Classical swine fever virus-specific cytotoxic T lymphocytes and identification of a T cell epitope

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Classical swine fever virus (CSFV) -specific cytotoxic T lymphocytes (CTL) were derived from peripheral blood mononuclear leukocytes of immunized NIH-minipigs (MHC d/d haplotype) after in vitro restimulation with infectious CSFV. Their cytotoxic activity was determined against CSFV-infected target cells obtained from simian virus 40 (SV40) large T antigen-transfected immortalized kidney cells of a syngeneic miniature swine. Experiments with separated effector cell populations revealed that the CSFV-specific cytotoxic activity was mediated by CD4-CD6+CD8+ MHC class I-restricted T lymphocytes. Infection of target cells with various vaccinia virus/CSFV recombinants led to the identification of a major antigenic site for CSFV-specific CTL near the cleavage site between the non-structural proteins p80 (NS3) and p10 (NS4a). Using synthetic overlapping nonapeptides which covered this protein region the sequence ENALLVALF is the first sequence to be identified as an MHC class I-restricted T cell epitope recognized by CSFV-specific CTL.

Introduction

Classical swine fever virus (CSFV), also known as hog cholera virus, is a small, enveloped RNA virus which is classified in the genus Pestivirus of the family Flaviviridae (Wengler, 1991). The genomes of pestiviruses consist of a single-stranded RNA of positive polarity which encodes one polyprotein (Collett et al., 1988a; Meyers et al., 1989; Moormann et al., 1990). Co- and post-translational processing gives rise to the individual viral proteins. At the N terminus of the polyprotein, an autoprotease (Npro) is followed by the structural proteins namely the viral core protein (C) and three glycoproteins (E0-E1-E2) (Collett et al., 1988b; Stark et al., 1990, 1993). Non-structural proteins are located in the C-terminal two-thirds of the pestiviral polyprotein.

The virus causes acute, subacute or chronic disease of swine, which under natural conditions is the only susceptible species, and leads to severe economic losses worldwide. Disease control is attempted by either vaccination or eradication. For vaccination against CSF, lapinized C-strains of CSFV and temperature-sensitive mutants have been developed and successfully used in several countries (Aynaud, 1988). However, the European Union banned vaccination in 1990 mainly because infections with either vaccine or field virus cannot be discriminated serologically. The latter aspect is being addressed by attempts to develop subunit or vector vaccines for protection of animals against CSF.

For the development of efficient vaccines, detailed information about the porcine immune response against CSFV is important. So far, mostly the humoral immune response of CSFV-infected animals has been investigated. Animals immunized with recombinant viruses expressing E0 or E2 protein are protected against lethal CSFV challenge infections (Rümenapf et al., 1991; van Zijl et al., 1991). Furthermore, the E2 glycoprotein (corresponding to E1 as cited by van Zijl et al., 1991 and Hulst et al., 1993) is known to induce neutralizing antibodies in swine (van Zijl et al., 1991). Immunization of swine with purified E2 glycoprotein indicates that this protein elicits at least a protective humoral immune response (Hulst et al., 1993).

The cellular immune response, especially virus-specific cytotoxic T lymphocytes (CTL), has been shown to represent a second important defence mechanism against certain virus infections (Ada & Jones, 1986; Byrne & Oldstone, 1984; Reddehase et al., 1988; Sethi et al., 1983; Yap et al., 1978). So far, only scarce information about cellular immunity to CSFV has been available. A
lymphoproliferative response to CSFV of T lymphocytes obtained from CSFV-immune animals was reported, but could not be attributed to particular viral proteins (Kimmann et al., 1993). Immunization of animals with recombinant vaccinia virus expressing several CSFV structural proteins failed to induce detectable neutralizing antibodies. Even so, these animals were protected against a lethal CSFV challenge (Rümenapf et al., 1991), suggesting the participation of virus-specific T lymphocytes in a protective immune response against CSFV.

This report focuses on the identification and characterization of virus-specific CTL directed against CSFV. Using target cells infected with vaccinia virus recombinants expressing individual viral proteins or parts thereof, we determined the viral proteins responsible for the induction of the virus-specific T lymphocyte response. Further experiments with synthetic peptides derived from CSFV proteins allowed the identification of a viral T cell epitope recognized by CSFV-specific CTL.

