JOURNAL OF VIROLOGY, Oct. 1997, p. 7253–7257 Vol. 71, No. 10
0022-538X/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Antiviral Immune Responses in Itk-Deficient Mice

MARTIN F. BACHMANN,[1]* DAN R. LITTMAN,[2]† AND X. CHARLENE LIAO[2]‡

Department of Pathology, University of Zurich, Zurich, Switzerland,[1] and Howard Hughes Medical Institute, Department of Microbiology and Immunology, University of California, San Francisco, California[2]

Received 28 January 1997/Accepted 20 June 1997

Mice lacking Itk, a T-cell-specific protein tyrosine kinase, have reduced numbers of T cells and reduced responses to allogeneic major histocompatibility molecules. This study analyzed antiviral immune responses in mice deficient for Itk. Primary cytotoxic T-lymphocyte (CTL) responses were analyzed after infection with lymphocytic choriomeningitis virus (LCMV), vaccinia virus (VV), and vesicular stomatitis virus (VSV). Ex vivo CTL activity was consistently reduced by a factor of two to six for the different viruses. CTL responses after restimulation in vitro were similarly reduced unless exogenous cytokines were added. In the presence of interleukin-2 or concanavalin A supernatant, Itk-deficient and control mice responded similarly. Interestingly, while LCMV was completely eliminated by day 8 in both Itk-deficient and control mice, VV cleared from *itk*$^{-/-}$ mice with delayed kinetics. Antibody responses were evaluated after VSV infection. Both the T-cell-independent neutralizing immunoglobulin M (IgM) and the T-cell-dependent IgG responses were similar in Itk-deficient and control mice. Taken together, the results show that CTL responses are reduced in the absence of Itk whereas antiviral B-cell responses are not affected.

The T-cell antigen receptor (TCR) recognizes peptides bound to the major histocompatibility complex (MHC) molecules. Such an interaction initiates a cascade of biochemical events involving protein tyrosine kinases. Recent biochemical and genetic evidence has established the importance of nonreceptor tyrosine kinases such as Lck, Fyn, and Zap70 in TCR signal transduction (38). A newly identified nonreceptor tyrosine kinase, Itk (interleukin-2-inducible T-cell kinase), has been shown to be phosphorylated after TCR stimulation (1). Itk is homologous to the Bruton's tyrosine kinase (Btk) expressed in B cells. Mutations in Btk are associated with X-linked agammaglobulinemia in humans and with X-linked immunodeficiency (*xid*) in mice (34, 36). In both cases, the defect is restricted to the B-cell lineage. Most X-linked agammaglobulinemia patients have few or no mature B cells, whereas mice with the *xid* mutation or with a null allele of Btk generated by gene targeting display a reduced number of mature B cells of 50% of the normal level (17, 18). These mature B cells are also impaired in responding to surface immunoglobulin (Ig) stimulation (18). Itk is specifically expressed in T cells and mast cells (10, 12, 13, 25, 33, 39). Recently, mice defective for Itk expression have been generated by gene targeting (24). Similar to *xid* mice with B-cell defects or mice with a targeted deletion of the Btk gene, Itk-deficient mice had reduced numbers of T cells, especially CD4$^+$ T cells. Mature T cells in Itk-deficient mice were hyporesponsive to cross-linking of the TCR complex. Study of T-cell maturation using TCR transgenic mice in the Itk-deficient background demonstrates that Itk plays an important role in the positive selection of thymocytes (24). In the Jurkat T-cell line, Itk has also been shown to be associated with CD28, a T-cell surface molecule crucial for providing a costimulatory signal for T-cell activation (1, 14). Upon cross-linking of CD28, Itk is tyrosine phosphorylated and the kinase activity of Itk is also increased (1). Furthermore, a recent study using an Lck-deficient Jurkat T-cell line suggests that Itk activation after CD28 cross-linking is dependent on Lck (11).

T lymphocytes play an important role in the battle with viruses. Engagement of the TCR, activation of the CD28 costimulatory molecule, and production of cytokines all contribute to immunity against viruses. The role of each of these T-cell effector functions varies with the virus and the host. To assess the responsiveness of Itk-deficient T cells in vivo, we used three different viruses: lymphocytic choriomeningitis virus (LCMV), vaccinia virus (VV), and vesicular stomatitis virus (VSV). LCMV and VV induce strong albeit reduced cytotoxic T-lymphocyte (CTL) responses in the absence of interleukin-2 (IL-2) (6, 9, 19) and CD28 (21), whereas the weaker VSV-specific response depends to a higher degree on T-cell help (4) and IL-2 (1a) and is completely dependent on CD28-mediated costimulation (21). After primary infection, LCMV is exclusively eliminated via CD8$^+$ CTLs in a perforin-dependent fashion (15), whereas protection from VV replication is more complex and involves both CD4$^+$ and CD8$^+$ T cells (8, 15, 16, 20, 29). Protection from primary VSV infection, on the other hand, is independent of CD8$^+$ T cells but is exclusively mediated by antibody (5, 22). VSV induces a strong T-cell-independent type I neutralizing IgM response peaking around day 4 which is followed by a T-cell dependent, long-lasting IgG response (2). Although protection from VSV replication can be mediated by the early T-cell-independent IgM antibodies, IgG antibodies seem to be more efficient and the presence of functional T helper cells is therefore beneficial for the survival of infected mice. Infection of Itk-deficient mice with these different viruses revealed a normally responsive B-cell compartment. In contrast, CTL responses were impaired against all three viruses, indicating an in vivo role of Itk for T- but not B-cell responses.

