A porcine CD8⁺ T cell clone with heterotypic specificity for foot-and-mouth disease virus

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Foot-and-mouth disease virus (FMDV)-specific T cell lines and clones have been obtained from a swine lymphocyte antigen (SLA) inbred miniature pig vaccinated with chemically inactivated virus. One of the clones obtained, CE3, showed a specific and heterotypic proliferation against infectious but not inactivated FMDV in the presence of syngeneic peripheral blood mononuclear cells (PBMC). Adherent cells from PBMC were sufficient to support specific activation of the clone and the proliferation was abolished when the contact between CE3 and adherent cells was prevented. Phenotypic characterization of CE3 cells revealed expression of CD2, CD25 (interleukin-2 receptor), SLA class I and SLA class II. Furthermore, the cells were highly CD8 positive but showed low expression of CD4. The expression of T cell receptor (TCR) α and β genes confirmed their T cell nature. Consistent with the CD8 phenotype, the proliferative response of CE3 was inhibited with MAbs to SLA class I and CD8. Altogether, these results indicate that CE3 is a porcine SLA class I-restricted CD8⁺ T cell clone, that recognizes a heterotypic FMDV antigen.

Introduction

Picornavirus infections represent good examples of antibody-mediated immunity to viruses. In recent years there has been increasing evidence of the relevance of the T cell-mediated immune response in protection and recovery from picornavirus infection (reviewed in Usherwood & Nash, 1995). The role of cellular immunity is particularly interesting for foot-and-mouth disease virus (FMDV), an antigenically diverse aphthovirus causing an economically important disease of farm animals, mainly cattle and swine (Bachrach, 1968; Domingo et al., 1990). Immunization with serotype-specific, chemically inactivated vaccines, is extensively used to control the disease in enzootic areas (reviewed in Barteling & Vreeswijk, 1991). A correlation has been established between high titres of circulating neutralizing antibodies and the capacity of vaccinated animals to resist experimental FMDV challenge. However, this correlation is not complete, and persistently infected animals usually show a good humoral antiviral response (McCullough et al., 1992; Collen, 1994).

Extensive information has been gained on the antigenic structure of B cell sites and their topology on the capsid surface (Acharya et al., 1989; Lea et al., 1994), and a continuous antigenic site located within the G–H loop of capsid protein VP1 (site A) (Strohmaier et al., 1982; Bittle et al., 1982; Pfaff et al., 1982) has been used as the basis for the development of recombinant and peptide vaccines (DiMarchi et al., 1986). However, these vaccines are poor immunogens and the protection they confer is lower than that induced by whole-virus inactivated vaccines (reviewed in Brown, 1992). This limitation could be due, at least in part, to the absence in their formulation of virus-specific T cell epitopes that can be efficiently recognized by host immune systems (Clarke et al., 1987; Glass & Millar, 1995). Only partial information is available to date on the characterization and functional role of the virus-specific T cells in the immune response of natural hosts to FMDV. There is evidence of a requirement for specific B cell–T cell interactions to elicit protective levels of anti-
FMDV antibodies in natural host species (reviewed in Collen, 1994) and for the involvement of antigen-presenting cells (APC) during in vivo protection (reviewed in McCullough et al., 1992). Induction of FMDV antibodies in mice is T cell dependent (Collen et al., 1989), and recent studies have characterized the lymphoproliferative response induced by the virus in cattle and swine (Collen & Doel, 1990; Sáiz et al., 1992). These studies have identified several T helper epitopes on capsid proteins recognized by individuals of these species (Glass & Millar, 1994; Collen et al., 1991; Rodríguez et al., 1994; Van Lierop et al., 1994). Cytotoxic T lymphocytes (CTL) appear to play an important role in the host immune response to most viral infections, including those of picornaviruses (Lindsey et al., 1991; Kutubudin et al., 1992, reviewed in Usherwood & Nash, 1995), by limiting replication and accelerating clearance of virus and by releasing cytokines with antiviral activity such as interferon-γ (Doherty et al., 1992). However, the role of CTL in FMDV infection remains largely unexplored.

