



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

JNI 02395

Trafficking of activated cytotoxic T lymphocytes into the central nervous system: Use of a transgenic model

Michael B.A. Oldstone^a and Peter J. Southern^b

^a Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, USA, and ^b Department of Microbiology, University of Minnesota, Minneapolis, MN, USA

(Received 10 February 1993)

(Accepted 23 February 1993)

Key words: Trafficking; Central nervous system; Cytotoxic T lymphocytes

Summary

We have used cell or tissue-specific promoters to express lymphocytic choriomeningitis virus (LCMV) proteins in selected cells in independent lines of transgenic mice. Upon adoptive transfers into these mice, MHC-restricted LCMV-specific cytotoxic T lymphocytes homed specifically to either the choroid plexus (SV40 promoter) or β cells of the islets of Langerhans (rat insulin promoter). The availability of promoters specific for neurons, oligodendrocytes and astrocytes makes this approach compelling for evaluating T cell trafficking into the CNS and for analyzing antigen presentation *in vivo* in the CNS.

Introduction

Damage to the brain during viral and/or autoimmune disease is often caused by cytotoxic T lymphocytes (CTL) or their products (Watanabe et al., 1983; Vandembark et al., 1985; Chan et al., 1989; Klavinskis et al., 1989; Sinha et al., 1990). Indeed, depletion of such cells prevents brain injury. Moreover, in a host that is T cell-depleted or deficient, reconstitution by adoptive transfer of activated antiviral or anti-self specific CTL leads to central nervous system (CNS) disease.

CTL recognize a unique amino acid sequence of a viral or self (or altered self) protein bound within the groove formed by the $\alpha 1$ and $\alpha 2$ arms of the major histocompatibility complex (MHC) glycoproteins (Zinkernagel and Doherty, 1974; Townsend et al., 1986; Oldstone et al., 1988; Van Bleek and Nathenson, 1990; Falk et al., 1991). CTL generated against lymphocytic choriomeningitis virus (LCMV) are CD8⁺ and restricted by MHC class I glycoprotein molecules

(Zinkernagel and Doherty, 1974; Oldstone, 1987, 1991). The peptides derived from LCMV Armstrong (ARM) proteins that act as 'CTL epitopes' and are restricted by MHC class I H-2^b, and H-2^d glycoproteins have been deciphered through the combined usage of genetic and biochemical techniques (Oldstone et al., 1988; Whitton et al., 1988a,b; Klavinskis et al., 1990; Yanagi et al., 1992). For example, H-2^b mice have three known CTL epitopes. They map to LCMV glycoprotein (GP) amino acids (aa) 34–43 (KAVYNFATC), GP aa 276–286 (SGVENPGGYCL) and nucleoprotein (NP) aa 396–404 (FQPQNGQFI) (Fig. 1). These peptides represent optimal binding and CTL recognition sequences (Gairin and Oldstone, 1992). At the clonal level, of over 40 CTL clones analyzed, 85% react with the GP-2 epitope aa 276–286 and only 3% with the NP epitope. For H-2^d mice, LCMV NP aa 119–127 (PQASGVYMG) is the optimal peptide, and > 96% of their CTL recognize this at both the clonal (analysis of over 50 clones) and primary immunization (7 days after initial inoculation) levels (Whitton et al., 1989; H. Lewicki, J.L. Whitton, M.B.A. Oldstone, unpublished observation, 1992). The lack of a GP epitope in H-2^d mice, under normal conditions, conveniently segregates H-2^d from H-2^b CTL responses (Oldstone et al., 1991; Fig. 1). When CTL recognize the viral peptide pro-

Correspondence to: M.B.A. Oldstone, Department of Neuropharmacology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA.

cessed by the appropriate MHC molecule at the target cell's surface, the result is target cell destruction.

The brain was formerly considered a privileged site whose borders disallowed the entry of lymphocytes. However, it is now clear from work of the Wekerle, Lampert, and Hickey laboratories, that activated T lymphocytes easily and rapidly enter the brain (Wekerle et al., 1986; Hickey et al., 1991), then pass through unless confronted by the appropriate peptide-MHC complex (Oldstone et al., 1986). Unfortunately, analysis of cells' movement in the CNS is complicated, at least in the course of viral infections, because viral gene products also frequently replicate at multiple CNS sites as well as other tissues. Therefore, we devised a strategy to direct trafficking of activated T lymphocytes to a specific CNS site or cell and also to evaluate in vivo the ability of such cells to present and process self, antiself, or viral antigens. Cell-specific promoter(s) and transgenic technology (Mucke et al., 1991, 1992; Rall et al., 1992) were combined with adoptive transfer of activated T lymphocytes (Oldstone et al., 1986; Joly et al., 1991) to implant and express viral or 'self' genes of interest in specific CNS cells. Here we document the expression of LCMV protein containing a CTL epi-

tope(s) in the choroid plexus using the SV40 promoter or in β cells of the islets of Langerhans using the rat insulin promoter (RIP). Upon adoptive transfer of MHC-restricted LCMV-specific CTL, T lymphocytes then homed directly to the choroid plexus or to β cells, respectively.

