Establishment and characterization of canine parvovirus-specific murine CD4⁺ T cell clones and their use for the delineation of T cell epitopes


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Canine parvovirus (CPV)-specific T cell clones were generated by culturing lymph node cells from CPV-immunized BALB/c mice at limiting dilutions in the presence of CPV antigen and interleukin-2 (IL-2). All isolated T cell clones exhibited the cell surface phenotype Thy1⁺, CD4⁺, CD8⁻ and proliferated specifically in response to CPV antigen. After stimulation with CPV antigen in culture the T cell clones produced IL-2 and proliferated in the absence of exogenous IL-2. Naive mice to which CPV-specific T cell clones had been adoptively transferred developed a CPV-specific delayed type hypersensitivity reaction upon simultaneous intracutaneous injection of CPV in their ears. The ability of recombinant viral fusion proteins, representing the VP₂ capsid protein of the antigenically closely related feline panleukopenia virus and of synthetic peptides derived from the amino acid sequence of the VP₂ of CPV, to stimulate these T cell clones enabled the identification of T cell epitopes.

Introduction

Canine parvovirus (CPV) is a member of the autonomous replicating parvoviruses and is aetiologically associated with enteritis and myocarditis in dogs. Since the virus was discovered in 1978 (Appel et al., 1979; Burtonboy et al., 1979; Carmichael et al., 1983; Gagnon & Povey, 1979; Osterhaus et al., 1980) live attenuated vaccines and, with limited success, inactivated vaccines have been used to prevent CPV infection and disease in dogs (Pollock & Carmichael, 1982a, b). In pups, especially susceptible to the disease, maternally derived antibodies interfere with the replication of live attenuated vaccines.

Synthetic peptides of CPV proteins, if presented in a proper immunogenic form, might induce immunity to CPV under circumstances where conventional live attenuated vaccines or inactivated vaccines cannot (Pollock & Carmichael, 1982a). Knowledge about the location and structures representing B cell and T cell epitopes on the proteins of the virus will be necessary for a better understanding of immunity to CPV in the dog and possibly for the development of a new generation of CPV vaccines, like synthetic peptide, recombinant DNA and idiotype vaccines. Identification of immunodominant helper T cell antigenic determinants should facilitate the development of a highly immunogenic carrier-free vaccine that induces T cell and B cell immunity. Although many reports have provided evidence that the specific antibody response is of major importance in the protection against infection with CPV, very little is known about the epitopes responsible for the induction of neutralizing antibodies. Studies using monoclonal antibodies have provided information about the natural variation of CPV (Parrish et al., 1985) and about B cell epitopes present on the viral proteins of CPV (Surleraux et al., 1987).

Although for the generation of an antibody response the activation of T helper (Th) cells is essential, the immune response to CPV mediated by T cells has so far not been studied. Since it has been demonstrated that immunodominant T cell epitopes exist and are recognized by T cell clones in association with major histocompatibility complex (MHC) molecules of different animal species (Sinigaglia et al., 1988; Hurwitz et al., 1984; Berzofsky et al., 1988), the generation of CPV-specific mouse T cell clones may provide tools for the delineation of epitopes of CPV recognized by canine T cells.

In the present study we describe the establishment and characterization of CPV-specific mouse T cell clones and their use for the delineation of T cell epitopes using Escherichia coli fusion products of VP₂ of the antigenically closely related feline panleukopenia virus (FPV)
and of synthetic peptides resembling CPV amino acid sequences.

Methods

Viruses and antigens. CPV (strain 780916) (Carmichael et al., 1981) was purified by immunoaffinity chromatography (IAC) from culture supernatant of infected A-72 cells, as previously described (Rimmelzwaan et al., 1987), and after dialysis against phosphate-buffered saline (PBS) was not further treated. Rabies virus (Pitman-Moore strain) and measles virus (Edmonston strain), propagated in dog kidney cells and Vero cells, respectively, were used as control antigens. Rabies virus antigen was prepared from a concentrated, purified virus suspension, inactivated with β-propiolactone according to standard methods (van Wezel et al., 1978). Measles virus antigen was purified by density centrifugation on sucrose and inactivated by u.v. irradiation (De Vries et al., 1988).

