Two Epitopes Shared by *Taenia crassiceps* and *Taenia solium* Confer Protection against Murine *T. crassiceps* Cysticercosis along with a Prominent T1 Response

ANDREA TOLEDO,1 GLADIS FRAGOSO,1 GABRIELA ROSAS,1 MARISELA HERNÁNDEZ,1 GOAR GEVKOIAN,1 FERNANDO LÓPEZ-CASILLAS,2 BEATRIZ HERNÁNDEZ,2 GONZALO ACERO,1 IRNA HUERTA,4 CARLOS LARRALDE,1 AND EDDA SCIUTTO1*

Instituto de Investigaciones Biomédicas, Instituto de Fisiología Celular, and Facultad de Medicina, UNAM, México D. F., and Facultad de Medicina, Benemérita Universidad Autónoma de Puebla, Puebla, México

Received 24 August 2000/Returned for modification 27 September 2000/Accepted 7 November 2000

*Corresponding author. Mailing address: Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), A.P. 70228, México D.F., 04510, México. Phone: (525) 6223818. Fax: (525) 6223369. E-mail: edda@servidor.unam.mx.

---

*Taenia crassiceps* recombinant antigens KETc1 and KETc12 have been shown to induce high level of protection against experimental murine *T. crassiceps* cysticercosis, an experimental model successfully used to test candidate antigens for use in vaccination against porcine *Taenia solium* cysticercosis. Based on the deduced amino acid sequence, KETc1 and KETc12 were chemically synthesized in linear form. Immunization with KETc1 induced 66.7 to 100% protection against murine cysticercosis, and immunization with KETc12 induced 52.7 to 88.1% protection. The elicited immune response indicated that both peptides contain at least one B-cell epitope (as demonstrated by their ability to induce specific antibodies) and one T-cell epitope that strongly stimulated the proliferation of T cells primed with either the free peptide or total cysticercal *T. crassiceps* antigens. The high percentage of spleen cells expressing inflammatory cytokines points to the likelihood of a T1 response being involved in protection. The protective capacity of the peptides and their presence in all developmental stages of *T. solium* point to these two epitopes as strong candidates for inclusion in a polyepitopic synthetic vaccine against *T. solium* pig cysticercosis.

---

Instituto de Investigaciones Biomédicas, Instituto de Fisiología Celular, and Facultad de Medicina, UNAM, México D. F., and Facultad de Medicina, Benemérita Universidad Autónoma de Puebla, Puebla, México

---

*Taenia solium* cysticercosis is a common parasitic disease of the central nervous system of humans in several countries in Latin America, Africa, and Asia, where it represents a major health and economic problem (2, 28). The life cycle of this parasite includes a larval phase (cysticercus) that affects both pigs and humans after the ingestion of *T. solium* eggs. The parasite’s life cycle is completed when humans consume improperly cooked cysticercotic pork and the adult intestinal tapeworm develops and, in turn, produces millions of eggs that are shed in human feces. In regions of endemic infection, transmission is clearly related to prevailing low standards of personal hygiene and environmental sanitation control (i.e., open air fecalization) in areas where rustic rearing of pigs is practiced by the rural population (pigs roaming about freely in search of edibles and/or deliberately fed with human feces [11]). Regrettably, control of transmission by general improvement of the social, economical, and educational status in developing countries or by proper and strict meat inspection programs is not within reach in the near future. However, since the pig is an indispensable intermediate host, transmission could be hindered by lowering the prevalence of pig cysticercosis through vaccination. Development of an effective vaccine to be used in pigs is being pursued by a number of scientists, with promising results (9, 15–17).