Materials and Methods

Transgenic mouse model

Transgenic mice were made as described earlier (Oldstone et al., 1991). The cDNA genes for the LCMV NP and GP coding regions were assembled from overlapping cDNA clones derived from the S RNA segment of LCMV (ARM clone 53b) (Dutko and Oldstone, 1983; Southern et al., 1987). The NP and GP cDNA genes were cloned into the RIP or the pSV2B expression vector as *Bam*HI-*Bgl*II fragments. The RIP vector was obtained from D. Hanahan (University of California, San Francisco). It consists of a 660 bp fragment that contains upstream regulatory elements in addition to the insulin promoter. Downstream are the SV40 t-antigen intron and SV40 polyadenylation

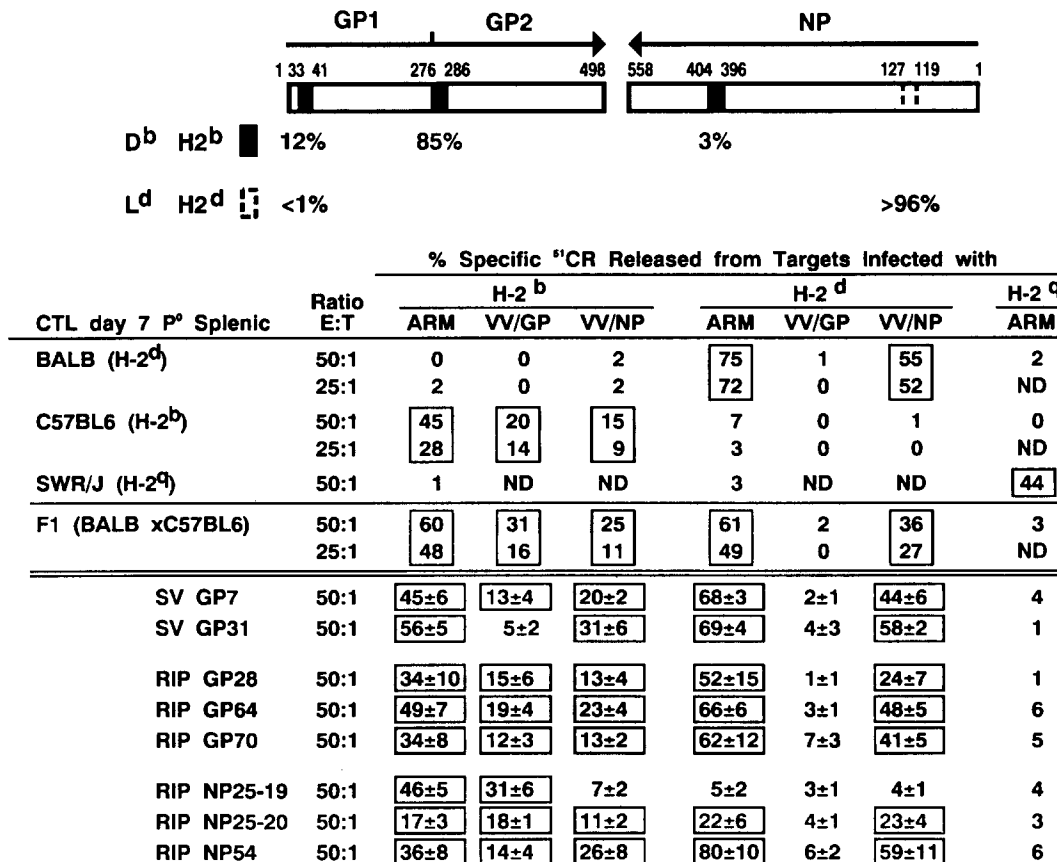


Fig. 1. Profile of LCMV-specific MHC-restricted CTL responses in normal H-2^d, H-2^b, and b x d mice and in b x d transgenic mice containing either SV40 LCMV GP or RIP GP and NP. The top of the Figure records the known LCMV ARM peptide sequences that are recognized by CTL from H-2^d and H-2^b mice. Numbers enclosed in boxes represent significant and specific lysis of target cells.

signal. The original upstream *Xho*I and the downstream *Hind*III cloning sites were converted to *Bgl*II site by linkers. The SV40 pSV2B vector is a derivative of the pSV2- β -globin vector. The upstream *Hind*III site, adjacent to the SV40 early promoter, was converted to *Bgl*II. pSV2B also contains the SV40 t-intron and early region polyadenylation signal.