T cell lines and clones are valuable tools for the analysis of the principles of the antiviral immune response in different species. FMDV-specific bovine CD4+ lymphocyte clones have been used to study the antigenic specificity of T cell recognition (Collen et al., 1991; Glass & Millar, 1994; van Lierop et al., 1992). The establishment of long-term T cell cultures from swine has presented various difficulties and it is only recently that a few stable lines have been described (Grimm et al., 1993; Dillender & Lunney, 1993). To study the lymphocyte populations involved in the porcine immune response to FMDV, and their possible role in the resolution of viral infection, we established a virus-specific T cell line derived from peripheral blood mononuclear cells (PBMC) of a vaccinated pig. The cells were expanded by in vitro restimulation with infectious virus and several stable clones were obtained by limiting dilution. A T cell clone derived from this cell line was further characterized and found to be CD8+ and SLA-restricted. It expressed T cell receptor (TCR) αβ genes and was specific for infectious FMDV.

### Methods

**Viruses.** A type C1 FMDV (C-S8) (Villanueva et al., 1983), either purified on a CsCl gradient or sedimented through a sucrose cushion (Sáiz et al., 1989), was used for pig immunization and for the establishment of T cell lines and clones, respectively. To study the antigenic specificity of T cell activation, FMDV stocks of serotypes C (C-S8 and C1 Oberbayer), A (A5 Westerwald) and O (O1 Kaufbeuren) were used to stimulate T cell cultures. Viruses were grown in BHK-21 cells and supernatants of infected cells were clarified and stored at −70 °C. Titres of the virus stocks were determined by plaque assay on BHK-21 cells (Domínguez et al., 1980), and are expressed as p.f.u./ml. When necessary, FMDV was inactivated with binary ethylenimine (BEI) as described by Bah nemann (1974).

**Animal immunization.** A 5-month-old, SLA inbred miniature pig (Sachs et al., 1976) of the SLA1/8 (c/d) phenotype was immunized intradermally with about 5 μg of BEI-inactivated, purified C-S8 FMDV (corresponding to 5 × 103 p.f.u.) in Freund's complete adjuvant. The animal was boosted 4 weeks later by a second equivalent dose in Freund's incomplete adjuvant and PBMC obtained 26 weeks later were used to establish FMDV-specific cell lines.

**Preparation of conditioned medium.** PBMC from the same animal were cultured for 48 h in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, 2% L-glutamine, 50 μg/ml mercaptoethanol, 100 μg/ml streptomycin, 100 U/ml penicillin, in the presence of 2.5 μg/ml concanavalin A (ConA) and 10 μg/ml phytohaemagglutinin (PHA). Supernatant was collected, concentrated five times through an Amicon filter (0.2 μm pore size) and stored in aliquots at −70 °C.

**Establishment of cell lines and cloning.** Isolation of PBMC from peripheral blood was performed as described previously (Sáiz et al., 1992). T cell lines were obtained following a modification of the procedure described by Taylor et al. (1987). Briefly, two separate cultures containing 4 × 105 PBMC/ml were incubated in supplemented RPMI medium in the presence of inactivated or infectious FMDV C-S8 (3 μg/ml), a viral concentration previously shown to induce significant in vitro proliferative responses of PBMC from immunized pigs (Sáiz et al., 1992). After 2 weeks of culture, the cells were supplemented with conditioned medium to a final concentration of 20%, and syngeneic irradiated (2000 rads) PBMC (2 × 106 cells/ml) were added as a source of APC and feeders. One week later, cells were harvested and cultured for a rest period of 1 week at 2 × 105 cells/ml in the presence of 2 × 106 irradiated PBMC cells/ml, and in the absence of viral antigen. Only the cell line restimulated with infectious virus was viable and was maintained for 33 days in culture. The cell line was maintained by alternating 1 week of antigen stimulation, using 3 μg/ml of FMDV C-S8, with 1 week of rest. After 6 weeks of culture, cells were cloned by limiting dilution in the presence of 20% conditioned medium, irradiated feeder cells and virus according to Taylor et al. (1987). Briefly, cells were seeded in 96-flat-bottom-well plates at 1, 5, 15 and 50 cells per well, using half a plate for each dilution. Cultures were scored for cell growth after 10–12 days and clones from dilutions with less than 10% of wells with growing cells were expanded and subcloned twice using the same procedure. Under these experimental conditions, Poisson statistics indicate that the probability of having obtained a clone derived from one single cell is effectively 100%. One of the clones obtained, CE3, was maintained as described above for cell lines and was characterized during this study. The specificity of CE3 clone was highly dependent on the maintenance of the culture conditions, and modifications of these conditions usually resulted in non-specific autocrine growth.