Because of the high costs of experimentation in pigs, murine cysticercosis caused by *Taenia crassiceps* has been used to test and select promising antigens before they are tested in pigs (13, 21). Thus, it has been shown that total *T. crassiceps* antigens can cross-protect pigs against *T. solium* cysticercosis. However, the effects of vaccination with whole-antigen extracts were strongly dose dependent; besides, some antigens were found to be protective while others led to facilitation of the infection (22). Such complications with the use of whole-antigen extracts led us to redirect our research to the identification of individual protective antigens (14, 26). Using recombinant DNA technology, several vaccine candidates were identified in murine *T. crassiceps* cysticercosis with crude lysates of the respective clones as the immunogen (13, 14). One of them, KETc7, which has a protective capacity confirmed by DNA immunization (1, 20), includes at least one protective epitope of 17 amino acids (GK1). GK1 is also expressed in *T. solium* oncospheres (25), the parasite’s developmental stage most vulnerable to immunological attack (19). Two additional protective clones, KETc1 and KETc12 (14), were also identified. Herein we report the protective capacity against *T. crassiceps* murine cysticercosis of the peptides deduced from these last two clones. Furthermore, we describe the localization of the peptides in each parasite stage of *T. solium* and *T. crassiceps*, the immune response they elicit in immunized mice—where T1 is most prominent—and propose them as additional components for a synthetic vaccine to be tested in pigs in an attempt to block *T. solium* transmission.

---

MATERIALS AND METHODS

Peptides. Two *T. crassiceps*-derived peptides (14) that are shared by *T. solium* (24), KETc1 [APMSTPSATSVR(G)] and KETc12 [GNLLSCL(G)], were synthesized by stepwise solid-phase synthesis with N-tert-butyloxycarbonyl derivatives of L-amino acids on phenyl-acetamidomethyl resin (Sigma Chemical Co., St. Louis, Mo.). The peptides were 95% pure as judged by high-pressure liquid
VOL. 69, 2001 CYSTICERCOSIS VACCINE 1767

Percentage of protection with respect to controls. 

Median (95% confidence interval) of individual parasite intensities in control mice injected with adjuvant alone or together with an immunizing peptide. Statistically significant differences between control and experimental mice were compared using analysis of variance followed by Tukey’s test.

**Individual number of cysticerci recovered 30 days after infection from each mouse.**

*Median (95% confidence interval) of individual parasite intensities in control mice.

### TABLE 1. Protective immunity against murine cysticercosis by immunization with KETc1 and KETc12 peptides

<table>
<thead>
<tr>
<th>Group of experimental mice</th>
<th>No. of cysticerci</th>
<th>Paramecium intensity</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>55, 48, 41, 47, 46</td>
<td>46.5 (41.5–51.5)</td>
<td></td>
</tr>
<tr>
<td>KETc1 Immunized</td>
<td>16, 6, 3, 10, 36, 10, 19, 13, 18</td>
<td>11.5 (8.5–22.9)**</td>
<td>75.3</td>
</tr>
<tr>
<td>KETc12 Immunized</td>
<td>22, 6, 2, 24, 27, 24</td>
<td>52.7 (41.2–59.6)</td>
<td>22.4</td>
</tr>
</tbody>
</table>

*Individual number of cysticerci recovered 30 days after infection from each mouse.*

**Paramecium intensity**

### FIG. 1. Antibody levels determined by ELISA in individual control (C) and immunized (I) mice against TcAg. The mean level of antibodies was significantly higher in immunized mice than in controls. O.D., optical density.

Chromatography in an analytical C18 reversed-phase column (3.9 by 150 mm; Delta Pak [Waters]). The correct amino acid sequence of each peptide was confirmed by protein sequencing on a pulsed-liquid-phase protein sequencer (Applied Biosystems) at the National Institute of Cardiology, Mexico City.

**Mice.** BALB/cAnN mice, previously characterized as susceptible to cysticercosis (3), were used in vaccine trials. The original murine stock was purchased from M. Bevan (University of Washington) and then bred and kept in our animal facilities by the “single-line breeding” system for more than 30 generations. All mice used were males that were 5 to 7 weeks of age at the beginning of the experiments. The experiments reported herein were conducted according to the protocols set forth in the **Guide for the Care and Use of Laboratory Animals,** Institute of Laboratory Animals Resources, National Research Council, Washington, D.C.

**Immunization of mice and serum collection.** Groups of six to nine BALB/cAnN mice were subcutaneously immunized with two doses of 10 μg of each individual peptide in saponin (Sigma Chemical Co.) per mouse at a concentration of 100 μg/mouse as described elsewhere (25). This dose was determined as optimal in collagen experiments (data not shown). Ten days later, the mice were given a booster with the same immunizing dose of the same peptide in the same adjuvant as used before. Immune sera were obtained from each individual mouse before and after each immunization and stored at −70°C until individually tested for the presence of specific antibodies.