After cloning and amplification, the RIP NP and RIP GP transcription units were isolated from flanking plasmid sequences and purified on a high resolution sucrose gradient. The SV NP and SV GP cassettes were excised from the plasmids and purified by preparative agarose gel electrophoresis (Oldstone et al., 1991).

Transgenic mice were generated using C57BL/6 [H-2^b] \times BALB/c [H-2^d] (b \times d) mice as a source of oocytes. Injected eggs were implanted in pseudopregnant CD1 females. Founder mice demonstrating integrated copies of the transgene were crossed to b \times d or C57BL/6 mice for one generation to produce the murine lines described. Thereafter each line was bred to at least the F3 generation to confirm transmission of the transgene. Mice utilized came from the vivarium of The Scripps Research Institute.

Mice carrying the transgene were identified by hybridization of DNA extracted from tail biopsies using LCMV-specific GP and NP probes (Southern et al., 1987). Mice were further characterized by Southern and Northern blot analysis and PCR (Southern et al., 1987; Oldstone et al., 1991; De la Torre and Oldstone, 1992).

Expression of LCMV GP or NP proteins was determined by adoptive transfer of LCMV-specific MHC-restricted CTL (Oldstone et al., 1986, 1991). For these studies, mice bearing the transgene received 5×10^7 LCMV-specific CTL i.p. The splenic CTL were obtained from H-2^b, H-2^d, or b \times d donors immunized 45–70 days earlier with 1×10^5 pfu of LCMV i.p. In other instances, transgenic mice received $1\text{--}2 \times 10^6$ LCMV GP (H-2^b [D^b] restricted) or $1\text{--}2 \times 10^6$ LCMV NP (H-2^d [L^d] restricted) CTL clones given i.p. or i.c. Details of adoptive transfer, as well as the generation and use of LCMV GP and NP CTL clones, are given elsewhere (Oldstone et al., 1988; Whitton et al., 1988a,b, 1989; Joly et al., 1991; Yanagi et al., 1992). At 1, 3, 7, and 10 days following transfer, pancreas, liver, and brain tissues were removed, fixed in Bouin's solution, and prepared for histological examination.

Cytotoxic T lymphocyte assay

LCMV-specific CTL activity was determined by a 5–9-h ⁵¹Cr release assay (13–16). Balb Clone 7 (H-2^d), MC57 (H-2^b) or SWR/J fibroblasts (H-2^q) cells were the targets (13–16). Uninfected target cells or target cells expressing viral epitopes were tested. The latter cells were infected 48 h earlier with LCMV ARM at moi 1 pfu/cell, or infected 10–14 h earlier with a

vaccinia virus recombinant expressing either LCMV GP or LCMV NP at moi 3 pfu/cell (Whitton et al., 1988b). Cells were labeled with ⁵¹Cr and resuspended at 10^5 ml⁻¹ in RPMI/5% FBS after which 10^4 target cells were added to each well of 96-well plates. All assays were done in triplicate and included controls for spontaneous and maximum ⁵¹Cr release from the targets. Percent specific CTL lysis was calculated at $100 \times (\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})$. The variance among triplicate samples was $< 10\%$.

To generate LCMV-specific CTL, mice were inoculated i.p. with 1×10^5 pfu of LCMV ARM. Seven days later, their spleens were removed and single lymphoid cell suspensions, free from red blood cells, prepared as described (Oldstone et al., 1988; Whitton et al., 1988b).

Antibody response

The antibody response to whole LCMV was assayed by using an ELISA with whole inactivated virus. Responses to NP or GP were evaluated with PAGE or immunofluorescence as described elsewhere (Oldstone et al., 1991).

Histology

Tissues taken for histological analysis were fixed in zinc formalin (10%) and stained with hematoxylin and eosin. In other instances, tissues were snap frozen in liquid nitrogen, sectioned on a cryomicrotome, and analyzed for expression of LCMV proteins (Joly et al., 1991; Oldstone et al., 1991).

