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Immunological control of murine gammaherpesvirus infection is independent of perforin

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Perforin-mediated cytotoxic T cell killing has been suggested to be of importance in the control of noncytopathic virus infections, based on studies with lymphocytic choriomeningitis virus (LCMV). We examined the role of perforin in a mouse model of gammaherpesvirus infection using transgenic perforin-deficient mice. Previous work from this laboratory has shown that CD8 T cells are essential for the resolution of the acute lung infection and control of latently infected B cells in murine gammaherpesvirus 68 infection. The absence of perforin did not significantly affect the kinetics of either the lytic lung infection or the latent spleen infection. Lymphocytes from both perforin-deficient and control mice secreted comparable levels of IFN-γ, IL-10 and IL-6. In addition, lymphocytes from both strains had similar levels of CD3ε-dependent cytotoxic activity in the spleen, draining lymph nodes and bronchoalveolar lavage. These data indicate that the lack of perforin has little affect on the ability of mice to control an experimental gammaherpesvirus infection.

Introduction

Cytotoxic T cells (CTLs) are known to be important in host defence against a number of viruses which infect the respiratory tract. CTLs can protect against lethal infection with influenza virus (Lukacher *et al.*, 1984; Taylor & Askonas, 1986) or Sendai virus (Kast *et al.*, 1991), and the clearance of these viruses is impaired in mice deficient in β_2-microglobulin (Eichelberger *et al.*, 1991*b*; Hou *et al.*, 1992; Bender *et al.*, 1992). It is thought that there are four major effector mechanisms through which CTLs act following recognition of virus-infected cells: (*a*) degranulation leading to the release of perforin and granzymes, which induce apoptosis in target cells; (*b*) engagement of Fas ligands on the CTL with Fas receptors on the target cell, also leading to target cell apoptosis; (*c*) surface-bound TNF-α which kills TNF-susceptible cells; and (*d*) the release of soluble factors such as IFN-γ, which can exert an antiviral effect in some virus infections without killing infected cells. Based on studies with lymphocytic choriomeningitis virus (LCMV) and a number of cytopathic viruses (vaccinia virus, Semliki Forest virus and vesicular stomatitis virus), it has been postulated that perforin is important in the CTL response to noncytopathic viruses, whereas other mechanisms such as cytokine secretion and antibody production are more important in the defence against cytopathic viruses (Kägi *et al.*, 1995).

Murine gammaherpesvirus 68 (MHV-68) is believed to spread naturally by the respiratory route. Following experimental intranasal inoculation the virus causes an acute infection in the lung, then remains in the host in a latent form within B lymphocytes (Sunil-Chandra *et al.*, 1992*a*, *b*, 1993, 1994). Based on genetic and pathological similarities, MHV-68 is proving to be a useful small animal model for studying gammaherpesvirus infection in man, notably the Epstein–Barr virus (EBV; Nash *et al.*, 1996). Clearance of the virus from the lung is known to be dependent on CD8 T cells (Ehtisham *et al.*, 1993), and depletion of these cells also results in elevated numbers of latently infected B cells in the spleen (Ehtisham *et al.*, 1993). Similarly, in EBV infection it is known that CTLs prevent the uncontrolled growth of immortalized B lymphocytes, and individuals lacking an adequate CTL response can develop lymphoproliferative disease (Crawford *et al.*, 1981; Yao *et al.*, 1985).

Given the importance of CD8 T cells in this infection, it was of interest to determine the extent to which these cells utilize perforin as an effector mechanism. To investigate this,

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transgenic perforin-deficient mice were infected with MHV-68 and the infection was compared with that in wild-type mice. Lymphocytes from perforin-deficient mice have been shown to have impaired natural killer (NK) cell and CTL activity (Kägi *et al.*, 1994*b*; Lowin *et al.*, 1994), although they can degranulate to release granzyme A (Lowin *et al.*, 1994) and killing by the Fas pathway remains intact (Lowin *et al.*, 1994; Walsh *et al.*, 1994*a*, *b*). CD8 T cells in these mice can still become activated (Walsh *et al.*, 1994*b*), and the recruitment and extravasation of T cells at inflammatory sites is unaffected (Kägi *et al.*, 1994*b*). CTLs from perforin-deficient mice could not efficiently lyse allogeneic fibroblasts, and poly(I·C)-elicited NK cells were unable to kill YAC-1 targets (Kägi *et al.*, 1994*b*). Perforin-deficient mice were not able to clear LCMV effectively (Kägi *et al.*, 1994*b*; Walsh *et al.*, 1994*b*), suggesting that lysis of infected cells by perforin was a necessary mechanism for the control of noncytopathic viruses such as LCMV. These mice are also unable to clear adenovirus (Yang *et al.*, 1995) and exhibit a delayed clearance of the JHM strain of mouse hepatitis virus (Lin *et al.*, 1997). Similarly, in the absence of perforin, listeria-infected mice were less able to mount appropriate protective responses upon secondary challenge with the bacterium (Kägi *et al.* 1994*a*).