**Parasites and cysticercal antigens.** The ORF strain of *T. crassiceps* (4) has been maintained by serial passage in BALB/cAnN female mice for 15 years in our animal facilities. Cysticerci for infection were harvested from the peritoneal cavity of mice 1 to 3 months after inoculation of 10 nonbudding small cysticerci (2 to 3 mm in diameter) per animal. The soluble antigens were recovered from similar cysticerci by a previously described procedure (18). Whole *T. solium* cysticerci were dissected from skeletal muscle of highly infected pork carcasses 2 to 4 h after slaughter in an abattoir in Zacatepec, Morelos, Mexico; embedded in optimum-cutting-temperature compound (Miles, Inc.), and frozen at −70°C until used in immunofluorescence assays (see below). Segments from *T. solium* tapeworm and eggs were obtained from the feces of an infected man in Puebla, Mexico. The tapeworm was recovered after treatment with a single oral dose (2 g) of niclosamide (Yomesan; kindly supplied by Bayer). After being washed in saline plus antibiotics (100 U of penicillin per ml plus 100 μg of streptomycin per ml), several gravid proglottids were separated for immunofluorescence assays.

**ELISA for antibody measurements.** *T. crassiceps* whole soluble antigens (TcAg) were obtained as previously described (18) and used as the source of antigens in an enzyme-linked immunosorbent assay (ELISA) to measure the antibody response induced by peptide immunization by using a procedure described elsewhere (25).

**Proliferation assay.** Spleen cells from control and KETc1- and KETc12-immunized mice were harvested 15 days after the second immunization and cultured in RPMI 1640 medium supplemented with l-glutamine (0.2 mM), nonessential amino acids (0.01 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and fetal bovine serum FBS (10%). The cells were cultured with the appropriate concentration of concanavalin A (ConA) (5 μg/ml), KETc1 (50 μg/ml), KETc12 (10 μg/ml), or TcAg (10 μg/ml) and incubated at 37°C in a 5% CO2 humidified atmosphere in flat-bottomed microtiter plates at a cell concentration of 2 × 10⁵ cells per 200 μl of final volume. Then 10³ peritoneal cells recovered from the
same mice were added to each well in a volume of 50 μl. Peritoneal cells were obtained by ex vivo lavage with 5 ml of RPMI 1640 medium. After 72 h, the cultured cells were pulsed (1 μCi per well) for a further 18 h with [methyl-3H] thymidine (Amersham Life Science, Little Chalfont, United Kingdom). Then all the cells were harvested and the amount of incorporated label was measured by counting in a 1205 β-plate spectrometer (Wallac).

Spleen cell phenotype analysis. After 3 days of in vitro culture with medium, TcAg, or peptides, splenocytes were harvested and CD8 and CD4 expression was determined by staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD8 (Pharmingen, San Diego, Calif.) and phycoerythrin-conjugated anti-CD4 (Pharmingen), respectively, by a previously reported procedure (25). Parallel samples of the cells were stained with the corresponding isotype control to account for nonspecific staining of the cells. Briefly, the cells were washed with phosphate-buffered saline (PBS) containing 10% gamma globulin-depleted FBS plus 0.02% NaN₃ and incubated with the indicated antibodies at 4°C for 30 min. After being washed, the splenocytes were resuspended in cold 1% formaldehyde in isotonic solution and analyzed with a FACScan instrument (Becton Dickinson, Palo Alto, Calif.). The results are expressed as a percentage of positive cells.