Results

Generation of transgenic mice and their CTL response to LCMV

To direct expression of LCMV proteins to the choroid plexus, we fused the SV40 enhancer region and promoter to cDNA encoding the NP or GP of LCMV ARM and inoculated the product into the germline of b \times d mice. This strategy was selected because of reports that the SV40 enhancer–promoter sequence fostered the expression of SV40 or human papilloma T antigen to the choroid plexus (Marks et al., 1988; Messing et al., 1988; Feigenbaum et al., 1992). Of 32 pups born from eggs inoculated with SV40 LCMV GP, six had integrated the LCMV GP DNA as documented by Southern hybridization analysis. From these mice, two lines were established in which LCMV GP was passed into progeny mice as detected by tail DNA dot blot hybridization. These murine lines were designated SVGP-7 and SVGP-31. Northern blot analysis was inconsistent in detecting LCMV RNA in several tissues analyzed (brain, choroid plexus, thymus, spleen, liver, kidney, and heart). Further, no LCMV

protein was observed in the choroid plexus by immunohistochemistry. In contrast, adoptively transferred LCMV-specific MHC restricted CTL clearly localized in the choroid plexus (Fig. 2). However, neither non-transgenic littermates (data not shown) nor other transgenic mice with LCMV expressed in cells other than the choroid plexus showed any sign of LCMV-

specific CTL in the choroid plexus (Fig. 2). Three of seven pups born from eggs inoculated with SV40 LCMV NP DNA were DNA positive but the transgene was not transmitted to other progeny and transgenic line(s) were not developed from this construct.

Figure 1 shows that SVGP-31 mice were unable to mount specific CTL responses to the LCMV GP but