**Flow cytometry analysis.** Assays were performed as described by Pescovitz et al. (1984), using monoclonal antibodies (MAb) against the following porcine markers: CD2 (MSA4) (Hammerberg & Schurig, 1986), CD4 (74-12-4), CD8 (76-2-11) and SLA class I (74-11-10) (Pescovitz et al., 1984); SLA class II (2E9/13) (kindly provided by R. Bulliados, CISA, INIA, Madrid); IgM (Mengeling, 1985); CD25 (IL-2 receptor) (Bailey et al., 1992). Cells (2.5 × 106–5 × 107) were washed twice with PBS containing 0.1% BSA and 0.01% sodium azide (FACS buffer) and incubated for 30 min at 4 °C with 50 μl of MAb supematants (1 : 2 dilution) or ascites (1 : 50 dilution). After washing twice in FACS buffer, cells were incubated for 30 min at 4 °C with 50 μl of fluorescein isothiocyanate (FITC-) conjugated rabbit anti-mouse F(ab)2 (Dako) diluted 1 : 40 in FACS buffer. Cells were washed twice in FACS buffer, fixed in 0.37% formalin and analysed with a flow cytometer (FACScan; Becton Dickinson).

**Proliferation assays.** Proliferation assays of the T cell clone were performed as described previously (Taylor et al., 1987), using cells grown for 1 week in the absence of infectious FMDV. Briefly, triplicates of 104 cells were incubated in 96-well plates with 105 irradiated syngeneic
PBMC and different FMDV concentrations in a total volume of 200 μl of supplemented RPMI medium. After 4 days at 37 °C, 1 μCi of [3H]thymidine was added to each well and the cultures were incubated for a further 18 h. The [3H]thymidine incorporation was then measured in a Microbeta counter (Pharmacia). The following controls were used to confirm the specificity of the proliferative response: (i) supernatants or clarified extracts of uninfected BHK-21 cells, and (ii) African swine fever or African horsesickness virus preparations, at a concentration of about 10^5 p.f.u./ml kindly provided by J. A. M. Escribano (CISA, INIA, Madrid) and M. Díaz-Laviada (CISA, INIA, Madrid), respectively. SLA restriction was assessed by incubation of CE3 cells with allogeneic feeder cells. Inhibition assays were carried out by adding 10 μg/well of each purified MAb during the incubation period. MAb 2E9/13 (anti-SLA class I) inhibited ConA but not PHA ionophore-induced proliferation of swine PBMC, while SLA class I, CD2, CD4 and CD8 MAbs did not affect these proliferations (N. Domenech, CISA, INIA, Madrid, personal communication). Plates (Transwell-COL; Costar), including a semipermeable membrane (0.4 μm pore size), were used to study the requirement for direct contact between CE3 cells and PBMC in the proliferative response.