MATERIALS AND METHODS

Mice. The generation of Itk-deficient mice has been described previously (24). The *itk*$^{-/-}$ mice and their control *itk*$^{+/-}$ littermates were of a mixed genetic background (50% C57BL/6, 50% 129/Sv). Mice were maintained under specific-pathogen-free conditions.

Detection of LCMV-specific CTL in vitro. Mice were immunized, and 8 days later, spleen cell suspensions were prepared and tested directly in a ^{51}Cr release, using peptide (derived from the LCMV glycoprotein, amino acids 33 to 42) GP33

* Corresponding author. Present address: Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, 610 University Ave., Toronto, Ontario M5G 2M9, Canada.

† Present address: Howard Hughes Medical Institute, The Skirball Institute of Biomolecular Medicine, New York University Medical Center, New York, NY 10016.

‡ Present address: Tularik Inc., South San Francisco, CA 94080.

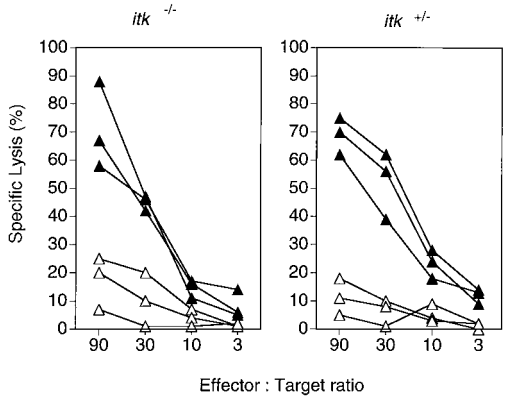

FIG. 1. Primary LCMV-specific CTL responses in Itk-deficient mice. Mice were immunized with LCMV (200 PFU), and virus-specific cytotoxicity was assessed in a ^{51}Cr release assay on day 8 of infection. EL-4 cells labeled with GP33 (closed triangles) or left untreated (open triangles) were used as target cells. Each line represents one mouse. Spontaneous release of ^{51}Cr was below 20%. One representative experiment of two is shown.

(27)-labeled EL-4 cells as target cells. Alternatively, cells were restimulated for 5 days with peptide GP33-labeled, irradiated (25 Gy) spleen cells (10^6 cells/well) at a density of 4×10^6 spleen cells in 2 ml of Iscove modified Dulbecco medium supplemented with 10% fetal calf serum. Restimulated spleen cells were resuspended in 0.5 ml of medium per culture well, and serial threefold dilutions of effectors were performed (referred to as dilution of standard culture) and tested in a conventional ^{51}Cr release assay, using GP33-labeled EL-4 cells as targets.

Detection of VV-specific CTL in vitro. Mice were immunized, and 6 days later, spleen cell suspensions were prepared and tested directly in a ^{51}Cr release assay, using VV-infected MC57G cells as targets.

Detection of VSV-specific CTL in vitro. Mice were infected with VSV (2×10^6 PFU), and spleens were removed 6 days later and tested directly in a ^{51}Cr release assay, using peptide (derived from the VSV nucleoprotein, amino acids 49 to 62, the major CTL target in *H-2*^{b} [28, 35])-pulsed EL-4 cells as target cells.

Serum neutralization test. Serum samples were collected from mice at specific time points after VSV infection. The sera were prediluted 40-fold in minimal essential medium containing 5% fetal calf serum and then heat inactivated for 30 min at 56°C. Serial twofold dilutions were mixed with equal volumes of VSV diluted to contain 500 PFU/ml. The mixture was incubated for 90 min at 37°C in an atmosphere with 5% CO_2. Then 100 μl of the serum-virus mixture was transferred onto Vero cell monolayers in 96-well plates and incubated for 1 h at 37°C. The monolayers were then overlaid with 100 μl of Dulbecco modified Eagle medium containing 1% methylcellulose. After incubation for 24 h at 37°C, the overlay was flicked off and the monolayer was fixed and stained with 0.5% crystal violet. The highest dilution of serum that reduced the number of plaques by 50% was taken as the titer. Due to the addition of an equal volume of virus, the titer of serum was considered to be one step higher. To determine IgG titers, undiluted serum was pretreated with an equal volume of 0.1 mM 2-mercaptoethanol in saline (30).