FPV fusion proteins. Expression of FPV capsid proteins in E. coli was accomplished in the plasmid ptrpLE', which contains the trp promoter and the Δ1413 deletion, which fuses the trp leader peptide to the trpE gene (Miozziari & Yanofsky, 1978). The plasmid ptrpLEFPV has the HpaII–EcoRI fragment (nucleotides 1652 to 3942; Carlson et al., 1985) containing the FPV capsid protein gene inserted in frame into the HpaII site in the LE' gene 40 codons downstream from the ATG of the LE' gene. Deletions of various lengths of the LEFPV fusion gene were constructed by digesting ptrpLEFPV with appropriate restriction enzymes and religating the products. The FPV sequences contained in the various deletion plasmids are: 1652 to 2287 (NeoEco63) and 1652 to 1976 (NeoEco36). All plasmids were maintained in E. coli strain HB101. Synthesis of the fusion proteins was induced in the bacteria by tryptophan starvation. The culture was collected by centrifugation (2500 g for 5 min) and the pellet was resuspended in 0.01 M-Tris–HCl buffer pH 8.0 containing 0.001 M-EDTA and was disrupted by sonication for a total of 3 min in 30 s bursts. The lysate was centrifuged for 15 min at 10000 g and the pellet containing the insoluble fusion proteins was dissolved in either SDS (0.5%) or urea (8 M) in 0.01 M-Tris–HCl pH 8.0 containing 0.001 M-EDTA. The extent of induction and the purity of the fusion proteins were analysed by SDS-PAGE according to Laemmli (1970).

Synthetic peptides. Peptides were synthesized by the solid-phase method (Erickson & Merrifield, 1976) using polystyrene resin and T-Boc-protected amino acids. All peptides were synthesized with a cysteine residue at the carboxy terminus to facilitate coupling to a carrier protein. The following side-chain-protecting groups were used: o-bromobenzoyloxycarbonyl for tyrosine and lysine; β-benzyl for threonine, serine, aspartic acid and glutamic acid; tosyl for arginine; n-formyl for tryptophan; and n-dinitrophenyl for histidine. The couplings were carried out with a 2.5- to 10-fold molar excess of the amino acid derivative and N,N′-dicyclohexylcarbodiimide. For asparagine and glutamine an equimolar amount of n-hydroxybenzotriazole was added. The peptides were cleaved from the resin with anhydrous hydrofluoric acid, extracted with ether and lyophilized.

Immunization. Female BALB/c mice (8 to 16 weeks of age), free from known pathogenic mouse viruses, including mouse parvoviruses, raised in the barrier maintained facilities of the Bilthoven laboratory, were immunized according to a method recently described by Ziola et al. (1987). Briefly, mice were injected intraperitoneally with 200 mg of cyclophosphamide (Asta-Werke) per kg mouse 2 days before immunization with antigen. Eight μg of IAC-purified CPV (25 μg/ml) was mixed with 100 μg dimethylidioctadecylammonium bromide (DDA) (Kodak) and injected into the hind leg muscles and footpads of each mouse.

T cell growth factor (TCGF). Interleukin-2 (IL-2)-containing medium was prepared according to the method of Gillis et al. (1978). Briefly, rat splenocytes were activated with phorbol myristate acetate (Sigma) for 24 h at 37 °C and stimulated with 5 μg/ml concanavalin A (Sigma) for 2 h. The cells were washed three times and cultured in RPMI medium supplemented with 10% foetal calf serum (FCS), antibiotics and glutamine for another 24 h. After centrifugation at 1200 g for 10 min the supernatant was collected and tested for IL-2 content using the IL-2-dependent CTLL line (Gillis et al., 1978).