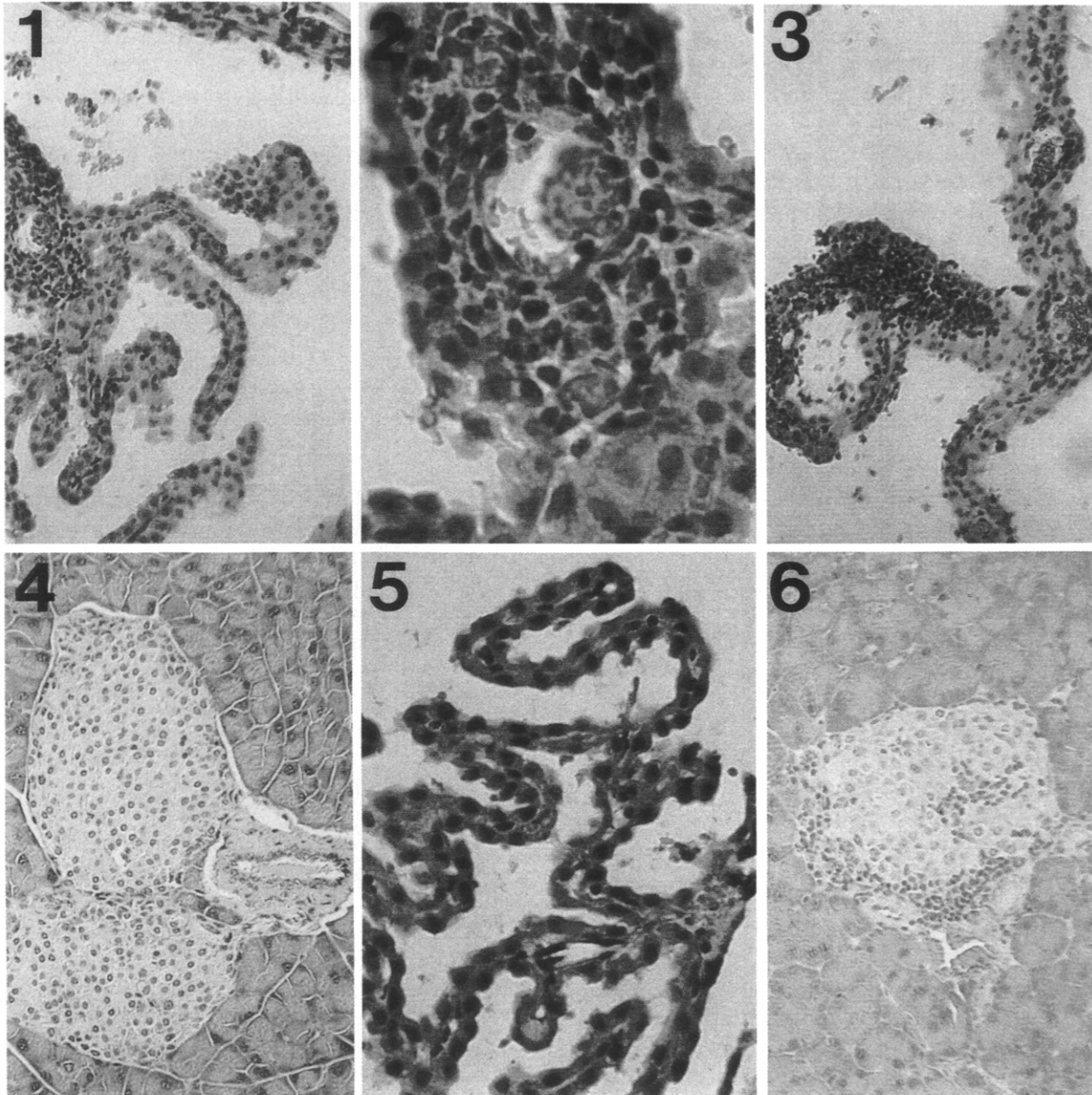


Fig. 2. Trafficking of LCMV-specific MHC-restricted CTL to the choroid plexus of transgenic mice expressing LCMV GP under the control of the SV40 promoter/enhancer region. Panels 1 and 2 are photomicrographs of the choroid plexus from a $b \times d$ mouse of transgenic line SV GP-31 that was inoculated i.v. with 5×10^7 LCMV-specific splenic CTL 5 days earlier. CTLs were generated in a $b \times d$ mouse inoculated with 1×10^5 pfu of LCMV and adoptively transferred 45 days later. Panel 3 is a photomicrograph of the choroid plexus from a $b \times d$ mouse of transgenic line SV GP-7 that was inoculated i.c. with 2×10^6 LCMV GP H-2^b-restricted CTL clone (228) (see Oldstone et al., 1988; Joly et al., 1991) 3 days earlier. Panel 4 is a photomicrograph of the pancreas from the mouse whose choroid plexus is shown in Panels 1 and 2. Note the absence of inflammatory cells in the islets of Langerhans. Inflammation did not occur when either this CTL clone (Panel 5) or 5×10^7 LCMV-specific splenic CTL were transferred i.c. or i.v., respectively. Trafficking of LCMV-specific MHC-restricted CTL to the islets of Langerhans cells occurred in mice from transgenic lines expressing LCMV NP or GP behind the RIP. Panel 6 is a photomicrograph from a RIP NP 25-19 $b \times d$ that received 2×10^6 CTL clones specific for LCMV NP. Similar results were obtained when 5×10^7 CTLs from spleens of $b \times d$ mice were transferred 45 days after a single inoculation of 1×10^5 pfu of LCMV.

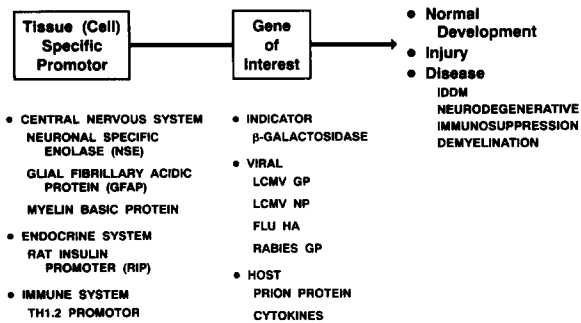


Fig. 3. A cartoon sketching an experimental strategy for studying CNS pathophysiology using the transgenic approach. HA, hemagglutinin; IDDM, insulin-dependent diabetes mellitus.

readily generated CTL responses to the viral NP. The SVGP-7 line mounted a modest but consistent CTL response to LCMV GP; all five mice in this group made CTL responses ($> 10\%$ specific ^{51}Cr release). Of over 32 individual SVGP mice tested, only one spontaneously made antibodies to LCMV. However, 28 days after challenge with 1×10^5 pfu of LCMV ARM i.p., ten of ten such mice made vigorous responses to LCMV. Of four randomly selected individual mice from each SV40 GP line, all made responses to both GP and NP of LCMV.

The generation of RIP LCMV NP and GP transgenic lines has been reported elsewhere (Oldstone et al., 1991). As with the SV40 LCMV transgenic mice, transmission of the LCMV gene was detected easily by Southern blot analysis. Although LCMV RNA was not consistently noted in Northern blot studies (Oldstone et al., 1991), the PCR method readily identified LCMV RNA in the pancreas (M. von Herrath et al., manuscript in preparation, 1993). Again, viral protein was not observed by immunohistochemistry but was documented by CTL recognition (Oldstone et al., 1991; Fig. 2). Three RIP GP (GP-28, GP-64, and GP-70) and three RIP NP (NP25-19, NP25-20, and NP-54) lines were selected for further study. All three RIP GP lines made LCMV-specific CTL to the viral GP and NP (Fig. 1). Similarly, RIP NP25-20 and NP-54 lines made CTL responses to LCMV GP and NP expressed behind a vaccinia virus promoter (Fig. 1). However, in contrast, as shown in Fig. 1, RIP LCMV NP25-19 failed to generate CTL to NP but made a vigorous CTL response to LCMV GP.