Enzyme-linked immunosorbent assay (ELISA). Ninety-six-well plates (Petra Plastic, Chur, Switzerland) were incubated with recombinant VSV nucleoprotein (0.1 μg/well) in 0.1 M NaH_2PO_4 (pH 9.6) at 4°C. Plates were then blocked with 2% bovine serum albumin in phosphate-buffered saline for 2 h and washed; and serial dilutions of serum samples were added to the wells and then the plates were incubated for 1 h. Plates were washed and incubated with goat anti-mouse IgG (Sigma). After 1 h, plates were washed and developed with ABTS (5 mg of 2,2′-azino-di-3-ethyl-benzthiazolinesulfonate and 20 μl of H_2O_2 in 50 ml of $NaHCO_3$ [pH 4.0]). Optical density was determined at 405 nm.

RESULTS

LCMV-specific CTL responses. Itk-deficient and control littermates were immunized with 200 PFU of LCMV strain WE. Eight days later, at the peak of the response, spleen cells were isolated and tested on EL-4 cells pulsed with peptide GP33, the major CTL epitope of LCMV in the *H-2*^{b} haplotype (27). CTL responses were slightly but consistently reduced in the absence of Itk (Fig. 1). Calculation of lytic units indicated that the response was reduced by a factor of about 2 (Table 1). Injection of LCMV into the footpads of mice leads to a local footpad swelling that is initially exclusively mediated by $CD8^+$ T cells (26). The amount of footpad swelling is therefore an additional readout for the induction of LCMV-specific CTLs. Mice were immunized in the footpads with 200 PFU of LCMV strain WE, and footpad swelling was monitored daily. Itk-deficient mice always developed the footpad swelling with a delay of 1 day, confirming the reduced CTL response observed in the ^{51}Cr release assay (Fig. 2).

TABLE 1. Itk-deficient mice mount two- to sixfold-reduced CTL responses after infection with LCMV, VV, or VSV[a]

Mouse group	CTL response (LU) against:		
	LCMV	VV	VSV
Control	806 ± 165	2,200 ± 690	925 ± 220
Itk deficient	437 ± 37.8	803 ± 176	150 ± 70

[a] Mean values for six (LCMV and VV) or four (VSV) mice are given. All three differences between Itk-deficient and control mice were statistically significant ($P < 0.05$; Student's *t* test). Lytic units (LU) were calculated for 30% specific lysis.

IL-2-deficient mice mount three- to ninefold-reduced but still efficient primary LCMV-specific CTL responses. In contrast, IL-2-deficient splenocytes fail to lyse target cells after 5 days of in vitro stimulation unless exogenous IL-2 is added (6, 9, 19). To determine whether Itk-deficient mice might exhibit a similar phenotype, spleen cells from LCMV-infected mice were restimulated with GP33 for 5 days. In contrast to a lack of IL-2, absence of Itk did not prevent in vitro restimulation of CTLs (Fig. 3). However, as observed in the primary assay, CTL responses were considerably reduced (Fig. 3). Addition of concanavalin A (ConA) supernatant completely overcame this reduction, and addition of IL-2 (50 U/ml) partly overcame it (Fig. 3).

VV-specific CTL responses. Mice were immunized with 2×10^6 PFU of VV strain WR, and lytic activity of splenocytes was tested 6 days later on VV-infected MC57G cells. As observed for LCMV, CTL responses were reduced about twofold in the absence of Itk (Fig. 4; Table 1). After in vitro restimulation for 5 days with VV-infected spleen cells, CTL activity in the absence of Itk was still reduced but clearly above the background level. If ConA supernatant was added during the in vitro cultivation, CTL activities of Itk and control splenocytes were comparable, while IL-2 had an intermediate effect (not shown).

VSV-specific CTL and antibody responses. VSV is a poorly virulent virus in mice and does not measurably replicate extraneuronally. Only if it reaches the central nervous system does it induce a lethal encephalitis (37). Probably due to this low virulence, VSV-specific CTL responses are most dependent on the presence of IL-2, T helper cells, and CD28. In fact, no

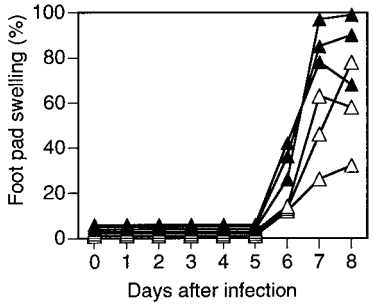

FIG. 2. Induction of local footpad swelling reaction after infection with LCMV. Itk-deficient (open triangles) and control (closed triangles) mice were immunized in the footpads with LCMV (200 PFU), and footpad swelling was assessed daily with a spring-loaded caliper. Each point represents the mean of two hind footpads. One representative experiment of two is shown.

