbs were purified by affinity chromatography (Protein G, Sepharose fast flow; Pharmacia Biotech AB, Uppsala, Sweden). Antibody concentration was measured by optical densitometry. The mAb VI22 neutralizes VSV-IN-D and has been previously described (12).

LCMV and VSV Titer and Neutralization Assay.

LCMV titers from tissue homogenates and vaccinia titers from ovaries were determined as previously described (13, 14). Anti-LCMV- and anti-VSV-neutralizing antibody titers were determined by in vitro reduction of infectious foci or plaques, respectively, as previously described (13, 15).

Cytotoxic Assay.

Spleen cells were restimulated in vitro for 5 d on either thioglycollate-induced (1 ml intraperitoneally 6 d before day 1 of restimulation) LCMV-infected (200 PFU intraperitoneally 4 d before day 1 of restimulation) peritoneal macrophages or on spleen cells loaded with the VSV-NP peptide p49-62 (16). Cytotoxic activity was assessed against peptide-loaded M C57G target cells (LCMV-GP33-41, reference 17; LCMV-NP396-408, reference 18; VSV-NP49-62) in a standard 51Cr-release assay (19). Spontaneous release was always <20%.

Results and Discussion

Neutralization of LCMV In Vivo.

Two newly selected LCMV-neutralizing mAbs, WEN3 and WEN4, were compared to the LCMV-neutralizing mAb KL25 (9) with respect to their neutralizing capacity in C57BL/6 mice. Intraperitoneal transfer of 200 μg of purified mAb led to LCMV-neutralizing serum antibody titers of 1/80 to 1/40 on days 1, 2, and 4 after mAb treatment. Mice were intravenously infected with 200 PFU of LCMV-WE 4 h after antibody treatment. On day 4 after infection, when the virus reaches maximal titers in naive mice, LCMV titers were determined in spleen. All mAb-treated mice had LCMV-WE titers below detection limits (Fig. 1A). Mice treated intraperitoneally with different doses of purified mAb WEN3 and intravenously infected with 200 PFU of LCMV-WE 4 h later were optimally protected after transfer of 200 μg of the mAb (Fig. 1B). Similar results were obtained after transfer of mAbs KL25 and WEN4 (data not shown). To exclude the possibility that LCMV in spleen was only masked by neutralizing mAbs the following experiment was performed: mice were treated with 200 μg of the mAb KL25 4 h before intravenous infection with 200 PFU of LCMV-WE; 5 d later, one group of mice was perfused with PBS under general anesthesia and then killed. Viral titers in spleen were determined. Irrespective of perfusion, all mAb-treated mice showed reduction of replicating virus below detection limit, whereas in untreated controls, high titers of replicating virus were present. Furthermore, we failed to detect neutralizing activity in organ homogenates of mice given 200 μg of the neutralizing mAb KL25 5 d before death (data not shown).

Virus-specific CTLs Are Induced in the Presence of Protective Levels of Neutralizing mAb.

Transfused LCMV-neutralizing mAbs have been demonstrated to protect against LCMV after systemic intravenous infection (4, 20). To test whether under such conditions induction of antiviral protective memory CTLs is still possible, CTL induction was analyzed after infection with LCMV-WE in the presence of neutralizing mAb; mice were treated intraperitoneally with 200 μg of mAb KL25 and infected intravenously with 200 PFU of LCMV-WE 4 h later. On day 20 after infection, lytic activity of spleen cells was tested in a 51Cr-release assay after 5 d restimulation in vitro, (Fig. 2, A–C). LCMV-specific CTL activities were only marginally reduced in mAb-treated mice when compared to untreated mice. Similar results were obtained after treatment with mAbs WEN3 and WEN4 (data not shown).