This report represents the first investigation of the role of perforin in an experimental herpesvirus infection.

Methods

■ **Virus.** Virus stocks were originally obtained from D. Blaskovic (Blaskovic *et al.*, 1980) and clone G2.4 was isolated (Efstathiou *et al.*, 1990*a*, *b*). Virus was propagated in either baby hamster kidney (BHK) or owl monkey kidney (OMK) cells and working stocks stored at −80 °C.

■ **Mice.** C57BL/6 mice were purchased from Bantin and Kingman (UK) or Jackson Laboratory (Bar Harbour, Maine, USA). Transgenic perforin-deficient mice (Kägi *et al.*, 1994*b*) were on a C57BL/6 background and obtained from H. Hengartner (Department of Pathology, University of Zürich, Switzerland). Mice were infected intranasally at 3–8 weeks of age.

■ **Assay for infectious virus and infective centres.** Virus plaque assays were performed on BHK cell monolayers as previously described by Sunil-Chandra *et al.* (1992*a*). To determine the number of latently infected cells an infective centre assay was utilized. Spleen cell suspensions were subjected to water lysis to remove red blood cells and then co-cultivated with BHK cells for 5 days. The number of spleen cells which reactivated virus to give rise to a plaque was calculated (Sunil-Chandra *et al.*, 1992*a*). The limit of detection of the plaque assay was 10 p.f.u. per organ. For infective centre assays the lowest dilution of spleen cells represented 1/10 of the total spleen cell number, and one infective centre on each of two duplicate plates was considered the minimum detection limit, i.e. 10 infective centres per spleen.

■ **Redirected cytotoxicity assay.** The cytotoxic activity of activated T cells from the spleen, cervical lymph node (CLN), mediastinal lymph node (MLN) and lung lavage (BAL) was determined using ^{51}Cr-labelled H-2^{d} FcR^{+} Fas^{+} P815 murine mastocytoma cells (Eichelberger *et al.*, 1991*a*; Cardin *et al.*, 1996). Target cells were pelleted and incubated with 0·1 mCi per 10^7 cells ^{51}Cr ($Na_2{}^{51}CrO_4$, Amersham) for 16–18 h at 37 °C before use. P815 cells were incubated with fresh effector cells in the presence or absence of the 2C11 mAb to CD3 [for 6 h in a ^{51}Cr-release assay as described previously by Eichelberger *et al.* (1991*a*)]. Data presented represents effector:target ratio of 40:1. For inhibition of killing by the Fas–Fas ligand pathway, anti-mouse Fas (Pharmingen, catalogue no. 15400D) was included in the assay at a concentration of 1/100.

■ **Cytokine assay.** Spleen, CLN or MLN cells were resuspended at a final density of 4×10^6 cells/ml in RPMI 1640 supplemented with 10% FCS, 10 mM HEPES, 1 mM glutamine and 10 µg/ml gentamicin and restimulated *in vitro* using 2×10^6 MHV-68-infected (0·01 m.o.i.) irradiated (3000 rads) splenocytes as a source of antigen-presenting cells (Sarawar *et al.*, 1996). Supernatants were harvested after 24 or 72 h and stored at −70 °C prior to assaying cytokines by sandwich ELISA as previously described by Sarawar & Doherty (1994). Maximal cytokine production was seen at 72 h in all cases. The detection limit was below 0·4 ng/ml for IL-10 and below 1 unit/ml for the other cytokines.