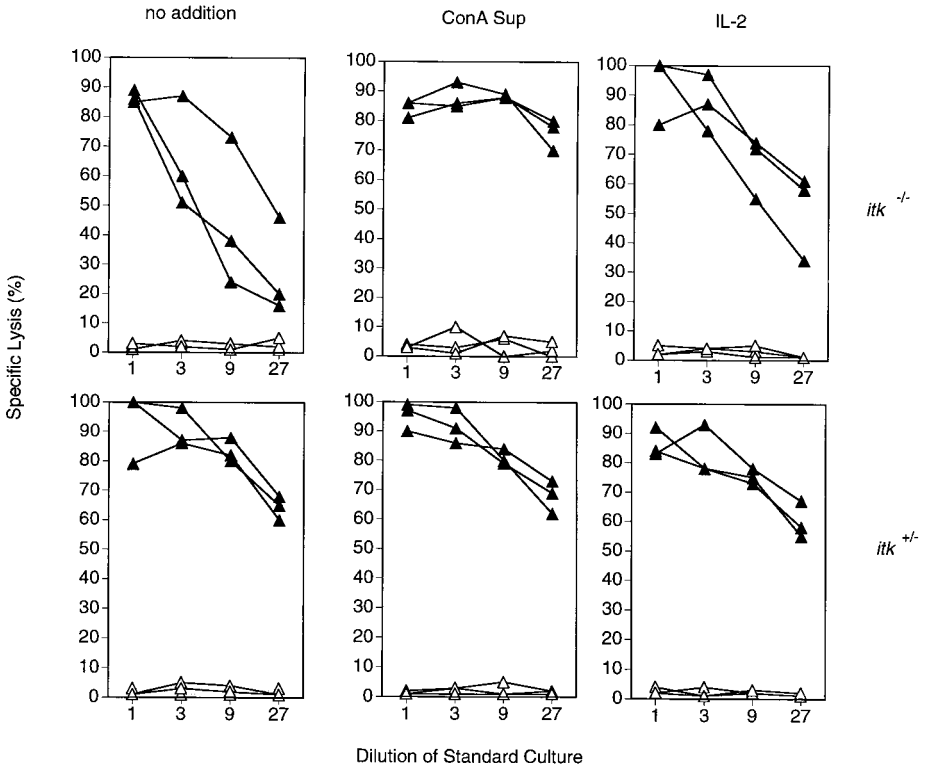

FIG. 3. In vitro restimulated secondary anti-LCMV CTL response in Itk-deficient mice. Itk-deficient (upper panel) and control (lower panel) mice were immunized with LCMV (200 PFU), and 8 days later, spleen cell suspensions were restimulated for 5 days with GP33-pulsed spleen cells in vitro either in the absence of exogenous cytokines (first column) or in the presence of ConA supernatant (second column) or IL-2 (50 U/ml) (third column). Cultures were tested on EL-4 cells labeled with GP33 (closed triangles) or left untreated (open triangles). Spontaneous release of ^{51}Cr was below 20%. One representative experiment of two is shown.

response could be observed in CD28-deficient mice (21). Itk-deficient and control mice were immunized with 2×10^6 PFU of VSV serotype Indiana, and 6 days later, spleen cells were tested in a ^{51}Cr release assay. The reduction of CTL responses was about sixfold with this virus in the absence of Itk (Fig. 5; Table 1). This reduction was also observed after in vitro restimulation (not shown). As observed for LCMV and VV, recombinant IL-2 partly restored the response and ConA supernatant fully restored it (not shown).

In a separate experiment, mice were immunized with 2×10^6 PFU of VSV, and neutralizing antibody titers in sera were determined at the indicated time points. Both the T-cell-independent IgM and T-cell-dependent IgG responses were comparable in Itk-deficient and control mice at all time points (Fig. 6). The glycoprotein of VSV is highly repetitive in the viral envelope and therefore strongly immunogenic (3). To assess the B-cell response against a less repetitive protein, an ELISA specific for the nucleoprotein of VSV was performed. As found for the neutralizing response, Itk-deficient mice mounted a normal VSV nucleoprotein-specific response (Fig. 6).