These findings were confirmed for infections of mice with VSV. Mice were intraperitoneally treated with 100 μg of the VSV-neutralizing mAb VI22 4 h prior to intravenous infection with 2 × 106 PFU VSV-IND. This transfer of mAb led to VSV-neutralizing serum titers of 1/20000, which has been shown to protect against lethal VSV infection (21). Similar to the LCMV infection experiments, mice treated with VSV-neutralizing mAb VI22 exhibited VSV-specific memory CTL activity comparable to untreated control mice (Fig. 2, D and E). Importantly, CTL induction in the presence of VSV-neutralizing mAbs was dose-dependent; although doses of 104 and 105 PFU of the abortively replicating VSV-IND intravenously induced VSV-specific mem-
Lytic activity of spleen cells from mAb-treated mice. C57BL/6 mice were intraperitoneally treated with 200 μg of the LCMV-neutralizing mAb KL25 (A–C), and the VSV-neutralizing mAb VI22 (D–I), or left untreated (A–I) and were intravenously infected with 200 PFU of LCMV-WE (A–C), 2 × 10⁶ PFU (D and E), 10⁴ PFU (F and G), or 10³ PFU (H and I) VSV-IND, respectively. At day 20 after infection, spleen cells were restimulated in vitro for 5 d, and CTL activity was determined in a standard 5-h Cr-release assay on MC57G target cells loaded with the LCMV-derived peptides GP33 (A) and NP396 (B), the VSV derived peptide NP49 (D, F, and H), or on unloaded target cells (C, E, G, I).

Shown are values of individual mice from one of three similar experiments. Spontaneous release was <20%.

Table 1. Effect of Protective LCMV-neutralizing Antibodies on Induction of Protective Cytotoxic T Cells

| Challenge infection with Vacc-G2 | Vacc-G2 (log PFU per ovary) |
|---------------------------------|-----------------------------|
| Vaccination                     |                             |
| Active                          | Passive                     |
| LCMV-WE                         | KL25                        | WEN3 |
| (200 PFU i.v.)                  | (200 μg i.p.)               | (200 μg i.p.) |
| Group                           |                              |      |
| 1                               | 6.2 ± 0.4                    |      |
| 2                               | 5.8 ± 0.8                    |      |
| 3                               | 6.1 ± 0.6                    | <1.7 |
| 4                               | +                            | <1.7 |
| 5                               | +                            |      |
| 6                               | +                            | 1.8 ± 0.2 |

Groups of four C57BL/6 mice were treated intraperitoneally (i.p.) as indicated with 200 μg of the LCMV-neutralizing mAbs KL25 or WEN3, or left untreated and primed intravenously (i.v.) with 200 PFU of LCMV-WE, or left uninfected. 10 d later, mice were challenged i.p. with 4 × 10⁶ PFU of Vacc-G2, and vaccinia titers in ovaries were determined 5 d after challenge. Shown are means of log vaccinia titers (± SEM) of four mice per group.

Table 1 shows the effect of protective LCMV-neutralizing antibodies on induction of protective cytotoxic T cells. The table compares the log PFU of Vacc-G2 per ovary in mice treated with different combinations of LCMV-neutralizing antibodies and vaccinia viruses.

Key findings:
- Active vaccination with LCMV-WE increased the protection against Vacc-G2 compared with control mice.
- Passive vaccination with LCMV-neutralizing antibodies alone did not provide protective effect.
- The combination of active vaccination with LCMV-neutralizing antibodies and passive vaccination with Vacc-G2 provided the highest level of protection.

Figure 2. Lytic activity of spleen cells from mAb-treated mice. C57BL/6 mice were intraperitoneally treated with 200 μg of the LCMV-neutralizing mAb KL25 (A–C) and the VSV-neutralizing mAb VI22 (D–I), or left untreated (A–I) and were intravenously infected with 200 PFU of LCMV-WE (A–C), 2 × 10⁶ PFU (D and E), 10⁴ PFU (F and G), or 10³ PFU (H and I) VSV-IND, respectively. At day 20 after infection, spleen cells were restimulated in vitro for 5 d, and CTL activity was determined in a standard 5-h Cr-release assay on MC57G target cells loaded with the LCMV-derived peptides GP33 (A) and NP396 (B), the VSV derived peptide NP49 (D, F, and H), or on unloaded target cells (C, E, G, I). Shown are values of individual mice from one of three similar experiments. Spontaneous release was <20%.