Results and Discussion

Respiratory infection with MHV-68 in perforin-deficient mice

C57BL/6 and perforin-deficient mice were infected intranasally with three different doses of MHV-68: $2·5\times10^3$, 1×10^4 and 4×10^5 p.f.u. Two experiments were performed using $2·5\times10^3$, two using 4×10^5 and one using 1×10^4 p.f.u. Similar results were obtained at all doses. Fig. 1 shows the lung virus titres after infection with 4×10^5 p.f.u. Taking all the results at different doses together there was no consistent difference in the kinetics of virus clearance from the lungs. Therefore, although CD8 T cells are known to be essential for clearance from the lung (Ehtisham *et al.*, 1993), the infection can be controlled in the absence of perforin.

Splenic infection in perforin-deficient mice

It has been shown previously (Sunil-Chandra *et al.*, 1992*a*; Usherwood *et al.*, 1996) that MHV-68 infection induces an increase in the number of lymphocytes in the spleen, resulting in a marked splenomegaly. Both T and B lymphocyte numbers rise during splenomegaly; the largest increase is in the CD8 T cell population (Usherwood *et al.*, 1996; Sarawar *et al.*, 1996). During this phase of the infection there is an increase in the number of latently infected cells, which then decreases to approximately 1–10 infective centres per 10^7 spleen cells. As shown in Fig. 2, the kinetics of the rise and fall in numbers of infective centres was similar in both perforin-deficient and C57BL/6 mice. Although mean infective centre levels were higher in the perforin-deficient mice than in controls on day 14 post-infection (p.i.), this difference was not significant, a finding which was reproduced in other experiments. No significant difference was seen in the degree of splenomegaly, and cytofluorimetric staining for CD4, CD8 and B220 showed no difference in the expansion of these lymphocyte subsets in the perforin-deficient and wild-type mice (data not shown). As in

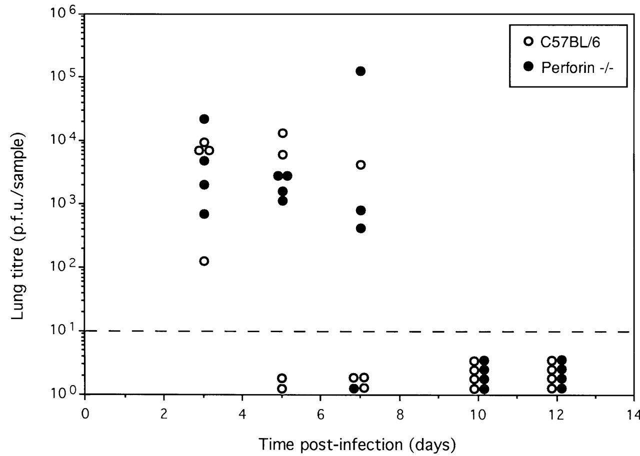

Fig. 1. Lung titres in MHV-68-infected perforin-deficient mice. Mice were infected with 4×10^5 p.f.u. MHV-68 intranasally. The lungs were removed at various times after infection, homogenized and assayed for virus content. Each point represents the lung titre from a single mouse. The limit of detection was 10 p.f.u. per organ.

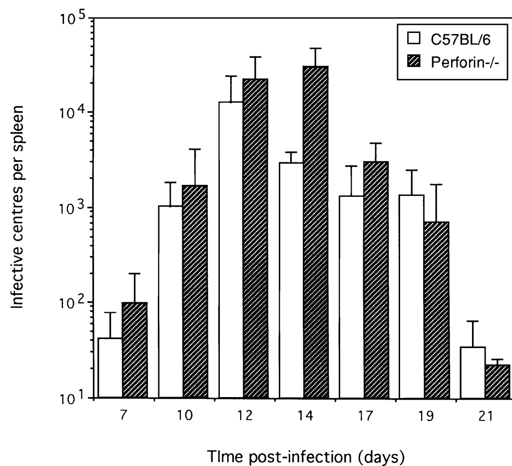

Fig. 2. Infective centre levels in MHV-68-infected perforin-deficient mice. Mice were infected with 4×10^5 p.f.u. MHV-68 intranasally. The spleens were removed at various times after infection and assayed for the number of latently infected cells using an infective centre assay. Each bar represents the mean from four mice, error bars show one standard deviation. The limit of detection was 10 infective centres per spleen.