Antiviral protection. The three viruses tested in this study differ with respect to how they are eliminated from the host after primary infection. Whereas LCMV is controlled via CTLs, VV replication is inhibited by both $CD4^+$ and $CD8^+$ T cells and VSV is exclusively eliminated by antibodies (5, 8, 15, 16, 20, 22, 29). Itk-deficient mice were completely resistant against VSV infection, and no symptoms of encephalitis developed. This is readily explained by the normal antiviral B cell response in these mice. Although the LCMV-specific CTL response was reduced in the absence of Itk, virus was completely eliminated by day 8 after infection, indicating that the diminished CTL response was still protective (not shown). In contrast, the protective VV-specific immune response was more seriously impaired, and viral titers were clearly elevated in Itk-deficient mice 6 days after infection (Fig. 7). Since Itk is expressed only in T cells, these results indicate that the virus was cleared with delayed kinetics due to the impaired T-cell response in the absence of Itk. This interpretation is supported

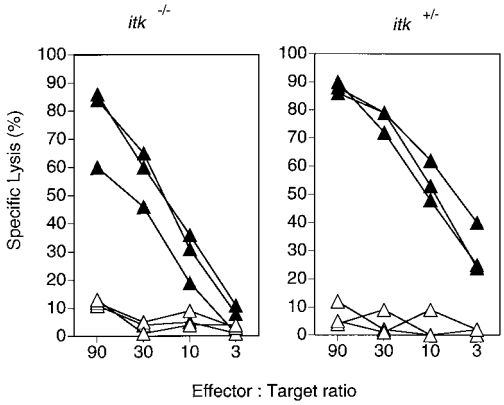

FIG. 4. Primary VV-specific CTL responses in Itk-deficient mice. Mice were immunized with VV (2×10^6 PFU), and virus-specific cytotoxicity was assessed in a ^{51}Cr release assay on day 6 of infection. MC57G cells infected with VV (closed triangles) or left untreated (open triangles) were used as target cells. Each line represents one mouse. Spontaneous release of ^{51}Cr was below 20%. One representative experiment of two is shown.

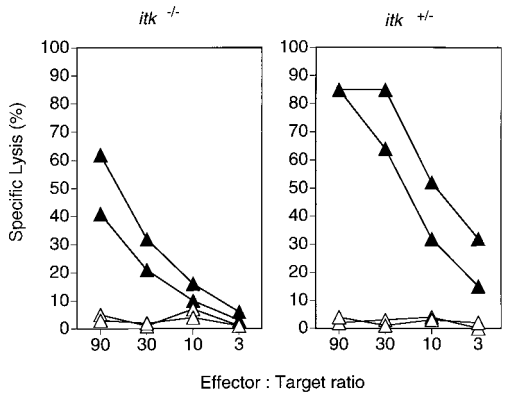

FIG. 5. Primary VSV-specific CTL responses in Itk-deficient mice. Mice were immunized with VSV (2×10^6 PFU), and virus-specific cytotoxicity was assessed in a ^{51}Cr release assay on day 6 of infection. EL-4 cells labeled with peptide (closed triangles) or left untreated (open triangles) were used as target cells. Each line represents one mouse. Spontaneous release of ^{51}Cr was below 20%. One representative experiment of two is shown.

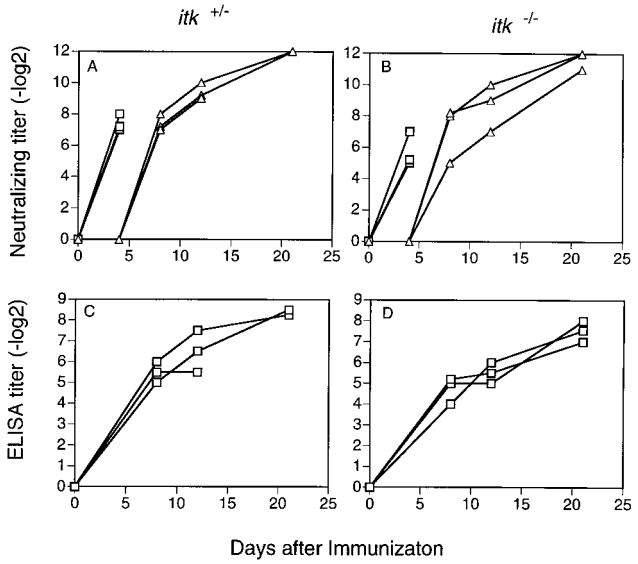

FIG. 6. VSV-specific antibody responses in Itk-deficient mice. Itk-deficient Control (A and C) and Itk-deficient (B and D) mice were immunized with VSV (2×10^6 PFU), and the neutralizing IgM (squares) and IgG (triangles) antibody response was assessed at the indicated time points from 40-fold-prediluted sera (A and B). Alternatively, VSV nucleoprotein-specific IgG responses were analyzed by ELISA (C and D). Each line represents one mouse. One representative experiment of two is shown.

by the finding that the LCMV-induced $CD8^+$ T-cell-mediated footpad swelling was delayed by 1 day (Fig. 2).

DISCUSSION

This study analyzed antiviral CTL and B-cell responses in Itk-deficient mice. While normal B-cell responses were mounted, CTL responses were, depending on the virus used for immunization, reduced by a factor of 2 to 6. This study provides the first evidence indicating that function of $CD8^+$ T cells in Itk-deficient mice is impaired.