Cloning of CPV-specific T cells. Seven days after immunization mice were sacrificed and the draining lymph nodes (LN) were removed. LN cell suspensions were prepared by forcing the lymph nodes through a nylon mesh. The cells were washed in Iscove's medium containing penicillin (100 units/ml), streptomycin (100 μg/ml) and 2 mM-glutamine. Lymphocytes were cloned directly from the suspension of the LN cell population by the limiting dilution technique. Different concentrations of cells from the LN cell suspension were incubated with 3 × 10^5-γ-irradiated (1500 rad) syngeneic spleen cells as antigen-presenting cells (APC) in Iscove's medium containing antibiotics, 2 mM-glutamine, 10% FCS (Boehringer) and 0.25 μg IAC-purified CPV per well in 96-well round-bottomed microtitre plates (Greiner Laborteknik). Growing clones were restimulated every 7 days with IAC-purified CPV in the presence of irradiated syngeneic spleen cells. Cloned cells were cultured in the presence of 5 to 10% TCGF as a source of IL-2.

Proliferation assay. Four to 7 days before testing, clones were grown in the presence of 3 × 10^5 irradiated spleen cells and IL-2, but in the absence of CPV. The proliferation assay was performed in 96-well round-bottomed plates (Greiner Laborteknik). Ten-thousand to 30000 cloned T cells were cultured together with 3 × 10^5 irradiated syngeneic spleen cells and antigen or control antigens in Iscove's medium containing antibiotics, glutamine and 10% FCS, with or without 2% IL-2-containing culture supernatant in a total volume of 150 μl. As a control T cells were cultured with irradiated spleen cells without antigen. After 2 days the cells were labelled with [3H]thymidine (1 μCi/well) (Amersham) for 16 h and harvested on glass fibre paper with a cell harvester (Skatron). Incorporation was measured in a scintillation counter. For bulk cultures 3 × 10^5 LN cells were cultured in the same medium, but without IL-2-containing culture supernatant and 8% autologous serum instead of FCS.

Phenotypic analysis of T cell clones. Cells were collected from culture plates by centrifugation through Lympholyte M (Cedarlane). After washing, cells were incubated with a monoclonal antibody (1 μg/10^6 cells) for 60 min at 4 °C in suspension. The monoclonal antibodies anti-Thy1.2, anti-Lyt1, anti-Lyt2 and anti-L3T4 were used (Becton Dickinson). The cells were washed in PBS containing 2% FCS and incubated with monoclonal anti-rat antibody (κ) conjugated to fluorescein isothiocyanate (1 μg/10^6 cells) at 4 °C. The cells were washed again in PBS supplemented with 2% FCS and resuspended in PBS with 2% FCS and 2% BSA (Boseral, Organon Teknika). Fluorescence was measured using a fluorescence-activated cell sorter (Becton Dickinson FACS systems).

IL-2 assay. T cells were treated as described for the proliferation assay and washed three times with medium without IL-2 before testing. Ten thousand T cells were co-cultured with 3 × 10^5 irradiated spleen cells as APCs in the presence of CPV and control antigen, or without antigen for 24 h at 37 °C. Culture supernatants were tested for their ability to induce proliferation of the IL-2-dependent CTLL line. Culture supernatant of MLA 144 cells was used as a positive control.
Identification of CPV T cell epitopes

After incubation for 16 h [3H]thymidine uptake was measured (Gillis et al., 1978).

Adoptive transfer of T cells and measurement of delayed type hypersensitivity (DTH). Two days after cyclophosphamide treatment CPV-specific or control mouse T cell clones (measles virus-specific T cell clones; De Vries et al., 1988) were transferred to naive recipients by intracutaneous injection of 2 × 10^6 T cells together with 0.6 μg CPV or 0.8 μg measles virus into the right ear, or T cells alone in the left ear. Ear swelling was measured at 24 h intervals after transfer with a Mitutoyo micrometer. Swelling was expressed in μm as the thickness of the right ear minus the thickness of the left ear.