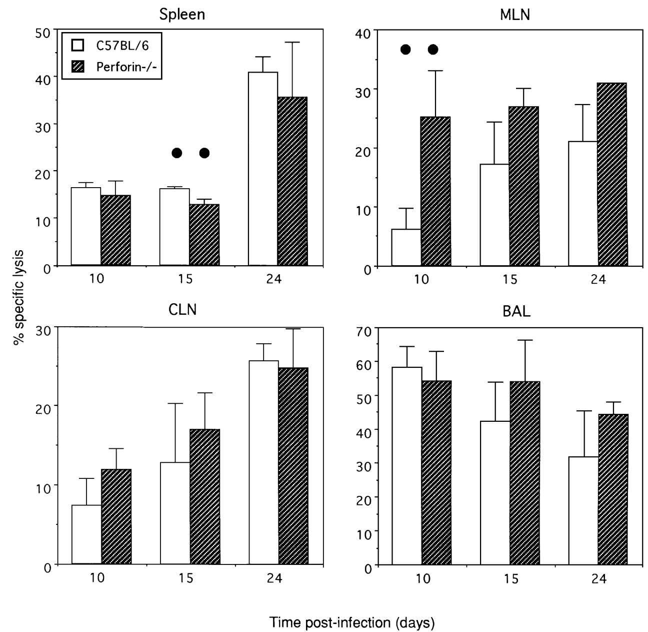

Fig. 3. Redirected CTL responses in MHV-68-infected perforin-deficient and C57BL/6 mice. Spleen, bronchoalveolar lavage (BAL), mediastinal lymph node (MLN) or cervical lymph node (CLN) lymphocytes were used in a redirected CTL assay where anti-CD3ε was used to cross-link freshly isolated effector cells with ^{51}Cr-labelled P815 target cells. Chromium release was measured after 6 h and percentage specific release calculated. For this experiment mice were infected intranasally with $2{\cdot}5 \times 10^3$ p.f.u., but similar results were obtained when an inoculum of 1×10^4 p.f.u. was used. Data shown was obtained at an effector:target ratio of 40:1. Each bar represents the mean percentage specific lysis from three perforin-deficient or C57BL/6 mice, error bars show one standard deviation. Black dots above the bars indicate a significant difference between the two readings ($P < 0{\cdot}05$ by Student's t test).

the lung infection, control of virus-infected cells in the spleen was perforin-independent.

CTL responses in intact and perforin-deficient mice

As the lack of perforin appeared to make little difference to the kinetics of virus clearance from the lungs or the rise and fall in numbers of latently infected cells in the spleen, it was of interest to determine whether the perforin-deficient mice mounted a CTL response following MHV-68 infection. In the absence of a virus-specific CTL assay for MHV-68, a CD3ε-dependent redirected assay was used to detect CTL activity in both perforin-deficient and control mice (Fig. 3). CTL activity in BAL was maximal at day 10 p.i., and subsequently decreased by day 24 p.i. Conversely, in the spleen, CLN and MLN responses continued to increase up to day 24 p.i. Despite differences between the killing observed by C57BL/6 and perforin-deficient mice at some time-points post-infection, overall the two strains displayed comparable CTL activity. Killing through the Fas–Fas ligand pathway was not responsible for the lysis induced by perforin-deficient lymphocytes since a blocking antibody specific for mouse Fas failed to inhibit killing (data not shown).

Cytokine secretion in intact and perforin-deficient mice

In vivo, CD8 T cell control of infected cells may not require cell lysis, and cytokine secretion by these cells may be sufficient to reduce the virus load, as is known to occur with hepatitis B virus (Guidotti *et al.*, 1996). It has been shown previously that lymphocytes from MHV-68-infected mice produce IL-6, IL-10 and IFN-γ, but not IL-4 or IL-5 (Sarawar *et al.*, 1996). Cytokine production in cultures from the MLN, CLN and spleen was measured in perforin-deficient mice.