Migration of LCMV-specific CTL to the choroid plexus in SV LCMV transgenic mice

Adoptive transfer of LCMV MHC-restricted CTL into SVGP transgenic mice resulted in cellular infiltration in the choroid plexus. As seen in Fig. 2, splenic CTL harvested from MHC compatible mice immunized 45-65 days earlier (panels 1, 2, 3) infiltrated the choroid

plexus but not the islets of Langerhans (panel 4) or liver (data not shown) of transfer recipients. These observations were recorded in mice from SVGP-7 and SVGP-31 lines (three to five mice per line). The responses were virus-specific, since CTL transferred into non-transgenic littermates or into transgenic mice in which the LCMV GP or NP was expressed in β cells of the islets of Langerhans did not infiltrate the choroid plexus (panel 5). However, these or similar LCMV-specific CTL transferred into RIP GP or NP transgenics (Oldstone et al., 1991; panel 6) migrated into the islets of Langerhans.

CTL clone 228 recognizes GP amino acid residues 276-286 of LCMV ARM and is restricted by the D end of H-2^b glycoprotein (Oldstone et al., 1988). Intracerebral transfer of this clone into SVGP-31 mice (Fig. 2, panel 3) led to an accumulation of lymphoid cells in the choroid plexus (three of four mice). Yet, inoculation i.p. with clone 228 did not yield lymphocytes in the choroid, presumably because of the IL-2 dependency of this cultured clone and the time required for its transit. When clone 228 was inoculated i.c. into non-transgenic littermates (four per group) or into RIP GP-64 mice (three per group) no lymphoid cells appeared in the choroid plexus. However, CTL clone 228 accumulated in the islets of Langerhans of RIP GP-64 mice (three of four).

Discussion

Here, using transgenic technology, we caused the expression of a foreign protein (LCMV GP) in a pre-specified compartment of the CNS and observed the trafficking to and accumulation of antiviral specific T cells at that CNS site. For these studies, we utilized the promoter and regulatory region of SV40, which has been shown to express its large T antigen and the T antigen of JC virus in the choroid plexus (Marks et al., 1988; Messing et al., 1988; Feigenbaum et al., 1992). We adapted the enhancer-promoter region of SV40 to express LCMV GP because of the specific GP CTL reagents available and knowledge of GP and NP LCMV CTL responses (Oldstone et al., 1988; Whitton et al., 1988a,b, 1989; Klavinskis et al., 1990; Gairin and Oldstone, 1992; Yanagi et al., 1992). After transferring LCMV-specific memory cells or cloned LCMV CTL into the transgenic mice, we demonstrated the accumulation of such cells specifically in the choroid plexus. These studies with SV40 LCMV GP transgenics extended observations by ourselves (Oldstone et al., 1991; Von Herrath et al., 1992) and others (Ohashi et al., 1991) that the trafficking of lymphocytes to the islets of Langerhans is involved in the etiology and pathogenesis of autoimmune insulin-dependent diabetes mellitus. Analysis of the pathophysiology of SV40 LCMV trans-

genics after LCMV challenge is to be reported elsewhere (P.S., unpublished observations).

A range of CNS-specific promoter–enhancer sequences is now available so that molecules representing modified self, self, or viral genes can be targeted to neurons, astrocytes, or oligodendrocytes. With this device, investigators in the Viral-Immunobiology Laboratory at Scripps are studying the expression of recorder genes like β -galactosidase, viral genes like LCMV GP or NP, rabies GP or HIV GP and mutated or self genes like prion protein, β -amyloid and cytokines (Campbell et al., 1992; Mucke et al., 1992; Rall et al., 1992, 1993; Toggas et al., 1993; Mucke, 1993; Mucke and Rockenstein, 1993) (Fig. 3). This approach should allow in vivo dissection of normal development as well as injury and disease of the CNS.

The SV40 LCMV GP line GP-31, generated CTL to LCMV NP but not GP. Additionally, one line, RIP LCMV NP25–19, generated CTL to LCMV GP but not NP. The RIP NP25–19 line expresses its transgene in the thymus as well as the β cells of the islets, suggesting that potentially NP reactive CTL are actively deleted (M. von Herrath and M.B.A. Oldstone, manuscript in preparation). The SV40 lines are under evaluation, but the observation of thymic hyperplasia and thymic tumors in transgenic mice in which SV40 early regulatory sequences express T antigen (Marks et al., 1988; Messing et al., 1988; Feigenbaum et al., 1992) suggests a similar scenario in which the thymuses of such mice express LCMV GP and delete LCMV GP-reactive CTL.