### Methods

**Cells and viruses.** Pig lymphoma cell line 38A1D was kindly provided by W. Schäfer, Max-Planck-Institut für Virusforschung, Tübingen, Germany. The cell line was grown in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) fetal calf serum (FCS). CSFV strain Alfort Tübingen was characterized by Meyers et al. (1989). CSFV strain Brescia was obtained from the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia, Italy. CSFV strain Riems was a gift of R. Ahl, Federal Research Centre for Virus Diseases of Animals, Tübingen, Germany. Vaccinia virus strain WR was provided by G. L. Smith, Dunn School of Pathology, Oxford OX1 3RE, UK.

**Construction of recombinant vaccinia viruses.** The vaccinia virus recombinant vector pGS62 (Crane et al., 1986) was obtained from G. L. Smith. CSFV strain Alfort Tübingen DNA fragments were inserted into the pGS62 vector by standard cloning procedures (Maniatis et al., 1982). The different constructs were used to obtain recombinant vaccinia viruses as described previously (Rümenapf et al., 1991).

**Monoclonal antibodies and antisera.** Anti-MHC class I monoclonal antibody (MAB) (MAb 2.27.3a, mouse IgG1, k) (Pescovitz et al., 1984) and anti-CD4 antibody (MAB 74-12-4, mouse IgG2b, k) (Pescovitz et al., 1984, 1985) were obtained from J. K. Lunney, USDA, ARS, Helminthic Diseases Laboratory, Beltsville, Maryland, USA). Anti-CD8 (MAB 11/295/33, mouse IgG2a, k) (Jonic & Koszinowski, 1984) and anti-CD6 antibodies (MAB a38b2, mouse IgG1, k) (Saalmüller et al., 1994a) were produced at the Federal Research Centre for Virus Diseases of Animals.

**Immunization of animals.** Three miniature swine of d/d haplotype (Sachs et al., 1976) were immunized with 4 × 10^6 TCID_50 of CSFV strain Riems by the intramuscular route (i.m.). After seroconversion, the animals were challenged at monthly intervals. For the first and second challenge, swine were inoculated i.m. with 2 × 10^6 TCID_50 CSFV Alfort Tübingen. Finally, the animals were infected i.m. and intranasally with 1 × 10^6 TCID_50 CSFV Brescia.

**Generation of effector cells.** Infected pigs were bled 1 to 2 weeks after each challenge infection. Peripheral blood mononuclear cells (PBMC) obtained by Ficoll Hypaque separation were seeded in 96-well round bottom microtitre plates at a concentration of 2 × 10^5/ml in RPMI 1640 supplemented with 10% v/v inactivated FCS and restimulated with infectious virus (5 × 10^5 TCID_50 CSFV Alfort Tübingen) for 3 to 5 days.

**Generation of target cells.** The cell lines A42 and B4Z (MAX cells) were established as syngeneic target cells for cytotoxicity assays. Cell suspensions from a kidney of a miniature swine (d/d haplotype) were obtained after digesting organ pieces with collagenase/dispease (No. 269638, Boehringer; 420 µg/ml RPMI 1640) and gently passing the cells through steel meshes. For transformation, MAX cells were lipofected with the plasmid pSVneo3 encoding the SV40 large T antigen under control of the SV40 promoter and a resistance marker for neomycin (Southern & Berg, 1982). The cloning of immortalized cells was performed by limiting dilution or with cloning cylinders in DME medium under G418 selection (2 µg/ml).

For use as antigen-expressing target cells in cytotoxicity assays 1 × 10^5 MAX cells were infected either with different CSFV strains (m.o.i. 0.5) for 2 days or with different vaccinia virus/CSFV recombinants (m.o.i. of 2-0) for 16 h. The cells were trypsinized and collected in 200 µl CTL assay medium (RPMI 1640 supplemented with 3% v/v FCS) for labelling with 100 µCi of Na_2^106CrO_4 for 90 min, washed three times and resuspended in CTL assay medium at a final concentration of 1 × 10^6 cells/ml.

For the peptide sensitization experiments, 1 × 10^5 non-infected radiolabelled target cells were incubated with 50 to 100 ng of peptide for 1 h (peptides were purchased from K. H. Wiesmüller, NMI Reutlingen, Germany).