Results

Isolation and specificity of T cell clones

Lymph node cells of CPV-immunized mice were seeded at a density of 10^4 cells per well and stimulated with IAC-purified CPV. After 6 to 8 days 14% of the wells showed cell growth. Numbers of wells showing cell growth did not increase with prolonged incubation up to 21 days. In a second cloning procedure 16% of the wells seeded at 3 × 10^4 cells per well contained proliferating cells after 8 days of culture. According to the Poisson distribution the growing T cells have a high probability (>98%) of being monoclonal.

Eleven clones were isolated that showed proliferative responses to IAC-purified CPV, indicating their specificity for CPV antigens. These responses were antigen dose-dependent, as is shown for five clones in Fig. 1. The specificity of the T cell clones for CPV was further established using different antigen preparations in the proliferation assay. As shown in Table 1 for six of the clones, the T cell clones could not be stimulated by measles virus or rabies virus antigens in concentrations that have been shown to be optimal for the stimulation of measles virus- and rabies virus-specific T cell clones, respectively (H. Bunschoten et al., unpublished; De Vries et al., 1988). Furthermore, the culture supernatant of non-infected A-72 cells, which were used to propagate CPV, was not able to induce proliferation, whereas culture supernatant of CPV-infected A-72 cells did show stimulation of T cell clones. Stimulation of rabies virus- or measles virus-specific T cell clones using IAC-purified CPV as an antigen did not result in proliferation of the clones (data not shown).

All T cell clones were found to exhibit the phenotype Thyl+, Lyt1+, CD4+, CD8-, as demonstrated by immunofluorescence (data not shown).

Determination of fine specificity of the CPV-specific T cell clones

In order to study whether the T cell clones specifically recognized sequences of VP2 of CPV, the capacity of E. coli fusion proteins representing different fragments of VP2 of FPV to stimulate CPV-reactive T cells was determined. As shown in Table 2, all fusion proteins tested were able to stimulate LN cells from mice immunized with CPV, indicating that T cell epitopes are present on each of these fusion products. The LN cells could not be stimulated by the product of the E. coli strain, which did not contain FPV sequences. Five out of six clones tested (P2, P8, P29, P35 and P45) could be stimulated by fusion protein LEFPV, representing the complete amino acid sequence of VP2 of FPV (amino acids 1 to 584). T cell clone P29 could also be stimulated by fusion protein HaeEcoA (1 to 351), which did not stimulate clones P2, P8 and P45. Stimulation with NcoEcoA6 (1 to 200) and NcoEcoA3 (1 to 95) did not result in a proliferative response of any of the T cell clones.

The fine specificity of the CPV-specific T cell clones was further determined with synthetic peptides derived from the CPV sequence (Reed et al., 1988). The results in Table 3 show that T cell clone P2 could be stimulated by peptide 6 (532 to 546), but not by the partially overlapping peptides 8 (522 to 536) and 9 (542 to 556), or any of the other peptides tested. T cell clones P8 and P45 could be stimulated by peptide 8 (522 to 536), but not by any of the other peptides tested, including partially overlapping peptides 10 (512 to 526) and 6 (532 to 546). T cell clone P35 was only stimulated by peptide 9 (542 to 556) and not by any of the other peptides, including...
Table 1. Evidence for CPV specificity of T cell clones

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>T cell clone*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29G</td>
</tr>
<tr>
<td>IAC-CPV 0.25 µg/well</td>
<td>9490 ± 3586</td>
</tr>
<tr>
<td>Culture supernatant of A-72 cells†</td>
<td>196 ± 53</td>
</tr>
<tr>
<td>Culture supernatant of CPV-infected A-72 cells†</td>
<td>4386 ± 85</td>
</tr>
<tr>
<td>RV 1.0 µg/well§</td>
<td>277 ± 91</td>
</tr>
<tr>
<td>MV-u.v. 0.8 µg/well§</td>
<td>369 ± 173</td>
</tr>
</tbody>
</table>