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neutralizing hyperimmune sera did not protect against fatal choriomeningitis after intracerebral infection with a low dose (10^2 PFU) of LCMV-WE (24). Earlier studies had shown that transfused LCMV-neutralizing hyperimmune sera against cytopathic viruses such as respiratory syncytial virus, rabies virus, and influenza virus, where preexistent neutralizing antibodies impaired induction of CTLs (26–29). Like VSV, and in contrast to LCMV, infections with these viruses are efficiently controlled by primary antibody responses. Preexistent antibody titers seem to very effectively neutralize virus, so that no or insufficient antigen is generated; thereby induction of CTLs is impaired in a dose-dependent manner.

Combinations of active and passive immunization are used in adults for antivaccinia virus vaccination, where immune sera are administered in parallel to active immunization if complications are expected. Similar strategies have been discussed for vaccinations against hepatitis virus A and B or for herpes simplex viruses. This study indicates that vaccination strategies that combine passive and active immunization are effective and may be especially advantageous for achieving protective immunity against viruses that tend to establish persistent infections and that are only well controlled by combined action of antibodies and CTLs (possibly including HIV; references 3, 30, 31).

**Table 2.** Effect of protective VSV neutralizing Antibody on Induction of Protective Cytotoxic T Cells and Helper T Cells

| Vaccination | Challenge infection with: |
|-------------|--------------------------|
|             | Vacc-IND-NP               | Vacc-IND-GP               |
| Group       | (2 x 10^6 PFU i.v.) | (200 µg i.p.) |
| (n = 4)     | V122                     | VI22                      |
| 1           | 6.4 ± 1.1                | 5.7 ± 1.4                |
| 2           | 3.1 ± 1.8                | 5.9 ± 0.2                |
| 3           | <1.7                     | <1.7                     |
| 4           | <1.7                     | 3.1 ± 1.1                |

Groups of four C57BL/6 mice were treated intraperitoneally (i.p.) with 100 µg of the VSV-neutralizing mAb V122 and/or were primed intravenously (i.v.) with 2 x 10^6 PFU of VSV-IND as indicated. 10 d later, mice were challenged i.p. with 4 x 10^6 PFU of Vacc-IND-NP or Vacc-IND-GP, and vaccinia titers in ovaries were determined 5 d later. Shown are means of log vaccinia titers (± SEM) of four mice per group.

**Figure 3.** No CTL-mediated choriomeningitis was detectable in mice pretreated with mAb and infected with LCMV-WE. 20 d before intracerebral challenge, groups of six C57BL/6 mice were treated with 200 µg of mAb KL25 (□), WEN3 (▲), and were intravenously primed with 200 PFU of LCMV-WE 4 h after treatment or were intravenously primed with 200 PFU LCMV-WE only (○). Control mice were treated with 200 µg of mAbs KL25 (■) or WEN3 (▲) 4 h before intracerebral challenge, or left completely untreated (●) before intracerebral challenge. All mice were challenged intracerebrally with 30–300 PFU of LCMV-WE, and survival was monitored twice daily.
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References

1. Haynes, B.F., G. Pataulea, and A.S. Fauci. 1996. Toward an understanding of the correlates of protective immunity to HIV infection. Science. 271:324–328.

2. Steiner, I. 1996. Human herpes viruses latent infection in the nervous system. Immunol. Rev. 152:157–173.

3. Plückthun, A. 1994. Human antibodies to HIV in vivo. Immunol. Rev. 137:275–288.

4. Battegay, M., S. Osmani, M. Schulz, H. Hengartner, and R.M. Zinkernagel. 1995. Early high affinity neutralizing anti-viral IgG without affinity maturation during a virus infection. Proc. Natl. Acad. Sci. USA. 92:1257–1261.