**CTL assays.** To achieve different effector to target cell ratios, effector cells were diluted in 96-well round bottom microtitre plates (triplicate cultures for each concentration in 100 µl volumes/well). Target cells (1 × 10^5) infected with CSFV or vaccinia virus/CSFV recombinants were added to each well in a volume of 100 µl. In peptide sensitization experiments, the peptide-loaded target cells were added to 1 × 10^5 effector cells. For the cytotoxicity assays, microtitre plates were centrifuged (100 g, 5 min) and incubated for 4 h at 37°C. After centrifugation (600 g, 10 min) supernatants were collected and the spontaneous chromium release of target cells included in the calculation described above was in all experiments less than 25% of the total chromium incorporation.

**Inhibition of CSFV-specific lysis by MABs.** For the characterization of MHC restriction of CSFV-specific T lymphocytes, blocking experiments were performed with MABs directed against the porcine CD4 (MAB 74-12-4), CD8 (MAB 11/295/33) and MHC class I antigen (MAB 2.27.3a). Effector cells were incubated with the respective MAB (hybridoma supernatant, 1:2 diluted in medium) of the total chromium incorporation. For use as antigen-expressing target cells in cytotoxicity assays 1 × 10^6 MAX cells were infected either with different CSFV strains (m.o.i. 0.5) for 2 days or with different vaccinia virus/CSFV recombinants (m.o.i. of 2-0) for 16 h. The cells were trypsinized and collected in 200 µl CTL assay medium (RPMI 1640 supplemented with 3% v/v FCS) for labelling with 100 µCi of Na_2^106CrO_4 for 90 min, washed three times and resuspended in CTL assay medium at a final concentration of 1 × 10^6 cells/ml.

**Multi-colour flow cytometric analysis.** Staining of PBMC for three-colour flow cytometric analysis was performed in a three-step procedure: (i) saturation of Fc-receptors by incubation with rabbit immunoglobulin (rlg; Jackson Laboratories, Avondale, PA, USA); (ii) incubation with MAB 74-12-4 (anti-CD4, mouse IgG2b), MAB a38b2
CSFV-specific CTL and T cell epitope

Immunomagnetic cell sorting. Purified CD4+ and CD4- or CD6+ and CD6- effector cell populations were obtained by immunomagnetic cell-sorting. Four days after in vitro restimulation PBMC of vaccinated pigs were labelled with: (i) MAb 74-12-4 (αCD4) or MAb a38b2 (αCD6); (ii) biotinylated F(ab')2 fragments of goat anti-mouse-IgG F(ab')2 (Jackson Laboratories); (iii) FITC-conjugated streptavidin (Jackson Laboratories) and (iv) biotinylated magnetic microparticles (Miltenyi Biotec, Bergisch Gladbach, Germany). Cell-sorting was performed with a magnet-activated cell sorter as recommended by the manufacturer (MACS, Miltenyi Biotec) and resulted in separation of highly purified antigen-positive and antigen-negative fractions, respectively. The purity of all sorted fractions used in the experiments was controlled by flow cytometric analyses. In order to ensure that the labelling procedure did not influence the reactivity of cytotoxic T lymphocytes, besides unlabelled cells, MAb-labelled unfractionated cells were also included in all cytotoxicity assays.

Results

Detection of CSFV-specific CTL response

The CTL activity of PBMC obtained from miniature pigs inoculated with CSFV was determined against syngeneic virus-infected MAX cells (Fig. 1). CSFV-specific CTL were induced after in vivo priming by infection of immunized swine (Fig. 1 b, c), whereas no virus-specific CTL activity could be detected in PBMC isolated from an uninfected animal (Fig. 1 a). A high ratio of CSFV-specific CTL, however, was only obtained after an in vitro cultivation period of effector cells with viral antigens (Fig. 1 c). The restimulation was only successful when the effector cells were incubated with infectious virus, but not when UV-inactivated virus was used (data not shown). These results indicate that in vivo priming and in vitro restimulation with newly synthesized viral antigens were required for clonal expansion of active CSFV-specific CTL. In addition, a booster effect was observed after in vivo restimulation achieved by repeated challenge infections of two immunized pigs. In another swine, a high CTL activity was already observed in effector cells collected after the first challenge (data not shown).