* Cloned T cells (10⁴) were cultured in the presence of 3 x 10⁵ autologous irradiated spleen cells as APCs and stimulated as indicated. Proliferation is expressed as the mean c.p.m. of triplicate cultures ± S.D.
† Culture supernatants of A-72 cells were added at a final dilution of 1:15.
§ RV, Rabies virus.
§ MV, Measles virus, u.v.-inactivated.
ND, Not done.

Table 2. Proliferative responses of CPV-reactive mouse T cell clones and LN cells to ptrpLEFPV fusion proteins

<table>
<thead>
<tr>
<th>Proteins expressed by</th>
<th>Amino acid residue of VP₃ expressed</th>
<th>29G</th>
<th>P2</th>
<th>P8</th>
<th>P35</th>
<th>P45</th>
<th>P29</th>
<th>LN cells†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ptrpLE</td>
<td>1-584</td>
<td>1591 ± 243†</td>
<td>2124 ± 350</td>
<td>1524 ± 69</td>
<td>1934 ± 485</td>
<td>1926 ± 342</td>
<td>857 ± 160</td>
<td>2306 ± 62</td>
</tr>
<tr>
<td>ptrpLEFPV</td>
<td>1-584</td>
<td>1814 ± 386</td>
<td>23649 ± 2317</td>
<td>72108 ± 7334</td>
<td>3844 ± 294</td>
<td>28666 ± 1182</td>
<td>7254 ± 428</td>
<td>22462 ± 2826</td>
</tr>
<tr>
<td>HaeEco</td>
<td>1-251</td>
<td>1815 ± 313</td>
<td>2245 ± 569</td>
<td>1487 ± 360</td>
<td>1764 ± 281</td>
<td>2403 ± 338</td>
<td>3407 ± 484</td>
<td>16386 ± 1815</td>
</tr>
<tr>
<td>NeoEcoA6</td>
<td>1-200</td>
<td>1540 ± 151</td>
<td>2283 ± 26</td>
<td>1536 ± 23</td>
<td>1976 ± 224</td>
<td>2186 ± 43</td>
<td>1395 ± 366</td>
<td>13326 ± 651</td>
</tr>
<tr>
<td>NeoEcoA3</td>
<td>1-95</td>
<td>1788 ± 262</td>
<td>1980 ± 66</td>
<td>1336 ± 25</td>
<td>1941 ± 385</td>
<td>2000 ± 231</td>
<td>962 ± 136</td>
<td>13969 ± 2432</td>
</tr>
</tbody>
</table>

* Cloned T cells (10⁴) were cultured with fusion proteins in the presence of 3 x 10⁵ autologous irradiated spleen cells as APCs.
† LN cells (10⁵) from CPV/DDA-immunized mice were cultured per well.
§ Fusion protein (2 µg) was added per well. Proliferation is expressed as the mean c.p.m. of triplicate cultures ± S.D.

Functional analysis of T cell clones

After stimulation with IAC-purified CPV all T cell clones produced IL-2, as shown in Table 4. When T cells were grown in the absence of CPV, or with control antigen, no IL-2 production could be detected. We also tested these clones for their ability to proliferate in the absence of IL-2-containing culture supernatant. In all cases the cells showed CPV-induced proliferation and addition of exogenous IL-2 did not enhance their proliferative responses (not shown).

Simultaneous subcutaneous injection of purified CPV with T cell clones P2 and 29G into the ears of syngeneic mice induced a strong DTH response, histologically characterized by infiltration of mononuclear cells at the site of inflammation (not shown). This response was significantly more pronounced than the response obtained when the T cell clones were inoculated in the presence of measles virus control antigen (Fig. 2). In the same experiment the measles virus-specific T cell clone 20F1 was shown to induce ear swelling in the presence of measles virus antigen, but not in the presence of CPV.