Cytokine measurements. For detection of intracellular cytokines, spleen cells were treated with medium, KETc1, KETc12, or TcAg and cultured for 60 h. To inhibit cytokine secretion, brefeldin A (2 μM) was added to the cells culture 10 h before the assay. At harvest, the cells were centrifuged at 500 × g for 10 min and washed twice in ice-cold PBS containing 10% gamma globulin-depleted FBS plus 0.02% NaN₃. CD3 and CD4 expression were determined by two-color fluorescence-activated cell sorting (FACS) as previously described (25). Briefly, the cells were stained with biotin anti-CD3 (Pharmingen) and then streptavidin-FITC (Sigma) was added. Intracellular cytokines were assayed by using a cytostain TM kit (Pharmingen) to fix and permeabilize the cells. To stain intracellular cytokines, fixed and permeabilized cells were incubated with phycoerythrin-conjugated monoclonal rat anti-IL-2, anti-IL-4, anti-IL-10. Intracellular cytokines were assayed by using a fluorescein isothiocyanate (FITC)-conjugated anti-IL-2, anti-IL-4, anti-IL-10, or anti-gamma interferon (INF-γ) (all from Pharmingen). Parallel samples of the cells were stained with isotype control to account for nonspecific staining of the cells. Then 10⁵ cells were analyzed with a CD3⁺ lymphocyte gate as defined by light scatter in a FACScan instrument. The results are expressed as a percentage of positive cells.

Experimental challenge. Metacestodes used in challenge infections were harvested from the peritoneal cavity of BALB/cAnFe female mice carrying the ORF strain of T. crassiceps cysticerci. Ten small (diameter, ca. 2 mm), nonbudding larvae were suspended in 0.5 M NaCl-0.01 M sodium phosphate buffer (pH 7.2) and intraperitoneally injected into each challenged mouse using a 27-gauge needle. After 3 days of in vitro culture with medium, CD8 and CD4 expression was determined by staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD8 (Pharmingen) and then streptavidin-FITC (Sigma) was added. Intracellular cytokines were assayed by using a cytoStain TM kit (Pharmingen) to fix and permeabilize the cells. To stain intracellular cytokines, fixed and permeabilized cells were incubated with phycoerythrin-conjugated monoclonal rat anti-IL-2, anti-IL-4, anti-IL-10, or anti-gamma interferon (INF-γ) (all from Pharmingen).

RESULTS

Protective effect of peptide immunization against T. crassiceps cysticercosis. The effect of peptide immunization on the number of cysticerci recovered from mice immunized with KETc1 and KETc12 or adjuvant alone (controls) is shown in Table 1: 66.7, 75.3 or 100% protection was induced using KETc1 as immunogen, and 52.7, 73.4, or 88.1% protection was induced using KETc12 as immunogen. Some mice were completely protected (no parasites) by immunization with either KETc1 or KETc12.

Antibody response induced by KETc1 and KETc12 immunization. To test for the presence of a B-cell epitope(s) within the two peptides, the levels of induced anti-KETc1 and anti-KETc12 specific antibodies were assessed. T. crassiceps cysticercal antigens (Fig. 1) as well as each of the peptides were used as antigens in ELISAs (data not shown). Figure 1 shows low but detectable levels of serum antibodies in both KETc1- and KETc12-immunized mice.

Immunolocalization of KETc1 and KETc12 in the parasite. Pooled sera with the highest antibody levels induced by KETc1 and KETc12 immunizations were used to immunolocalize the native antigen in both T. crassiceps and T. solium (Fig. 2 and 3). KETc1 and KETc12 were expressed in the tegument of T. crassiceps cysticerci, albeit with different distributions. KETc1 was restricted to the tegument of both cysticerci (Fig. 2E and F), while in T. solium it was found in the most external part of the tegument and also in the cuticular folds of the spiral canal (Fig. 2F). KETc12 (Fig. 2G and H) was very abundant in both metacestodes. Nevertheless, the T. crassiceps tegument showed an intensely positive wall surface and parenchyma, especially around the calcareous corpuscles (Fig. 2G). KETc12 was also detected in the oncosphere of the egg as numerous points (Fig. 3G), in contrast to KETc1, which was almost negative. Both epitopes were present in tapeworm tissue: KETc1 was very abundant on the most external side of the tegument (Fig. 3F), and KETc12 was distributed along the tegument’s depth (Fig. 3H). When sera from infected mice were used, all structures were fluorescent (Fig. 3C and D). The specificity of these antibody reactions was demonstrated by the lack of reactivity of normal mouse serum with the used tissues (Fig. 3A and B and 3A and B).
TABLE 2. T-cell proliferative response of splenocytes from control and KETc1- and KETc12-immunized mice