**Determination of TCR usage.** Total RNA was isolated by using a commercial kit (RNAgents; Promega) based on the method described by Chomczynski & Sacchi (1987). RNA was dissolved in DEPC-treated water, quantified by A260 measurement and stored in aliquots at −20 °C until use. Total RNA (2–10 μg) was reverse transcribed using 100 U MMLV-RT (Promega) and an appropriate reverse primer (0.4 μM) in 50 μl of PCR mixture (0.4 mM-dNTPs, 1 mM-MgCl₂, 10 mM-Tris-HCl, 50 mM-KCl, 0.005% Tween 20, 0.005% NP40) for 1 h at 37 °C. After this, 20 pmol of forward primer and 2.5 U of thermostable DNA polymerase (PfuTurbo; Epicentre Technologies) was added to the mixture and the amplification was carried out for 30–35 cycles (denaturation: 94 °C, 45 s; annealing: 60 °C, 45 s; extension: 72 °C, 2 min) followed by a final extension step of 7 min at 72 °C. Amplification products were analysed on agarose gels stained with ethidium bromide. The synthetic oligonucleotides (purchased from Ramon Comet) used for the specific RT–PCR amplification of the different swine TCR genes are shown in Table 4.

**Results**

**Specificity of the proliferative response of CE3 clone**

PBMC from a FMDV-immunized SLA inbred pig were used to generate virus-specific T cell lines and clones as described in Methods. The ability of one of the clones obtained, CE3, to proliferate in response to FMDV was investigated. CE3 cells proliferated in the presence of infectious C-S8 FMDV, but not with BEI-inactivated virus, showing a typical T cell proliferation dose-dependent curve with an optimal concentration between 5 × 10^5 and 5 × 10^6 p.f.u./ml (Fig. 1).

FMDV has different serotypes and subtypes among which important antigenic differences exist. To characterize the antigenic specificity of the CE3 response, heterologous FMDV isolates of serotypes A, O and C were used to induce CE3 proliferation. The response observed was heterotypic, being significantly induced by each of the C1, A5 and O1 viruses tested. The specificity of the FMDV recognition by CE3 was confirmed as no proliferative response could be detected when supernatants or extracts of uninfected BHK-21 cells were added to CE3 cultures (data not shown). In addition, African swine fever virus or African horsesickness virus did not induce proliferation (Fig. 1b).

**Characterization of FMDV recognition by CE3 cells**

To assess whether the recognition of FMDV antigens by CE3 cells was mediated by a conventional T cell receptor–antigen interaction, the following experiments were performed. The requirement for accessory cells for CE3 FMDV-specific proliferation was analysed in cultures containing either syngeneic or allogeneic irradiated PBMC. Significant proliferative responses were only obtained when syngeneic PBMC were present, suggesting SLA restriction of the antigen recognition by CE3 cells (Table 1).

The role of macrophages as APC for viral antigens is well established. Recently, these cells have also been associated with the multiplication and spread of FMDV (Baxt & Mason, 1995). Therefore, we determined whether isolated syngeneic macrophages could serve as APC for CE3 recognition. Macrophage-enriched cultures, obtained from syngeneic PBMC by washing out non-adherent cells, were sufficient to support CE3 activation in the presence of infectious virus (Table 1). At this point, we had evidence of the requirement for infectious FMDV as well as the presence of syngeneic adherent PBMC or macrophages for the CE3 activation. Classical antigen recognition by T cells depends on a physical interaction between APC and the effector T cell. To examine whether this was the case here, a proliferation assay was carried out in which CE3 cells were separated from the PBMC by a semipermeable membrane, which allowed diffusion of soluble factors and virus but not cell–cell interaction. As shown in Table 2, the proliferative response was significantly inhibited when direct contact between APC and T cells was prevented.

To characterize the interaction between CE3 cells and the APC and to examine SLA restriction, MAbs against porcine CD4, CD8, SLA class I and SLA class II were used to inhibit the FMDV-specific proliferation of CE3. The results presented in Table 3 show that proliferation was strongly inhibited by MAbs to CD2 and CD8. MAbs to SLA class I and class II partially inhibited proliferation and a weak inhibition was observed with a MAb to CD4. The inhibition observed with MAbs to class I and CD8, together with the requirement for syngeneic PBMC, suggest that the recognition of FMDV antigen by CE3 cells is SLA class I-restricted and CD2 as well as CD8 molecules are involved in the cellular interaction.