Discussion

In the present study CPV-specific CD4⁺ mouse T cell clones were isolated from the lymph nodes of mice inoculated with CPV and DDA after pretreatment with cyclophosphamide, according to a method previously described (Smith & Ziola, 1986; Ziola et al., 1987). This method was chosen since it requires only small amounts of viral antigen for the induction of antigen-specific T cell responses (Smith & Ziola, 1986; Ziola et al., 1987). It should be realized that this protocol introduces a bias towards the generation of predominantly CD4⁺ T cells. The antigen-induced IL-2 production and the induction of a DTH response upon transfer of CPV-specific T cell clones P2 and 29G on challenge with CPV, indicate that...
Table 3. Proliferative responses of CPV-reactive mouse T cell clones to synthetic peptides of CPV VP2*

<table>
<thead>
<tr>
<th>Peptide Positions</th>
<th>Sequence</th>
<th>Clone P2</th>
<th>Clone P8</th>
<th>Clone P35</th>
<th>Clone P45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 μg/well</td>
<td>5 μg/well</td>
<td>2 μg/well</td>
<td>10 μg/well</td>
</tr>
<tr>
<td>17</td>
<td>MSDGAVQPDDGG</td>
<td>1.1 ± 0.7</td>
<td>1.4 ± 1.4</td>
<td>0.6 ± 0.7</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>18</td>
<td>PDGQPQAVRNERA</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>19</td>
<td>STGTFNNQTEFKFLE</td>
<td>1.2 ± 1.6</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>EITANSSRLVHLN</td>
<td>0.9 ± 0.8</td>
<td>2.6 ± 2.3</td>
<td>1.1 ± 0.4</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>131</td>
<td>NVVLTNSCESATQPPTK</td>
<td>1.0 ± 0.5</td>
<td>0.5 ± 0.4</td>
<td>N/T</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>135</td>
<td>ARSETLGFYPWP</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.4</td>
<td>N/T</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>136</td>
<td>NVVLKTVSESATQPPTK</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.4</td>
<td>N/T</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>137</td>
<td>APVCCQNCPCGQLFKVAPNLTNEYPDASA</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>138</td>
<td>KLVEKAKRASHTWN</td>
<td>1.14 ± 1.20</td>
<td>15.9 ± 4.4</td>
<td>144 ± 209</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>139</td>
<td>STWNPQIQMSINVD</td>
<td>0.9 ± 0.7</td>
<td>0.7 ± 0.3</td>
<td>15 ± 0.9</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>140</td>
<td>SINVQNFYNY</td>
<td>1.2 ± 0.8</td>
<td>0.8 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>141</td>
<td>KIVYKQFAPKLY</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.9</td>
<td>0.2 ± 0.1</td>
<td>1.1 ± 0.4</td>
</tr>
</tbody>
</table>

* Cloned T cells (10⁴) were cultured in the presence of 3 x 10⁵ autologous irradiated spleen cells as APCs and stimulated with synthetic peptides as indicated. Proliferation is expressed as the mean c.p.m. ± s.d. of triplicate cultures.

† N/T, Not tested.
the T cell clones belong to the Th1 subset of Th cells (Cher & Mosmann, 1987; Mosmann et al., 1986).

Although it has been suggested that T cells belonging to this subset of inflammatory Th1 cells are not able to provide help for a specific antibody response (Bottomly, 1988), recent experiments have demonstrated that B cells can conjugate to either type of Th cell (Sanders et al., 1988). Furthermore, it has been demonstrated that Th1 clones may also function as classical Th cells able to provide help for a specific antibody response (De Kruyff et al., 1989) (for review see Mosmann & Coffman, 1989). For the initial determination of CPV specificity of the T cell clones, IAC-purified CPV preparations were used. This procedure yields highly purified CPV preparations, as demonstrated by electron microscopy and analysis by SDS gel electrophoresis (Rimmelzwaan et al., 1987). Induction of a dose-dependent proliferative T cell response by IAC-purified CPV was a strong indication that the clones were CPV-specific. This specificity was further confirmed by showing that the T cell clones could be stimulated by culture supernatant of CPV-infected A-72 cells and not by culture supernatant of non-infected A-72 cells, measles virus or rabies virus antigens, which were used as control antigens.