MHC class I-restriction of CSFV-specific CTL

Virus-specific CTL recognize processed viral antigens expressed as a complex with MHC class I molecules on the surface of infected cells. This recognition process is mediated by the T cell receptor complex and associated CD8 molecules (Zinkernagel & Doherty, 1979). Involvement of CD8 molecules in virus antigen-specific recognition was demonstrated by inhibition of CSFV-specific lysis using anti-CD8 MAb (11/295/33), which reduced the cytotoxic activity of the effector cells to the level of non-specific lysis of non-infected target cells.
In contrast, the anti-CD4 MAb 74-12-4 had no effect on virus-specific cytotoxic activity. Participation of the MHC class I molecule in restricted lysis was also demonstrated by inhibition of CSFV-specific lysis using the MAb 2.27.3a directed against porcine class I MHC. Furthermore, only targets with matched d/d alleles were lysed by CSFV-specific CTL obtained from an immunized swine sharing these alleles, whereas infected target cells obtained from outbred swine with non-matched MHC class I alleles were not recognized by these effector cells (data not shown). These results underline the MHC restriction of the CSFV-specific CTL used in our experiments.

Phenotypic characterization of CSFV-specific CTL

The phenotype of virus-specific CTL was characterized using a combination of three-colour flow cytometric analysis and CTL-activity studies with MACS-separated effector cell populations. First, the expression of T lymphocyte differentiation antigens CD4, CD6 and CD8 on the effector cells was studied after labelling of the cells with the respective MAb. Four populations were identified by their CD4 and CD8 antigen expression as shown in the contour plot (Fig. 3a). Besides the CD4CD8\(^+\) (Fig. 2).
CSFV-specific CTL and T cell epitope

Analyses of the cytotoxic activity of MACS-separated effector cell populations revealed an enrichment of cytotoxic activity in the CD4⁺ fraction, whereas no target cell lysis by CD4⁺ effector cells could be detected (Fig. 4). Separation of effector cells according to their expression of CD6 antigen demonstrated that the CD6⁻ fraction comprised effector cells with virus-specific cytotoxic activity, as indicated by the efficient lysis of infected target cells. The non-specific lysis of non-infected target cells by purified CD6⁺ CTL was significantly reduced in comparison with non-separated control groups. In addition, blocking experiments demonstrated that the specific lysis of virus-infected cells by CD6⁺ effector cells could almost be completely abolished by an anti-CD8 antibody, implying that the CSFV-specific
cytotoxic activity was indeed MHC class I-restricted. No such effect was seen using CD6+ effector cells suggesting that their lytic activity was not MHC class I-restricted (data not shown).

Taken together, these results demonstrated that CSFV-specific T lymphocytes belong to the CD4+CD6+CD8+ T lymphocyte subpopulation, representing the phenotype of classical MHC class I-restricted CTL.

**Mapping of CTL epitopes using vaccinia virus/CSFV recombinants**

In order to define epitopes on viral proteins which are recognized by CSFV-specific T lymphocytes, recombinant vaccinia viruses were used as a vector system for the expression of different viral proteins in target cells. Taken together, the recombinants made up 75% of the CSFV genome, namely the coding sequences for the three glycoproteins (E0, E1, E2), the nucleocapsid protein (C) and certain non-structural proteins [auto-

The expression of CSFV proteins in infected MAX cells was demonstrated by SDS-PAGE and immunoblotting assays (data not shown). First, recombinant vaccinia viruses expressing the N-terminal autoprotease and core protein (Vac-Npro/C), the three glycoproteins (Vac-E0/1/2) or the non-structural proteins p125, p10 and the N-terminal part of p30 (Vac-p125*) were tested. CSFV-specific CTL lysed only target cells infected with the vaccinia virus p125* recombinant (Fig. 6a). The lysis of syngeneic cells expressing the N-terminal autoprotease and core protein or the glycoproteins showed no significant differences when compared to the lysis of wild-type vaccinia virus-infected cells (Vac-WR), indicating that none of these proteins contained appropriate epitopes recognized by the cytotoxic effector cells. In order to localize the CTL epitope(s) of the CSFV non-structural proteins, additional recombinants with 3’ terminal truncated sequences compared to the coding sequence of Vac-p125* were constructed (Fig. 5; Vac-p125*V1 to Vac-p125*V4). Testing these recombinants in cytotoxicity assays, a T cell reactivity could be identified against an area located near the putative cleavage site between p125/p80 and p10, comprising amino acid positions 2168 to 2285 (Meyers et al., 1989) (data not shown). The vaccinia virus recombinant Vac-p80*V1, which encodes the 3’ terminal two-thirds of Vac-p125*V1 represented the starting point for two additional recombinants with 3’ terminal truncations of different lengths (Vac-p80*VA and Vac-p80*VX) as well as the recombinant Vac-p80*VZ encoding only the 3’ terminal part (Fig. 5); all these recombinants were tested in a third series of experiments. Fig. 6(b) demonstrates that only target cells infected with the recombinant Vac-p80*VZ were lysed by CSFV-specific CTL as efficiently as the positive controls Vac-p125* and Vac-p80*V1, whereas target cells expressing C-terminal truncated protein products of Vac-p80*V1 or Vac-p80*VZ, respectively, were not recognized by effector cells.