Itk is expressed in T cells, mast cells, and human but not mouse natural killer cells (10, 12, 13, 33, 39). Itk-deficient mice have a perturbed T-cell development and exhibit reduced numbers of peripheral T cells (24). The reduction in numbers of mature T cells was mainly caused by a reduction in the numbers of $CD4^+$ T cells to about 50% of the normal level. The numbers of $CD8^+$ T cells in Itk-deficient mice, on the average, are not significantly different from those in control $itk^{+/-}$ or $itk^{+/+}$ mice (24). In assays using purified $CD4^+$ T cells from Itk-deficient mice and control littermates, reduced proliferative responses to allogeneic MHC stimulation and to anti-TCR cross-linking were found in the absence of Itk (24). Although T cells from Itk-deficient mice also showed reduced proliferative responses to certain differences in MHC class I molecules (22a), the function of $CD8^+$ T cells in Itk-deficient mice has not been studied in great detail. The present study used three viruses to address the antiviral effector functions in Itk-deficient mice. Of the three viruses used, LCMV and VV can induce an efficient CTL response in the absence of T helper cells (4). Our results suggest an inherent defect of $CD8^+$ T cells of Itk-deficient mice in antiviral function.

We do not believe that the reduced numbers of mature T cells in Itk-deficient mice are the explanation for our results. First, numbers of $CD8^+$ T cells generally are not lower in Itk-deficient mice than in control $itk^{+/-}$ or $itk^{+/+}$ mice. However, a twofold reduction in CTL responses after LCMV and VV infection was observed. Second, the sixfold-reduced CTL response in VSV-infected mice seems difficult to explain in terms of a simple reduction in available T-cell numbers. In addition, after restimulation of cells for 5 days in vitro in bulk cultures, small differences in CTL precursor frequencies before restimulation cannot be detected, in particular—as is the case here—if restimulation is performed early after immunization when CTL precursor frequencies are not limiting. Thus, Itk-deficient $CD8^+$ T cells seem to have an inherent defect.

The T-cell defect may be located in the TCR signaling or CD28 costimulatory pathway, and it is difficult at present to distinguish between the two possibilities. However, the observed phenotypes do not resemble the defect in IL-2-deficient mice, where primary responses have been reported to be threefold (6, 19) to ninefold (9) reduced. Importantly, however, and in contrast to Itk-deficient mice, secondary in vitro responses were completely absent (6, 19). The T-cell defect of CD28-deficient mice is also different (31), because the mice fail to generate any CTL activity against VSV (21). Together with the earlier finding that CD28-dependent signals do not seem to be impaired in Itk-deficient mice but in fact are even increased (23), these results suggest that perhaps an altered TCR signaling rather than CD28 costimulatory pathway is responsible for the phenotypes seen with Itk-deficient mice in viral infections. The fact that exogenously applied cytokines could restore in vitro CTL responses to normal levels may also be compatible with the view that TCR-mediated signals are impaired in the absence of Itk. It has been shown earlier for allospecific and more recently for LCMV-specific T-cell responses that in vitro proliferation and induction of lytic activity against low-affinity ligands may depend on the presence of exogenous IL-2 (7, 32).

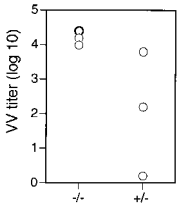

FIG. 7. Protection from VV replication. Itk-deficient and control mice were infected with VV (2×10^6 PFU), and viral titers were determined in lungs 6 days later. One representative experiment of two is shown.

Thus, both in the absence of Itk and upon stimulation with a low-affinity ligand, only a weak signal is generated and the T-cell responses may therefore depend more on the presence of a second signal, e.g., one from IL-2.

Antiviral protection against LCMV and that against VV differed with respect to their dependence on Itk. While Itk-deficient mice apparently normally eliminated LCMV, protection against VV was impaired. This difference cannot be definitively explained. However, it is tempting to speculate that the explanation may lie in how the viruses are eliminated from the host. While LCMV is controlled in a perforin-dependent fashion by $CD8^+$ CTLs, both $CD4^+$ and $CD8^+$ T cells interfere with VV replication (8, 15, 16, 20, 29). In addition, protection against VV is independent of perforin and is mediated by cytokines (16). Thus, it may be possible that Itk is important for the generation of the normal cytokine pattern crucial for protection from VV infection while the perforin-mediated arm of antiviral protection is less affected. Taken together, the results of this study reveal impaired CTL responses against three different viruses in the absence of Itk, demonstrating an important, though not essential, role of Itk in antiviral immunity.

ACKNOWLEDGMENTS

We thank R. M. Zinkernagel, H. Hengartner, A. Oxenius, and T. Fehr for helpful discussions.