**Phenotypic characterization of CE3**

The presence of porcine surface markers on CE3 cells was analysed by flow cytometry (Fig. 2). CE3 cells showed a phenotype characteristic of activated T lymphocytes: they expressed CD2, SLA class I and class II, and CD25 (interleukin-2 receptor) antigens and were negative for IgM. In addition, the clone was highly positive for CD8 and showed a low expression of CD4. These results are consistent with the
Fig. 1. Specificity of the proliferative response of CE3 cells to FMDV. Cells were incubated with APC and different virus preparations. Data indicate the [3H]thymidine incorporation by CE3 cells on the fourth day of culture (see Methods). (a) Requirement for infectious virus. Infectious C8 FMDV (■), BEI-inactivated C8 FMDV (●). (b) CE3 stimulation by different FMDV serotypes and other viruses. C1 Oberbayern (■), A5 Westerwald (●), O1 Kaufbeuren (○), African swine fever and African horsesickness viruses (□). Data are expressed as Δc.p.m (c.p.m. sample - c.p.m. medium alone), and the standard errors never exceeded 20% of the mean value. The backgrounds of the assays (proliferation without viral antigens) were: (a) 9979 ± 626 c.p.m.; (b) 3198 ± 987 c.p.m.

Table 1. Proliferative response of CE3 cells to FMDV with different PBMC

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>[3H]Thymidine incorporation (c.p.m. ±σm,n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic PBMC</td>
<td>9979±2494</td>
</tr>
<tr>
<td>Allogeneic adherent</td>
<td>3090±241</td>
</tr>
<tr>
<td>Syngeneic adherent</td>
<td>4912±1228</td>
</tr>
<tr>
<td>Control + FMDV</td>
<td>79412±2977</td>
</tr>
<tr>
<td>Control + FMDV*</td>
<td>3650±414</td>
</tr>
<tr>
<td>Control + FMDV†</td>
<td>69189±1078</td>
</tr>
</tbody>
</table>

* PBMC from a SLA a/a inbred pig.
† Plastic adherent feeder cells were obtained by repeated washes of feeder cells following 1 h incubation at 37 °C in the wells.
‡ FMDV was used at the optimum proliferative concentration (5 × 10^6 p.f.u./ml) as described in Methods.

Table 2. Effect of a semipermeable membrane between CE3 and irradiated syngeneic PBMC in the proliferation to FMDV

<table>
<thead>
<tr>
<th>FMDV</th>
<th>Membrane</th>
<th>[3H]Thymidine incorporation (c.p.m. ±σm,n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>No</td>
<td>34922±2906</td>
</tr>
<tr>
<td>Infectious</td>
<td>Yes</td>
<td>5455±511</td>
</tr>
<tr>
<td>Inactivated</td>
<td>No</td>
<td>1784±477</td>
</tr>
<tr>
<td>Inactivated</td>
<td>Yes</td>
<td>2593±533</td>
</tr>
</tbody>
</table>

(Fig. 3). The specificity of the primers was confirmed by partial sequencing of the amplification products (data not shown) or by amplification of plasmids containing cDNA of each of the TCR genes (Thome et al., 1993).

Expression of TCR genes on CE3 cells

To determine the expression of TCR in the CE3 clone we performed RT–PCR on RNA obtained from the cloned cells, using the specific oligonucleotides shown in Table 4. Only primers specific for constant segments of swine TCR α and β (but not δ) genes generated DNA products of the expected size, showing the presence in CE3 cells of TCR αβ transcripts.