In a first attempt to locate T cell epitopes on VP2 of CPV we used bacterial fusion products of VP2 of FPV, a virus antigenically closely related to CPV, with an amino acid homology of 99% for the capsid genes (Reed et al., 1988). VP2 was chosen since the viral capsid consists predominantly of VP2 (85%), which contains the whole amino acid sequence of VP3 and most of the amino acid sequence of VP1. Based on the reactivity of the T cell clones and LN cells from CPV-immunized mice, at least three different regions containing Th cell epitopes could be identified. The C-terminal region within amino acid sequence 352 to 584 was recognized by T cell clones P2, P8, P35 and P45. These clones only proliferated upon stimulation with the fusion protein LEFPV, representing the whole amino acid sequence of FPV, but not with fusion proteins representing smaller parts of VP2. T cell clone P29 responded to stimulation with fusion proteins LEFPV and HaeEcoA (amino acids 1 to 351), but not to stimulation with NeoEcoA6 (1 to 200) or NeoEcoA3 (1 to 95), indicating that an epitope within region 200 to 351 is recognized by clone P29. T cell clone 29G did not respond to any of the fusion proteins tested, which may indicate that clone 29G recognized an epitope present on VP1 and not on VP2, or that the epitope recognized is present only on CPV and not on FPV. Alternatively, it cannot be excluded that higher concentrations of antigen are required to stimulate this clone and, due to toxicity, these could not be met by adding bacterial fusion protein. LN cells from CPV-immunized mice responded to stimulation with all fusion proteins tested, suggesting...
that a T cell epitope is present in the amino-terminal amino acid sequence 1 to 95. From this study it is not clear whether sequence 96 to 200 contains T cell epitopes. The level of the response of the LN cells to LEFPV confirm that T cell epitopes are present in the amino acid sequence 1 to 95. From this study it is not compared with the response to other fusion proteins may confirm that T cell epitopes are present in the amino acid sequence 352 to 584. In order to map T cell epitopes of CPV more precisely, synthetic peptides were used to stimulate CPV-specific T cell clones. It has been shown that T cell epitopes in proteins are likely to be located in sequences of high amphipathicity (Berzofsky et al., 1986; Margalit et al., 1987). Plotting of the amphipathicity values of VP2 of CPV indeed provided evidence that regions of high amphipathicity are present in the sequence of peptides 8, 6 and 9 (data not shown). Although we realize that different species express different T cell repertoires and that T cell epitopes that have been found to be immunogenic in mice may not be immunogenic in dogs, several examples exist of inter-species cross-reactive epitopes for Th cells. Such epitopes have been identified in the gp120 envelope proteins of human immunodeficiency virus (Berzofsky et al., 1988), influenza virus haemagglutinin (Hurwitz et al., 1984) and the circumsporozoite protein of the malaria parasite Plasmodium falciparum (Sinigaglia et al., 1988). Preliminary experiments indicate that peptides 8, 6 and 9 may represent immunodominant T cell epitopes in dogs. These peptides were used to stimulate peripheral blood mononuclear cells from nine CPV-immune dogs in vitro. Five of these dogs showed a strong proliferative response in vitro after stimulation with peptides 8, 6 and 9 (G. F. Rimmelzwaan et al., unpublished observations). Experiments are now being conducted to examine these responses at the clonal level. The identification of immunodominant T cell epitopes in dogs may contribute to the construction of a carrier-free synthetic peptide vaccine.

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Identification of CPV T cell epitopes

1101


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