This work was supported by the Swiss National Science Foundation (31-32179.91), the Human Frontier Science Program, the Kanton Zürich, and the Damon Runyon-Walter Winchell Cancer Research Fund (X.C.L.). D.R.L. is an investigator of the Howard Hughes Medical Institute.

REFERENCES

1. **August, A., S. Gibson, S. Kawakami, T. Kawakami, G. B. Mills, and B. Dupont.** 1994. CD28 is associated with and induces the immediate tyrosine phosphorylation and activation of the Tec family kinase ITK/EMT in the human Jurkat leukemic T cell line. Proc. Natl. Acad. Sci. USA **91:**9347–9351.

1a. **Bachmann, M. F.** Unpublished data.

2. **Bachmann, M. F., H. Hengartner, and R. M. Zinkernagel.** 1995. T helper cell-independent neutralizing antibody responses against vesicular stomatitis virus: possible role of antigen patterns in B cell induction? Eur. J. Immunol. **25:**3445–3451.

3. **Bachmann, M. F., R. U. Hoffmann, T. M. Kündig, K. Burki, H. Hengartner, and R. M. Zinkernagel.** 1993. The influence of antigen organization on B cell responsiveness. Science **262:**1448–1451.

4. **Bachmann, M. F., and T. M. Kündig.** 1994. In vivo versus in vitro assays for assessment of T- and B-cell function. Curr. Opin. Immunol. **6:**320–326.

5. **Bachmann, M. F., T. M. Kündig, C. P. Kalberer, H. Hengartner, and R. M. Zinkernagel.** 1994. How many specific B cells are needed to protect against a virus? J. Immunol. **152:**4235–4241.

6. **Bachmann, M. F., B. Sadlack, W. Muller, H. Hengartner, R. M. Zinkernagel, and I. Horak.** 1995. Antiviral immune responses in mice deficient for both interleukin-2 and interleukin-4. J. Virol. **69:**4842–4846.

7. **Bachmann, M. F., E. Sebzda, T. M. Kündig, A. Shahinian, D. Speiser, T. W. Mak, and P. S. Ohashi.** 1996. T cell responses are governed by avidity and costimulatory thresholds. Eur. J. Immunol. **26:**2017–2022.

8. **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.

9. **Cousens, L. P., J. S. Orange, and C. A. Biron.** 1995. Endogenous IL-2 contributes to the T cell expansion and IFN-γ production during lymphocytic choriomeningitis virus infection. J. Immunol. **155:**5690–5699.

10. **Desidero, S., and J. D. Siliciano.** 1994. The Itk/Btk/Tec family of protein-tyrosine kinases. Chem. Immunol. **59:**191–210.

11. **Gibson, S., A. August, D. Ranch, B. Dupont, and G. B. Mills.** 1996. Functional Lck is required for optimal CD28-mediated activation of the TEC family tyrosine kinase EMT/ITK. J. Biol. Chem. **271:**7079–7083.

12. **Gibson, S., B. Leung, J. A. Squire, M. Hill, N. Arima, P. Gross, D. Hogg, and G. B. Mills.** 1993. Identification, cloning and characterization of a novel human T cell specific tyrosine kinase located at the hematopoietin complex on chromosome 5q. Blood **82:**1561–1572.

13. **Heyeck, S. D., and L. J. Berg.** 1993. Developmental regulation of a murine T cell-specific tyrosine kinase gene, Tsk. Proc. Natl. Acad. Sci. USA **90:**669–673.

14. **June, C. H., J. A. Bluestone, L. M. Nadler, and C. B. Thompson.** 1994. The B7 and CD28 receptor families. Immunol. Today **15:**321–331.

15. **Kagi, D., B. Ledermann, K. Burki, P. Seiler, B. Odermatt, K. J. Olsen, E. Podack, R. M. Zinkernagel, and H. Hengartner.** 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature **369:**31–37.

16. **Kagi, D., P. Seiler, J. Pavlovic, K. Burki, R. M. Zinkernagel, and H. Hengartner.** 1995. The roles of perforin-and Fas-dependent cytotoxocity against cytopathic and non-cytopathic viruses. Eur. J. Immunol. **25:**3256–3262.

17. **Kerner, J. D., M. W. Applby, R. N. Mohr, S. Chien, D. J. Rawlings, C. R. Maliszewski, O. N. Witte, and R. M. Perlmutter.** 1995. Impaired expansion of mouse B cell progenitors lacking Btk. Immunity **3:**301–312.

18. **Khan, W. N., F. W. Alt, R. M. Gerstein, B. A. Malynn, I. Larsson, G. Rathbun, L. Davidson, S. Muller, A. B. Kantor, and L. A. Herzenberg.** 1995. Defective B cell development and function in Btk-deficient mice. Immunity **3:**283–299.

19. **Kündig, T., H. Schorle, M. F. Bachmann, H. Hengartner, R. M. Zinkernagel, and I. Horak.** 1993. Immune responses in interleukin-2 deficient mice. Science **262:**1059–1061.