Discussion

Our results indicate that in vitro stimulation of PBMC from vaccinated pigs allowed the selection of virus-specific T cell lines and clones. Suitable conditions for the establishment of a T cell line, including weekly restimulations with FMDV, were explored. In vitro stimulation of immune PBMC with infectious
Table 3. Inhibition of the proliferative response of CE3 cells to FMDV by monoclonal antibodies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Δc.p.m. ± s_m, *</th>
<th>Inhibition (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + ‡</td>
<td>9,364 ± 495</td>
<td>–</td>
</tr>
<tr>
<td>Anti-CD2</td>
<td>285 ± 39</td>
<td>97</td>
</tr>
<tr>
<td>Anti-CD8</td>
<td>283 ± 37</td>
<td>97</td>
</tr>
<tr>
<td>Anti-CD4</td>
<td>7,587 ± 460</td>
<td>19</td>
</tr>
<tr>
<td>Anti-SLA I</td>
<td>4,293 ± 971</td>
<td>54</td>
</tr>
<tr>
<td>Anti-SLA II</td>
<td>4,543 ± 143</td>
<td>51</td>
</tr>
<tr>
<td>Anti-IPNV§</td>
<td>8,802 ± 331</td>
<td>6</td>
</tr>
</tbody>
</table>

* Δc.p.m. is expressed as sample c.p.m. – c.p.m. of medium without FMDV.
† Percentage of inhibition was calculated as [(Δc.p.m. control – Δc.p.m. sample)/Δc.p.m. control] x 100.
‡ CE3 cells and irradiated syngeneic PBMC incubated with FMDV, as described in Methods, in the absence of MAb.
§ An irrelevant MAb against infectious pancreatic necrosis virus.

Fig. 2. Flow cytometry histograms of CE3 cells stained with MAb against swine surface molecules. Background (grey curves) corresponds to the fluorescence obtained directly with FITC-conjugated antibody.
### Table 4. Primers used for PCR amplification of TCR RNAs

<table>
<thead>
<tr>
<th>Primer*</th>
<th>Sequence (5' → 3')†</th>
<th>Expected size (bp)§</th>
<th>Source††</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR-Cα (f)</td>
<td>cccgggatccCCACTGTGTACCAGCTGAGA</td>
<td>425</td>
<td>(1)</td>
</tr>
<tr>
<td>TCR-Cα (r)</td>
<td>cccgggatccTCAACTGGACCACAGCCGAG</td>
<td>460</td>
<td></td>
</tr>
<tr>
<td>TCR-Cβ (f)</td>
<td>CCATCGGAAGCGGAGATCTCC</td>
<td>374</td>
<td>(2)</td>
</tr>
<tr>
<td>TCR-Cβ (r)</td>
<td>ggccacgcgtcgacggatccGAAGCCACAGTCTGCTTTGCC</td>
<td>396</td>
<td></td>
</tr>
<tr>
<td>TCR-Cγ (f)</td>
<td>cccgggatccGTCAGCCTCCTGCCAAACCAT</td>
<td>494</td>
<td>(2)</td>
</tr>
<tr>
<td>TCR-Cγ (r)</td>
<td>cccgggatccTCCTCATGCCAGTCAGCCT</td>
<td>350</td>
<td></td>
</tr>
</tbody>
</table>

* f, forward primer; r, reverse primer.
† The lower case letters indicate additional nucleotides introduced for cloning purposes.
§ Predicted size of the fragment amplified.
†† (1) Consensus sequence obtained after alignment of constant segment sequences of TCR α genes of human, mouse, rat, sheep and cow, obtained from the EMBL database. (2) Thome et al. (1993).

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Macrophages has been observed in the absence of antiviral antibodies (Baxt & Mason, 1995; Wardley et al., 1980; A. Rodriguez and others, unpublished results). Accordingly, macrophage-enriched cultures from syngeneic PBMC were assayed in the proliferation experiments and proven to be sufficient to sustain virus-specific activation of CE3.

In addition, the results suggest that CE3 cells recognize in a conventional way an FMDV antigen presented in the context of SLA molecules, since prevention of CE3-APC contact abolished proliferation. This hypothesis is consistent with the restriction observed in the response when allogeneic PBMC are used and with the inhibitions achieved with MAbs to CD8. The T cell lineage of CE3 cells was confirmed by the detection of the expression of TCR αβ genes by PCR.