Acknowledgements

This is Publication Number 7798-NP from the Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA. This work was supported in part by USPHS grants NS-12428 and AI-09484. Dale McFarlin was a friend, scientific collaborator and associate (of MBO) for over the last two decades.

References

Campbell, I.L., Oldstone, M.B.A. and Mucke, L. (1992) Neurologic disease induced in transgenic mice by the astrocyte-specific expression of interleukin-6. *Society for Neuroscience*, Anaheim, CA, October 25–30.

Chan, W., Javanovic, T. and Lukic, M. (1989) Infiltration of immune T cells in the brain of mice with herpes simplex virus-induced encephalitis. *J. Neuroimmunol.* 23, 195.

De la Torre, J.C. and Oldstone, M.B.A. (1992) Selective disruption of growth hormone transcription machinery by viral infection. *Proc. Natl. Acad. Sci. USA* 89, 9939–9943.

Butko, F.J. and Oldstone, M.B.A. (1983) Genomic and biological

variation among commonly used lymphocytic choriomeningitis virus strains. *J. Gen. Virol.* 64, 1689–1698.

Falk, K., Rotzschke, O., Stevanovic, S. et al. (1991) Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351, 290–296.

Feigenbaum, L., Hinrichs, S.H. and Jay, G. (1992) JC virus and simian virus 40 enhancers and transforming proteins: Role in determining tissue specificity and pathogenicity in transgenic mice. *J. Virol.* 66, 1176–1182.

Gairin, J.E. and Oldstone, M.B.A. (1992) Design of high-affinity major histocompatibility complex-specific antagonist peptides that inhibit cytotoxic T lymphocyte activity: Implications for control of viral disease. *J. Virol.* 66, 6755–6762.

Hickey, W.F., Hsu, B.L. and Kimura, H. (1991) T-lymphocyte entry into the central nervous system. *J. Neurosci. Res.* 28, 254–260.

Joly, E., Mucke, L. and Oldstone, M.B.A. (1991) Viral persistence in neurons explained by lack of major histocompatibility complex class I expression. *Science* 253, 1283–1285.

Klavinskis, L.S., Whitton, J.L. and Oldstone, M.B.A. (1989) Efficiency and effectiveness of cloned virus specific cytotoxic T lymphocytes in vivo. *J. Immunol.* 143, 2013–2016.

Klavinskis, L.S., Whitton, J.L., Joly, E. and Oldstone, M.B.A. (1990) Vaccination and protection from a lethal viral infection: Identification, incorporation and use of a cytotoxic T lymphocyte glycoprotein epitope. *Virology* 178, 393–400.

Marks, J., Lin, J., Miller, D., Lozano, G., Herbert, J. and Levine, A.J. (1988) The expression of viral and cellular genes in papillomas of the choroid plexus induced in transgenic mice. *Prog. Clin. Biol. Res.* 284, 163–186.

Messing, A., Pinkert, C.A., Palmiter, R.D. and Brinster, R.L. (1988) Developmental study of SV40 large T antigen expression in transgenic mice. *Oncogene Res.* 3, 87–97.

Mucke, L., Oldstone, M.B.A., Morris, J.C. and Nerenberg, M.I. (1991) Rapid activation of astrocyte-specific expression of GFAP-lacZ transgene by focal injury. *New Biologist* 3, 465–474.

Mucke, L., Forss-Petter, S., Goldgaber, D., Johnson, W., Picard, E., Rockenstein, E. and Abraham, C. (1992) Transgenic models to study the pathogenic role of mutated and non-mutated forms of human amyloid proteins in the development of Alzheimer's disease (AD). Third International Conference on Alzheimer's Disease and Related Disorders, Abano Terme (Padova), Italy, July 12–17.

Mucke, L. (1993) Transgenic models to study diseases of the nervous system: An in vivo approach to dissect complex pathogenetic networks. *Proc. Symp. Immunology II*, Freiburg, Germany, in press.

Mucke, L. and Rockenstein, E.M. (1993) Prolonged delivery of transgene products to specific brain regions by migratory astrocyte grafts. *Transgene*, in press.

Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., Malissen, B., Zinkernagel, R.M. and Hengartner, H. (1991) Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 65, 305–317.

Oldstone, M.B.A., Blount, P., Southern, P.J. and Lampert, P.W. (1986) Cytoimmunotherapy for persistent virus infection: Unique clearance pattern from the central nervous system. *Nature* 321, 239–243.

Oldstone, M.B.A. (1987) *Arenaviruses: Biology and Immunotherapy*, Current Topics in Microbiology and Immunology. Springer-Verlag, Berlin.