These results allowed the localization of a T cell epitope for CSFV-specific CTL between amino acids 2223 and 2285.
Mapping of CTL epitopes using synthetic peptides

In order to map the T cell epitope more accurately we used synthetic nonapeptides and pentadecapeptides overlapping by 8 and 12 amino acids, respectively. These peptides were composed of the amino acid region 2223 to 2285, which had been shown to possess a CTL epitope (see above). In a first series of experiments, mixtures of 10 to 12 nonapeptides were used for loading target cells. The results of chromium release assays indicated that CSFV-specific CTL recognized only target cells incubated with peptides 51 to 62 (Fig. 7a). For further investigation, each of the peptides 51 to 62 was tested separately. Fig. 7(b) shows the results of chromium
release assays, demonstrating that MAX cells loaded with peptide 58 (ENALLVALF, amino acid positions 2276 to 2284) were lysed by CSFV-specific CTL. In similar experiments using pentadecapeptides instead of nonapeptides a higher lysis of target cells loaded with the peptide STAENALLVALFGYV (amino acid positions 2273 to 2287) compared to the lysis of target cells incubated with other pentadecapeptides was observed (data not shown). This peptide contains the amino acid sequence of the nonapeptide identified above.

**Discussion**

Until now, very little information was available concerning CTL responses in the course of pestiviral infections (Woldehiwet & Hussin, 1994). The current paper describes for the first time the existence of porcine, virus-specific CTL directed against CSFV and their recognition of a virus-specific T cell epitope.

CSFV-specific CTL were isolated from immunized miniature swine of the MHC d/d haplotype. No measurable CTL activity was detectable in non-immunized animals, indicating a low frequency of CSFV-specific CTL in non-sensitized swine, which is consistent with results from other species and viral systems. On the other hand, a reasonable virus-specific CTL response was obtained after repeated challenge infections and subsequent *in vitro* restimulation of T lymphocytes primed *in vivo* with infectious virus. The restimulation was performed by incubation of complete PBMC fractions with infectious virus, thereby warranting that besides virus-specific T lymphocytes, antigen-presenting cells and cytokine-producing cells also necessary for efficient restimulation were included in the cultures. The exclusive lysis of autologous but not of heterologous target cells as well as the blocking of virus specific lysis by monoclonal antibodies against CD8 or MHC class I antigen indicated that the recognition of virus-infected
targets by CSFV-specific CTL is a classical MHC class I-restricted process. Although inhibition with MAb 2.27.3a (anti-MHC class I) was only partial, the result is consistent with previously described inhibition of CTL activity in other studies (Martins et al., 1993). The virus-specific CTL described here belong to the CD4+CD6+CD8+ T lymphocyte subpopulation as demonstrated in chromium release assays with separated effector cell populations. For two reasons we expected to identify CD8+ cytotoxic T lymphocytes: (i) the use of infectious virus in the restimulation protocol led to synthesis of viral proteins in the cytoplasm of infected cells; this allows the antigen presentation through the MHC class I pathway and results in a stimulation of mainly CD8+ T cells. In contrast, UV-inactivated virus, which would favour MHC class II presentation, failed to restimulate primed PBMC in vitro. (ii) The MAX cells used as target cells express MHC class I but not MHC class II antigens. Therefore antigen presentation to CD4+ T lymphocytes does not occur under these conditions.