Attempts to set up experimental conditions that would enable a CTL assay of CE3 cells have been unsuccessful. Infection of syngeneic primary cultures of kidney cells resulted in a strong and rapid cytopathic effect (within 5 h p.i.) which hampered the detection of specific ⁵¹Cr release. The use of macrophages as target cells did not result in significant specific lysis (data not shown), probably because their susceptibility to FMDV is too low to allow the CTL-mediated lysis of a significant percentage of the ⁵¹Cr-labelled cells.

Certain characteristics make the porcine immune system different from that of other species. Among swine peripheral lymphocytes, typical single CD4⁺ and CD8⁺ subpopulations coexist with CD4⁺/CD8⁺ double-positive cells (unique to swine) as well as a double-negative population CD4⁻/CD8⁻ that is subdivided in CD2⁺ and CD2⁻ lymphocytes (Saalmüller et al., 1994; Pescovitz et al., 1994). FACS analysis of CE3 cells has revealed the expression of CD2 and IL-2 receptor markers. In addition, the CE3 clone expresses CD4 surface marker. However, the staining intensity of CE3 cells with anti-CD4 is lower than that normally found among CD4⁺ PBMC, while signal with CD8 MAb is in the same range as that found in CD8⁺ PBMC (R. Bullido, personal communication). Therefore, the CE3 clone is not a typical double-positive T cell, since these cells normally exhibit strong intensity of the CD4 marker and a low CD8 intensity (Pescovitz et al., 1994). MAb to the CD8 and CD2 markers strongly inhibited CE3 proliferation. Conversely, the MAb to CD4 did not significantly affect the response. In addition, both SLA class I and class II MAbs significantly inhibited proliferation. The inhibition observed with class II MAb is probably due to a mechanism similar to that resulting in the inhibition exerted by this MAb in ConA stimulation of swine PBMC (N. Domenech, personal communication). Altogether, these results indicate that CE3 recognition of FMDV antigen is class I-restricted and hence CD8 acts as a co-receptor.

In spite of the considerable antigenic diversity of FMDV, the CE3 response is heterotypic, a characteristic also found in T cell epitope recognition among other picornaviruses (Kutubuddin et al., 1992). Cloning of different FMDV polypeptides in vaccinia virus is now in progress in our laboratory to generate suitable target cells for CTL assays, and to provide us with a tool for the identification of the polypeptide bearing the FMDV epitope recognized by CE3 cells. In addition, we are investigating other possible biological activities of CE3 cells such as the secretion of soluble antiviral interleukins (Zinkernagel, 1996). CE3 cells might become activated upon rec-
ognition of target cells that do not support a fully productive FMDV infection, but which are able to present viral antigens, as could be the case for macrophages.

A class I (CD8+)-restricted response is generally induced by endogenously synthesized antigens, such as those generated during viral infections, while induction of a class II (CD4+) response is mediated by exogenous soluble proteins. Consequently, in order to induce a CD8 cell response, the strategy is usually the inoculation of an infectious or partially inactivated virus which can replicate in the host. Thus, it was unexpected to find that the phenotype of the clone obtained in this study corresponded to that of a CD8+ T cell, since it was selected from a PBMC culture from an animal vaccinated with inactivated virus. However, it has been shown that purified proteins or peptides can induce class I-restricted CTL populations in several systems (Aichele et al., 1990; Schirmbeck et al., 1995) suggesting that our results may not be so unusual. The protocol for establishment of the cell line and maintenance of this clone, by using infectious virus for the in vitro restimulation, together with the ability of FMDV to infect, to some extent, APC, may have favored the selection of a population enriched in CD8+ T cells. According to these results, the induction of a wide humoral and cellular response to FMDV could be induced by the inclusion of not only B and CD4 but also CD8 epitopes in synthetic peptide-based vaccines.

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