20. **Kündig, T. M., H. Hengartner, and R. M. Zinkernagel.** 1993. T cell dependent interferon gamma exerts an antiviral effect in the central nervous system but not in peripheral solid organs. J. Immunol. **150:**2316–2321.

21. **Kündig, T. M., A. Shahinian, K. Kawai, H.-W. Mittrucker, E. Sebzda, M. F. Bachmann, T. W. Mak, and P. S. Ohashi.** 1996. Duration of TCR stimulation determines costimulatory requirements. Immunity **5:**41–52.

22. **Lefrancois, L.** 1984. Protection against lethal viral infection by neutralizing and nonneutralizing monoclonal antibodies: distinct mechanisms of action in vivo. J. Virol. **51:**208–214.

22a. **Liao, X. C.** Unpublished data.

23. **Liao, X. C., S. Fournier, N. Killeen, A. Weiss, J. P. Allison, and D. R. Littman.** 1996. Itk negatively regulates induction of T cell proliferation by CD28 costimulation. J. Exp. Med. **186:**221–228.

24. **Liao, X. C., and D. R. Littman.** 1995. Altered T cell receptor signaling and disrupted T cell development in mice lacking Itk. Immunity **3:**757–769.

25. **Mano, H., K. Mano, B. Tang, M. Koehler, T. Yi, D. J. Gilbert, N. A. Jenkins, N. G. Copeland, and J. N. Ihle.** 1993. Expression of a novel form of Tec kinase in hematopoietic cells and mapping the gene to chromosome 5 near Kit. Oncogene **8:**417–424.

26. **Moskophidis, D., and F. Lehmann-Grube.** 1989. Virus-induced delayed-type hypersensitivity reaction is sequentially mediated by $CD8^+$ and $CD4^+$ T lymphocytes. Proc. Natl. Acad. Sci. USA **86:**3291–3295.

27. **Pircher, H. P., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, and R. M. Zinkernagel.** 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. Nature **346:**629–633.

28. **Puddington, L., M. J. Bevan, and L. Lefrancois.** 1986. N protein is the predominant antigen recognized by vesicular stomatitis virus-specific cytotoxic T cells. J. Virol. **60:**708–717.

29. **Ramshaw, I., J. Ruby, A. Ramsay, G. Ada, and G. Karupiah.** 1992. Expression of cytokines by recombinant vaccinia viruses: a model for studying cytokines in virus infections in vivo. Immunol. Rev. **127:**157–182.

30. **Scott, D. W., and R. K. Gershon.** 1970. Determination of total and mercaptoethanol-resistant antibody in the serum sample. Clin. Exp. Immunol. **6:**13–18.

31. **Shahinian, A., K. Pfeffer, K. P. Lee, T. M. Kündig, P. S. Ohashi, C. B. Thompson, and T. W. Mak.** 1993. Differential T cell costimulatory requirements in CD28-deficient mice. Science **261:**609–612.

32. **Sprent, J., E. K. Gao, and S. R. Webb.** 1990. T cell reactivity to MHC molecules: immunity versus tolerance. Science **248:**1357–1363.

33. **Tanaka, N., H. Asao, K. Ohtani, M. Nakamura, and K. Sugamura.** 1993. A novel tyrosine kinase gene inducible in T cells by interleukin 2. FEBS Lett. **324:**1–5.

34. **Tsukada, S., D. J. Rawlings, and O. N. Witte.** 1994. Role of Bruton's tyrosine kinase in immunodeficiency. Curr. Opin. Immunol. **6:**623–630.

35. **van Bleek, G. M., and S. G. Nathenson.** 1990. Isolation of an endogenously processed immunodominant viral peptide from class I $H\text{-}2K^b$ molecule. Nature **348:**213–215.

36. **Vetrie, D., I. Vorechovsky, P. Sideras, J. Holland, A. Davies, F. Flinter, L. Hammarstrom, C. Kinnon, R. Levinsky, M. Bobrow, C. I. E. Smith, and D. R. Bently.** 1993. The gene involved in X-lined agammaglobulinemia is member of the src family of protein-tyrosine kinases. Nature **361:**226–233.

37. **Wagner, R. R.** 1987. The rhabdoviruses, 1st ed. Plenum Press, New York, N.Y.

38. **Weiss, A., and D. R. Littman.** 1994. Signal transduction by lymphocyte antigen receptors. Cell **76:**263–274.

39. **Yamada, N., Y. Kawakami, H. Kimura, H. Fukamachi, G. Baier, A. Altman, T. Kato, I. Yoshimasha, and T. Kawakami.** 1993. Structure and expression of novel protein tyrosine kinases, emb and emt, in hematopoietic cells. Biochem. Biophys. Res. Commun. **192:**231–240.