<table>
<thead>
<tr>
<th>Source of cells</th>
<th>Media</th>
<th>ConA</th>
<th>Peptide</th>
<th>TcAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1,086 ± 370</td>
<td>183,643 ± 16,482</td>
<td>1,342 ± 248</td>
<td>754 ± 113</td>
</tr>
<tr>
<td>KETc1 mice</td>
<td>2,734 ± 1,364</td>
<td>119,030 ± 2,675</td>
<td>7,289 ± 2,675&lt;sub&gt;b&lt;/sub&gt;</td>
<td>9,941 ± 1,802&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>KETc12 mice</td>
<td>1,560 ± 262</td>
<td>155,125 ± 9,816</td>
<td>6,321 ± 2,021&lt;sub&gt;b&lt;/sub&gt;</td>
<td>10,450 ± 2,238&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± standard deviation of the [3H]thymidine incorporated after 3 days of culture of splenocytes from three individual mice assayed separately. The data are representative of three repeat experiments, each performed in triplicate.

<sup>b</sup> Significantly increased proliferative responses were achieved when cells from immunized mice were stimulated with both peptides and whole TcAg. Comparisons between immunized and control values were considered statistically significant at (<em>P</em> < 0.05).

**Assessment of T-cell epitopes on the KETc1 and KETc12 peptides.** The proliferative response of spleen cells from mice immunized with KETc1 or KETc12 or saponin alone is reported in Table 2. Spleen cells from mice injected in vivo with free peptides or saponin were stimulated in vitro with the corresponding peptide, TcAg, or ConA in previously determined optimal concentrations. Table 2 shows that in vitro stimulation with KETc1 or KETc12, as well as with cysticercal antigens, induced a significantly greater proliferative response in cells from immunized mice than in those from control mice. Cells from mice injected with saponin (controls) showed no proliferative response above background levels.

Figure 4 shows that stimulated cells increased from 3.5 or 4.5% to 8–16.3% when the cells were primed with TcAg or the appropriate peptide, respectively. Stimulated cells were enriched in both CD4<sup>+</sup> and CD8<sup>+</sup> cells by factors of 1.2 to 2.0 for CD4<sup>+</sup> and 3.9 to 4.9 for CD8<sup>+</sup>.

The proportion of cells capable of producing IL-2, IL-4, IL-10, and INF-γ was determined by FACscan analysis after intracellular staining for cytokines. The results are shown in Table 3. The proportion of cells producing IL-2 and INF-γ were significantly higher in KETc1-, KETc12-, or TcAg-stimulated cells than with media alone and more so in immunized than in control mice. The levels of IL-4- and IL-10-expressing cells were also increased but to a lesser extent.

**DISCUSSION**

Our results show that KETc1 and KETc12 induce protection against experimental murine <em>T. crassiceps</em> cystercerosis and that both are B- and T-cell epitopes. The protective capacity and the immunity induced by these two peptides closely resemble those induced by GK1, a previously reported protective epitope also shared by <em>T. crassiceps</em> and <em>T. solium</em> (25). It should be noted that there are two forms of expressing protection; i.e., reduction in parasite intensity and proportion of totally parasite-ridden mice. Because <em>T. crassiceps</em> cysticeri multiply asexually in the peritoneal cavity of infected mice, the reduction in parasite intensity is highly dependent on the time of assessment after infection; the effects of vaccination tending to disappear in late infections. Sterile immunity is attained only if the initial inoculum is totally destroyed by the immune system response. These and previous results are in accordance with the notion that if a single <em>T. crassiceps</em> cysticercus evades or survives the initial immune attack of the murine host, it will multiply and eventually reach very high parasite intensities indeed (3). This initial immune attack would appear must successful if a strong T1 response is induced, as we have shown here and others have shown previously (23). In older infections, when massive parasite intensities (>10<sup>5</sup>) are achieved, T2 responses predominate and perhaps downregulate T1 (23). These two peptides would appear to touch off the protective T1 response more efficiently than the T2 response.