Oldstone, M.B.A., Whitton, J.L., Lewicki, H. and Tishon, A. (1988) Fine dissection of a nine amino acid glycoprotein epitope, a major determinant recognized by lymphocytic choriomeningitis virus specific class I restricted H-2D^b cytotoxic T lymphocytes. *J. Exp. Med.* 168, 559–570.

Oldstone, M.B.A. (1991) Molecular anatomy of viral persistence. *J. Virol.* 65, 6381–6386.

- Oldstone, M.B.A., Nerenberg, M., Southern, P., Price, J. and Lewicki, H. (1991) Virus infection triggers insulin dependent diabetes mellitus in a transgenic model: Role of anti-self (virus) immune response. *Cell* 65, 319–331.
- Rall, G.F., Mucke, L. and Oldstone, M.B.A. (1992) Transgenic mice which express histocompatibility molecules targeted to unique cells of the central nervous system. American Society for Virology, Cornell University, Ithaca, New York, July 11–15.
- Rall, G.F., Mucke, L. and Oldstone, M.B.A. (1993) Targeting specific host and viral genes to cells of the central nervous system: Transgenic studies. NIH AIDS Postdoctoral Fellows Meeting, Albuquerque, NM. March 29–April 4.
- Sinha, A.A., Lopez, M.T. and McDevitt, H.O. (1990) Autoimmune diseases: The failure of self tolerance. *Science* 248, 1380–1387.
- Southern, P.J., Singh, M.K., Riviere, Y., Jacoby, D.R., Buchmeier, M.J. and Oldstone, M.B.A. (1987) Molecular characterization of the genome S RNA segment from lymphocytic choriomeningitis virus. *Virology* 157, 145–155.
- Toggas, S.M., Rockenstein, E. and Mucke, L. (1993) The effects of cerebral HIV-1 gp120 expression in transgenic mice. NIH AIDS Postdoctoral Fellows Meeting, Albuquerque, NM. March 29–April 4.
- Townsend, A.R.M., Rothbard, J., Gotch, F.M., Bahadur, G., Wraith, D. and McMichael, A.J. (1986) The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 44, 959–968.
- Van Bleek, G.M. and Nathenson, S.G. (1990) Isolation of an endogenously processed immunodominant viral peptide from the class I H-2K^b molecule. *Nature* 348, 213–216.
- Vandenbark, A., Offner, H., Reshef, T., Fritz, R., Chou, C. and Cohen, I. (1985) Specificity of T lymphocyte lines for peptides of myelin basic protein. *J. Immunol.* 135, 229–233.
- Von Herrath, M., Paulk, J., Lewicki, H., Tishon, T. and Oldstone, M.B.A. (1992) Virus induced autoimmunity: Ability of various LCMV strains to cause insulin dependent diabetes mellitus in a RIP-LCMV-ARM transgenic mouse model. American Society for Virology, Cornell University, Ithaca, NY, July 11–15.
- Watanabe, R., Wege, H. and ter Meulen, V. (1983) Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. *Nature* 305, 150–153.
- Wekerle, H., Linington, C. and Meyermann, R. (1986) Cellular immune reactivity within the CNS. *Trends Neurosci.* 9, 271.
- Whitton, J.L., Gebhard, J.R., Lewicki, H., Tishon, A. and Oldstone, M.B.A. (1988a) Molecular definition of a major cytotoxic T lymphocyte epitope in the glycoprotein of lymphocytic choriomeningitis virus. *J. Virol.* 62, 687–695.
- Whitton, J.L., Southern, P.J. and Oldstone, M.B.A. (1988b) Analyses of the cytotoxic T lymphocyte responses to glycoprotein and nucleoprotein components of lymphocytic choriomeningitis virus. *Virology* 162, 321–327.
- Whitton, J.L., Tishon, A., Lewicki, H., Gebhard, J., Cook, T., Salvato, M., Joly, E. and Oldstone, M.B.A. (1989) Molecular analyses of a five amino acid cytotoxic T lymphocyte (CTL) epitope: An immunodominant region which induces nonreciprocal CTL cross-reactivity. *J. Virol.* 63, 4303–4310.
- Yanagi, Y., Tishon, A., Lewicki, H., Cubitt, B.A. and Oldstone, M.B.A. (1992) Diversity of T-cell receptors in virus-specific cytotoxic T lymphocytes recognizing three distinct viral epitopes restricted by a single major histocompatibility complex molecule. *J. Virol.* 66, 2527–2531.
- Zinkernagel, R.M. and Doherty, P.C. (1974) Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* 248, 701–702.