5. Battegay, M., S. Cooper, A. Althage, J. Baenziger, H. Hengartner, and R.M. Zinkernagel. 1991. Quantification of lymphocytic choriomeningitis virus with an immunological focus assay in 24 or 96 well plates. J. Virol. Methods. 33:191–198.

6. Binder, D., and T.M. Kündig. 1991. Antiviral protection by CD8 versus CD4 T cells: CD8 T cells correlating with cytotoxic activity in vitro are more efficient in antivaccinia virus protection than CD4-dependent interleukins. J. Immunol. 146:4301–4307.

7. Charan, S., A.W. Hügin, A. Cerny, H. Hengartner, and R.M. Zinkernagel. 1986. Effects of cyclosporin A on humoral immune response and resistance against vesicular stomatitis virus in mice. J. Virol. 57:1139–1144.

8. van Bleek, G.M., and S.G. Nathenson. 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I H-2K molecule. Nature. 348:213–216.

9. Schulz, M., P. Aichele, M. Vollweide, F.W. Bobe, F. Cardinaux, H. Hengartner, and R.M. Zinkernagel. 1989. MHC-dependent T cell epitopes of LCMV nucleoprotein and their protective capacity against viral disease. Eur. J. Immunol. 19:1657–1667.

10. Zinkernagel, R.M., T.P. Leith, H. Hengartner, and A. Althage. 1985. Susceptibility to lymphocytic choriomeningitis virus isolates correlates directly with early and high cytotoxic T cell activity, as well as with footpad swelling reaction, and all three are regulated by H-2K<sup>b</sup> molecule. J. Exp. Med. 162:2125–2141.

11. 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.

12. Bachmann, M.F., U. Kalinke, A. Althage, G. Freer, C. Burkhardt, H.-P. R oost, M. Aguet, H. Hengartner, and R.M. Zinkernagel. 1997. The role of antibody concentration and avidity in antiviral protection. Science. 276:2024–2027.

13. Zinkernagel, R.M., S. Cooper, J. Chambers, R.A. Lazzarin, H. Hengartner, and H. Arnhelte. 1990. Virus-induced autoantibody response to a transgenic viral antigen. Nature. 344:653.
23. Bachmann, M.F., R.U. Hoffmann, T.M. Kündig, K. Burk, H. Hengartner, and R.M. Zinkernagel. 1993. The influence of antigen organization on B cell responsiveness. Science. 262:1448–1451.

24. Cole, G.A., N. Nathanson, and R.A. Prendergast. 1972. Requirement for theta-bearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. Nature. 238:335–337.

25. Doherty, P.C., and R.M. Zinkernagel. 1975. Capacity of sensitized thymus-derived lymphocytes to induce fatal lymphocytic choriomeningitis is restricted by the H-2 gene complex. J. Immunol. 114:30–33.

26. Bangham, C.R.M. 1986. Passively acquired antibodies to respiratory syncytial virus impair the secondary cytotoxic T-cell response in the neonatal mouse. Immunology. 59:37–41.

27. Xiang, Z.Q., and H.C. Ertl. 1992. Transfer of maternal antibodies results in inhibition of specific immune responses in the offspring. Virus Res. 24:297–314.

28. Yap, K.L., and G.L. Ada. 1979. The effect of specific antibody on the generation of cytotoxic T lymphocytes and the recovery of mice from influenza virus infection. Scand. J. Immunol. 10:325–332.

29. Leung, K., R.B. Ashman, C. Hildebrandt, J. Ertl, and G.L. Ada. 1980. Selective suppression of the cytotoxic T cell response to influenza virus in mice. Eur. J. Immunol. 10:803–810.

30. Planz, O., P. Seller, H. Hengartner, and R.M. Zinkernagel. 1996. Specific cytotoxic T cells eliminate cells producing neutralizing antibodies. Nature. 382:726–728.

31. Koup, R.A., J.T. Safrit, Y. Cao, C.A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D.D. Ho. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. 68:4650–4655.