The immunologic assays performed in our experiments indicate the immune mechanisms involved in infection control. It has been repeatedly stated that protection induced by vaccination against <em>T. crassiceps</em> murine cystercerosis is T1 related whereas antibodies and other T2 molecules are less effective (1). In this study, results point in the same direction: while antibodies are erratically and weakly induced by both KETc1 and KETc12, IL-2 induction is noticeably increased 5.7- to 10.1-fold in immunized mice relative to control mice. The same is true for IFN-γ, the characteristic inflammatory cytokine, which activates macrophages in the vicinity of the parasite and triggers their well-known damaging effects (25). In addition to the preponderance of the inflammatory interleukins IFN-γ and IL-2, the low profiles of IL-4 and IL-10, which inhibit the proliferation of the T2 responses, could well explain the low levels of antibodies elicited by both peptides. Protective immunity in the context of a T1 response has also been related to innate resistance conditions (23, 27). Despite the low levels of specific antibodies induced by both peptides, their possible protective role cannot be excluded. This is of particular relevance considering the recent finding of the capacity of anti-GK1 antibodies to block <em>T. solium</em> cysticercus conversion to tapeworms (5). The fact that more than 50% of human neurocysticercosis patients make antibodies against KETc1 and KETc12 (8) strengthens our interest in these two epitopes.

Based on the different anatomic distribution of KETc1 and
KETc12 in *T. solium* cysticerci and in oncospheres, which also differ from that of GK1, it would appear that all three peptides should be included in a vaccine against pig cysticercosis to maximize the number of targets. In spite of the risk associated with extrapolating from *T. crassiceps* and mice to *T. solium* and pigs, optimism about vaccine development prevails because of the many examples of effective immunity induced against different cestodes in diverse hosts. Also, the extensive similarities among cestode infections in terms of their natural history, pathology, and antigenic composition all point to possibly similar effects of vaccination (6, 7, 10). In fact, different sources of protective antigens have been successfully used as vaccines against porcine cysticercosis, one using recombinant antigen from *Taenia ovis* (12) and the other using antigen from *T. crassiceps* itself (13).

In the hope of increasing the efficiency of vaccination, it is advisable that KETc1 and KETc12 plus GK1, all of which induce high levels of protection in the murine model of cysticercosis and are present at all stages of *T. solium* development, be considered as candidates for inclusion in a mixed poly-

**FIG. 4.** Flow cytometer analysis of spleen cells from KETc1- and KETc12-immunized mice with or without in vitro stimulation with the respective peptide or antigen (TeAg) R1 denotes the region of proliferating cells in the SSC/FSC plot (side-scatter/forward-scatter plot), and the number below indicates the percentage of cells in this region. CD4⁺ and CD8⁺ cell percentage expression was determined in the defined R1 gate.
epitopic synthetic vaccine to be used against *T. solium* cysticercosis in pigs.

**ACKNOWLEDGMENTS**

This work was supported by Dirección General de Asuntos de Personal Académico (IN212798) and Dirección General de Inter cambio Académico, Universidad Nacional Autónoma de México; CONACYT (G25955M), México; Fundación Miguel Alemán, México; ANUIES (M99S03); and the British Council.

We thank Felipe Massó for performing the peptide sequence analyses, Mercedes Baca for administrative support, Ismael Ramírez Jiménez for technical support, and Isabel Pérez Montfort for help in the translation of the manuscript.

**REFERENCES**


**TABLE 3. Percentage of CD3**

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>INF-γ</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>INF-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (media)</td>
<td>1.4</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>1.6</td>
<td>0.9</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Peptide</td>
<td>8.1</td>
<td>4.4</td>
<td>3.0</td>
<td>11.2</td>
<td>9.4</td>
<td>3.2</td>
<td>4.8</td>
<td>14.5</td>
</tr>
<tr>
<td>Cystercal antigens</td>
<td>13.0</td>
<td>4.0</td>
<td>3.6</td>
<td>6.7</td>
<td>16.2</td>
<td>2.6</td>
<td>4.2</td>
<td>17.0</td>
</tr>
</tbody>
</table>

* Pooled splenocytes from three KETc1 or KETc12 immunized mice were analyzed for intracellular cytokines 60 h after in vitro stimulation.

* The percentages of CD3⁺ cells expressing the four different interleukins (not included) were below 1% in splenocytes from control mice with and without in vitro stimulation. Data are representative of two different experiments using different mice.

**Editor:** J. M. Mansfield