Interestingly, the effector cells showed a high nonspecific lysis of non-infected target cells which has been previously described in conjunction with other viral infections of swine (Martins et al., 1993). This nonspecific lysis may be due to either non-specific activity of the effector cells or lymphokine-activated killer cells (LAK). As is known, a high LAK activity can be observed especially in virus-infected pigs (Scholl et al., 1989). Separation of effector cells which differed with regard to their expression of the CD6 antigen allowed us to attribute the non-specific non-MHC-restricted lysis to CD6+ cells and discriminated this cell population from CSFV-specific CTL, which showed a CD6+ phenotype and lysed virus-infected targets in a MHC class I-restricted process.

In order to develop effective vaccines, information about relevant T cell epitopes capable of stimulating virus-specific CTL and/or T helper cells and thereby eliciting a cellular immune response would be advantageous. For the screening of CTL epitopes it is an established method to use recombinant viruses expressing individual protein products of the viral genome after infection of appropriate target cells. Several vector systems have been used successfully for the identification of target proteins for virus-specific CTL (Bennink & Yewdell, 1990; Berensci et al., 1993; Li et al., 1993; Lovett et al., 1993; Moss, 1991). By using vaccinia virus/CSFV recombinants for a coarse mapping of virus-specific T cell epitopes, we observed that only syngeneic target cells expressing non-structural proteins of CSFV were killed by CSFV-specific CTL. Results of chromium release assays performed with recombinants encoding truncated sequences of the non-structural proteins p125, p10 and p30 of CSFV showed that one T cell epitope is located between amino acid positions 2223 to 2285 near to the putative cleavage site of p125/p80 and p10. Screening of this region with the aid of synthetic overlapping peptides led to identification of the nonapeptide ENALLVALF as an epitope presented in context with MHC molecules of d/d haplotype and recognized by CSFV-specific CTL. So far, other peptides involved in cellular immune responses of swine have not been described. The appropriate size of peptides presented by porcine MHC class I molecules as well as typical anchor sequences of these peptides have to be investigated in more detail.

For flaviviruses as well as for hepatitis C virus, the two other genera of the family Flaviviridae, T cell epitopes recognized by virus-specific CTL have been predominantly located on the NS3 protein which represents the protein corresponding to p80 (Erickson et al., 1993; Kurane et al., 1991; Lobigs et al., 1994). However, data about T cell epitopes from other members of the genus Pestivirus are not available.

In our experiments, additional T cell epitopes were not detected on other proteins of CSFV. It seems likely that the T cell epitope near the cleavage site between p80 and p10 is immunodominant for the induction of CSFV-specific CTL in d/d haplotype swine. However, additional T cell epitopes located in the so far not investigated C-terminal part of the CSFV polyprotein or epitopes not identified by the conditions used in our experiments may exist. It should be mentioned that so far only the CSFV-specific immune response of miniature pigs of the d/d haplotype has been investigated. Inbred swine with other MHC determinants and especially outbred swine may present different epitopes in combination with the appropriate MHC alleles. Nevertheless, immunodominance for one viral protein has also been described in other viral systems, for instance the immediate early protein of the murine cytomegalovirus (Reddehase & Koszinowski, 1984).

The antiviral effect of CTL is known from several virus systems where CTL can block the outgrowth of virus (Doherty et al., 1992). Accordingly, the induction of virus-specific CTL may protect swine against classical swine fever. As an effective vaccine has to induce protection against antigenically different virus strains it should contain conserved immunogenic epitopes. Alignment of the amino acid sequences for several CSFV (Meyers et al., 1989; Moormann et al., 1990; Müller, 1993) and bovine viral diarrhoea virus (BVDV) strains (Collett et al., 1988 a; De Moerlooze et al., 1993; N. Tautz, unpublished data) showed that the identified T cell epitope is highly conserved among pestiviruses. Furthermore, testing our CTL for crossreactivity with different pestivirus strains revealed that CSFV-specific CTL recognized not only a broad spectrum of CSFV
strains but also an epitope located in the same non-structural protein region of a BVDV isolate as identified for CSFV Alfort Tübingen (data not shown). The described T cell epitope presented by MHC class I molecules to CTL may therefore be useful as a component of a recombinant vaccine against CSFV and possibly other pestiviruses. As such a vaccine will allow serological discrimination of immunized and field virus-infected animals it would offer a good alternative to the eradication programme presently used by the European Union. The contribution of the T cell epitope identified in these studies to the induction of a protective immune response against CSFV is currently under investigation.

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