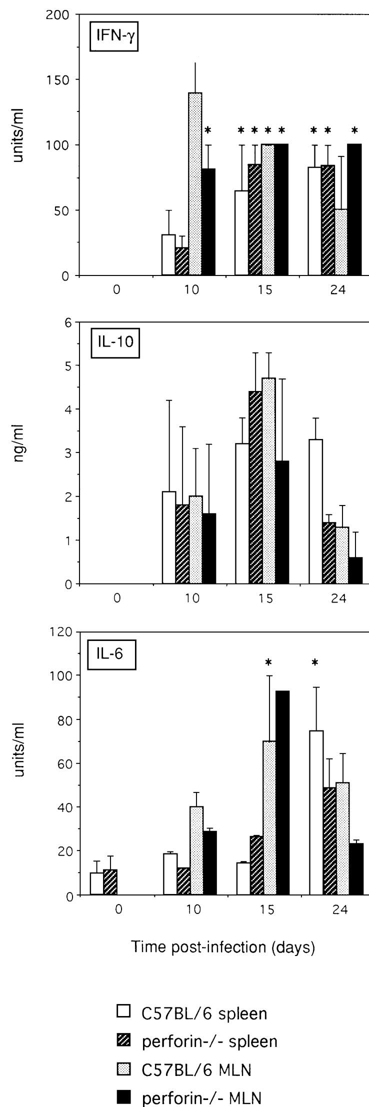

Fig. 4. Cytokine secretion from spleen and mediastinal lymph node (MLN) following MHV-68 infection. Lymphocytes from MLN or spleen were cultured with MHV-68-infected irradiated splenocytes. Supernatants were harvested after 72 h and assayed by sandwich ELISA. Each bar represents the mean reading from three mice, error bars show one standard deviation. The detection limit was below 0·4 ng/ml for IL-10 and below 1 unit/ml for other cytokines. Mice were infected intranasally with $2{\cdot}5\times10^3$ p.f.u. Asterisks represent readings which were beyond that of the standard in the ELISA assays.

Fig. 4 shows the production of these cytokines from MLN and spleen at various times after infection; cytokine production from CLN was similar to MLN (data not shown). Cells from the draining lymph node (MLN) produced more IL-6 and IFN-γ than spleen cells at early time-points (days 10 and 15). However, as the infection developed in the spleen, cytokine production from the MLN fell and production rose from spleen cells. Little or no IL-2, IL-4 or IL-5 was detected. Unstimulated cultures or MHV-68-infected irradiated antigen-presenting cells did not produce detectable amounts of cytokine (data not shown). Despite some differences at individual time-points, cytokine production from perforin-deficient mice was very similar to that of control C57BL/6 mice.

The absence of perforin has been shown to have little effect on the clearance of vesicular stomatitis virus, Semliki Forest virus (Kägi *et al.*, 1995) or influenza virus (R. A. Tripp, S. R. Sarawar, D. Kägi, H. Hengartner & P. C. Doherty, unpublished results). In contrast, lymphocytic choriomeningitis virus, which establishes a noncytopathic persistent infection, cannot be cleared in perforin-deficient mice. MHV-68 infection is cytopathic in the lung, but the virus establishes a latent non-cytopathic infection in B lymphocytes. As we have shown, perforin is not required to control MHV-68 infection in either lung or lymphoid tissue, demonstrating that perforin-dependent T cell effector functions are not required for immunity to all viruses with noncytopathic components to their life cycle. The role of the Fas–Fas ligand route of CTL killing was not addressed in this work, but it was not responsible for the CD3ε-dependent killing of target cells by perforin-deficient effectors. $CD8^+$ T cells may control the infection through the secretion of soluble factors such as chemokines or cytokines. This is thought to be the method by which $CD4^+$ T cells control influenza virus infection in β_2-microglobulin-deficient mice (Topham *et al.*, 1996) as there is no requirement for direct contact between infected epithelial cells and immune $CD4^+$ T cells. Whether this is also true for $CD8^+$ T cells is unclear. These cells do require direct antigen presentation by infected respiratory epithelial cells (Hou & Doherty, 1995). However, this could lead to the release of soluble factors in addition to direct cell killing. CTLs have been shown to act in this manner in a mouse model of hepatitis B virus infection (Guidotti *et al.*, 1996), where the secretion of IFN-γ and TNF-α activates intracellular virocidal pathways without killing infected cells. It has recently been demonstrated that CD8 T cell immunity to murine rotavirus does not involve the perforin, Fas or IFN-γ effector functions, so it is clear that additional antiviral mechanisms are present (Franco *et al.*, 1997). The precise mechanism(s) responsible for the action of MHV-68-specific CTLs is unclear, and it is possible that the mechanism used to counter the lytic infection in the lung is different to that used in the control of latently infected B lymphocytes.

Concluding remarks

Perforin-mediated lysis of virus-infected cells is thought to be an important tool employed by cytotoxic T lymphocytes during infection. It is, however, expendable in the host defence against certain cytopathic viruses, and we have shown that this is also true in MHV-68 infection. This illustrates that, although perforin-mediated CTL killing is essential in some non-cytopathic virus infections, such as LCMV, this cannot be generalized to other viruses capable of establishing a latent infection.

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References

Bender, B. S., Croghan, T., Zhang, L. & Small, P. A., Jr. (1992). Transgenic mice lacking class I major histocompatibility complex-restricted T cells have delayed viral clearance and increased mortality after influenza virus challenge. *Journal of Experimental Medicine* **175**, 1143–1145.

Blaskovic, D., Stancekova, M., Svobodova, J. & Mistrikova, J. (1980). Isolation of five strains of herpes viruses from two species of free living rodents. *Acta Virologica* **24**, 468.

Cardin, R. D., Brooks, J. W., Sarawar, S. R. & Doherty, P. C. (1996). Progressive loss of $CD8^+$ T cell-mediated control of a γ-herpesvirus in the absence of $CD4^+$ T cells. *Journal of Experimental Medicine* **184**, 863–871.

Crawford, D. H., Edwards, J. M. B., Sweny, P., Hoffbrand, A. V. & Janossy, G. (1981). Studies on long-term T-cell-mediated immunity to Epstein–Barr virus in immunosuppressed renal allograft recipients. *International Journal of Cancer* **28**, 705–709.

Efstathiou, S., Ho, Y. M. & Minson, A. C. (1990*a*). Cloning and molecular characterization of the murine herpesvirus 68 genome. *Journal of General Virology* **71**, 1355–1364.

Efstathiou, S., Ho, Y. M., Hall, S., Styles, C. J., Scott, S. D. & Gompels, U. A. (1990*b*). Murine herpesvirus 68 is genetically related to the gammaherpesviruses Epstein–Barr virus and herpesvirus saimiri. *Journal of General Virology* **71**, 1365–1372.

Ehtisham, S., Sunil-Chandra, N. P. & Nash, A. A. (1993). Pathogenesis of murine gammaherpesvirus infection in mice deficient in CD4 and CD8 T cells. *Journal of Virology* **67**, 5247–5252.

Eichelberger, M., Allan, W., Carding, S. R., Bottomly, K. & Doherty, P. C. (1991*a*). Activation status of the $CD4^-CD8^-$ $\gamma\delta$-T cells recovered from mice with influenza pneumonia. *Journal of Immunology* **147**, 2069–2074.

Eichelberger, M., Allan, W., Zijlstra, M., Jaenisch, R. & Doherty, P. C. (1991*b*). Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted $CD8^+$ T cells. *Journal of Experimental Medicine* **174**, 875–880.

Franco, M. A., Tin, C., Rott, L. S., Van Cott, J. L., McGhee, J. R. & Greenberg, H. B. (1997). Evidence for CD8+ T-cell immunity to murine rotavirus in the absence of perforin, Fas and gamma interferon. *Journal of Virology* **71**, 479–486.

Guidotti, L. G., Ishikawa, T., Hobbs, M. V., Matzke B., Schreiber, R. & Chisari, F. V. (1996). Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* **4**, 25–36.

Hou, S. & Doherty, P. C. (1995). Clearance of Sendai virus by $CD8^+$ T cells requires direct targeting to virus-infected epithelium. *European Journal of Immunology* **25**, 111–116.

Hou, S., Doherty, P. C., Zijlstra, M., Jaenisch, R. & Katz, J. M. (1992). Delayed clearance of Sendai virus in mice lacking class I MHC-restricted $CD8^+$ T cells. *Journal of Immunology* **149**, 1319–1325.

Kägi, D., Ledermann, B., Burki, K., Hengartner, H. & Zinkernagel, R. M. (1994*a*). $CD8^+$ T cell mediated protection against an intracellular bacterium by perforin-dependent cytotoxicity. *European Journal of Immunology* **24**, 3068–3072.

Kägi, S., Ledermann, B., Bürki, K., Seiler, P., Odermatt, B., Olsen, K. J., Podack, E. R., Zinkernagel, R. M. & Hengartner, H. (1994*b*). Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. *Nature* **369**, 31–37.

Kägi, D., Seiler, P., Pavlovic, J., Ledermann, B., Burki, K., Zinkernagel, R. M. & Hengartner, H. (1995). The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and non-cytopathic viruses. *European Journal of Immunology* **25**, 3256–3262.

Kast, W. M., Roux, L., Curren, J., Blom, H. J. J., Voordouw, A. C., Meloen, R. H., Kolakofsky, D. & Melief, C. J. M. (1991). Protection against lethal Sendai virus infection by *in vivo* priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. *Proceedings of the National Academy of Sciences, USA* **88**, 2283–2287.

Lin, M. T., Stohlman, S. A. & Hinton, D. R. (1997). Mouse hepatitis virus is cleared from the central nervous system of mice lacking perforin-mediated cytolysis. *Journal of Virology* **71**, 383–391.

Lowin, B., Beermann, F., Schmidt, A. & Tschopp, J. (1994). A null mutation in the perforin gene impairs cytolytic T lymphocyte- and natural killer cell-mediated cytotoxicity. *Proceedings of the National Academy of Sciences, USA* **91**, 11571–11575.

Lukacher, A. E., Braciale, V. L. & Braciale, T. J. (1984). *In vivo* effector function of influenza virus-specific cytotoxic T lymphocyte clones is highly specific. *Journal of Experimental Medicine* **160**, 814–826.

Nash, A. A., Usherwood, E. J. & Stewart, J. P. (1996). Immunological features of murine gammaherpesvirus infection. *Seminars in Virology* **7**, 125–130.

Sarawar, S. R. & Doherty, P. C. (1994). Concurrent production of interleukin-2, interleukin-10 and gamma interferon in the regional lymph nodes of mice with influenza pneumonia. *Journal of Virology* **68**, 3112–3119.

Sarawar, S. R., Cardin, R. D., Brooks, J. W., Mehrpooya, M., Tripp, R. A. & Doherty, P. C. (1996). Cytokine production in the immune response to murine gammaherpesvirus-68. *Journal of Virology* **70**, 3264–3268.

Sunil-Chandra, N. P., Efstathiou, S., Arno, J. & Nash, A. A. (1992*a*). Virological and pathological features of mice infected with murine gammaherpesvirus 68. *Journal of General Virology* **73**, 2347–2356.

Sunil-Chandra, N. P., Efstathiou, S. & Nash, A. A. (1992*b*). Murine gammaherpesvirus 68 establishes a latent infection in mouse B lymphocytes *in vivo*. *Journal of General Virology* **73**, 3275–3279.

Sunil-Chandra, N. P., Efstathiou, S. & Nash, A. A. (1993). Interactions of murine gammaherpesvirus 68 with B and T cell lines. *Virology* 193, 825–833.

Sunil-Chandra, N. P., Arno, J., Fazakerley, J. & Nash, A. A. (1994). Lymphoproliferative disease in mice infected with murine gammaherpesvirus 68. *American Journal of Pathology* **145**, 818–826.

Taylor, P. M. & Askonas, B. A. (1986). Influenza nucleoprotein-specific cytotoxic T cell clones are protective *in vivo*. *Immunology* **58**, 417–420.

Topham, D. J., Tripp, R. A., Sarawar, S. R., Sangster, M. Y. & Doherty, P. C. (1996). Immune $CD4^+$ T cells promote the clearance of influenza virus from major histocompatibility complex class II−/− respiratory epithelium. *Journal of Virology* **70**, 1288–1291.

Usherwood, E. J., Ross, A. J., Allen, D. J. & Nash, A. A. (1996). Murine gammaherpesvirus-induced splenomegaly: a critical role for CD4 T cells. *Journal of General Virology* **77**, 627–630.

Walsh, C. M., Glass, A. A., Chiu, V. & Clark, W. R. (1994*a*). The role of the Fas lytic pathway in a perforin-less CTL hybridoma. *Journal of Immunology* **153**, 2506–2514.

Walsh, C. M., Matloubian, M., Liu, C.-C., Ueda, R., Kurahara, C. G., Christensen, J. L., Huang, M. T. F., Young, J. D.-E., Ahmed, R. & Clark,

W. R. (1994*b*). Immune function in mice lacking the perforin gene. *Proceedings of the National Academy of Sciences, USA* **91**, 10854–10858.

Yang, Y., Xiang, Z., Ertl, H. C. J. & Wilson, J. M. (1995). Upregulation of class I major histocompatibility complex antigens by interferon γ is necessary for T-cell-mediated elimination of recombinant adenovirus-infected hepatocytes *in vivo*. *Proceedings of the National Academy of Sciences, USA* **92**, 7257–7261.

Yao, Q. Y., Rickinson, A. B., Gaston, J. S. H. & Epstein, M. A. (1985). *In vitro* analysis of the Epstein–Barr virus: host balance in long-term renal allograft recipients. *International Journal of Cancer* **35**, 43–49.

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