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Identification of T cell epitopes within a 23-kD antigen (P24) of *Toxoplasma gondii*

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SUMMARY

Among the potentially vaccinating antigens, the products excreted/secreted by the parasite *T. gondii* have been demonstrated to be excellent candidates. The molecular cloning of one of these antigens (P24) present in excreted/secreted antigens (ESA) has recently been carried out in our laboratory. The recombinant antigen P24 corresponds to a native molecule of 23 kD. We were interested in determining the main epitopes of the P24 antigen eliciting a T lymphocyte response using synthetic peptides derived from the primary structure of P24. Five peptides: 64–79, 88–109, 170–193, 194–208 and 231–250 were synthesized according to their hydrophobicity, mobility and accessibility profiles. The presence of T lymphocyte epitopes in these peptides has been examined in the rat model. The determination of T cell epitopes was carried out using T lymphocytes from infected rats, and from ESA and P24 expression vaccine virus immunized rats. The results showed that the stimulation of T cells with these peptides varied according to the period after *Toxoplasma* infection. The main T cell stimulation was obtained with the 88–109, 170–193 and 194–208 peptides. When Fisher rats were immunized with ESA, a most significant stimulation was achieved with the 170–193 and 194–208 peptides. In addition, T lymphocytes primed with P24 expressed vaccine virus immunization were more stimulated with the 88–109 and the 194–208 peptides. This study showed that P24-derived peptide-specific T cells were elicited in the three experimental situations, although no antibody response against the 23-kD native antigen was evidenced in the Fisher rat model. However, the native antigen (presented by irradiated parasites) can induce a proliferative response of the 170–193 peptide-specific T lymphocytes, confirming that this peptide contains an important T cell epitope. The adoptive transfer into athymic rats of T helper cells recovered from 170–193 peptide-immunized Fisher rat conferred a significant protection to infected nude rats despite the fact that no antibody production was observed.

Keywords *Toxoplasma gondii* P24 T cell epitopes synthetic peptides

INTRODUCTION

In attempts to understand how the host responds to *T. gondii* infection, several experiments have indicated that T cell subsets must play an important role in cell-mediated immunity against toxoplasmosis. This was observed in mice, hamsters and guinea pigs (Frenkel, 1967; Lindberg & Frenkel, 1977; Pavia, 1986a, 1986b). With the athymic rat model, we have also shown that T cells were essential for the resistance against infection by this parasite (Santoro *et al.*, 1987; Duquesne *et al.*, 1990).

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The coccidian protozoa *T. gondii* is an obligate intracellular parasite of humans and animals. Because this parasite can cause important fetal lesions in congenital toxoplasmosis and toxoplasmic encephalitis in immunocompromized individuals, including transplant recipients (Luft *et al.*, 1983) and patients with AIDS (McCabe & Remington, 1988), a vaccine against *T. gondii* would be highly desirable for these populations at risk.

Among the potential vaccinating antigens, the molecules excreted secreted by the parasite seem to be excellent candidates. We have shown that sera (Darcy *et al.*, 1988) and T cells (Duquesne *et al.*, 1990) from Fisher rats immunized with excreted/secreted antigens (ESA) can protect athymic rats that are otherwise highly susceptible to *T. gondii* infection.

Recently in our laboratory, a molecule of 23 kD has been cloned (Cesbron-Delauw *et al.*, 1989). This secreted antigen is

recognized by serum from rabbits immunized with ESA and by sera from chronically infected humans. The recombinant proteins were immunogenic in mice, producing antibodies against the native P23. Immunocytochemical analysis located the native antigen in the dense granules of both tachyzoite and bradyzoite forms and showed that it is secreted into the parasitophorous vacuole.

For a strategy of vaccination, it would be interesting to produce a vaccine of a defined molecular composition. This molecular vaccine needs to include all the components capable of eliciting a protective immune response, notably the B cell as well as the T cell epitopes. In this regard, the determination of the primary structure of P23 (Cesbron-Delauw *et al.*, 1989) has allowed the selection and synthesis of peptides.

In the present study, the identification of the T cell epitopes of the 23-kD antigen has been undertaken by using five synthetic peptides selected according to their probable epitopic structure. Among the five peptides, several showed a significant importance in T cell-mediated immunity.

MATERIALS AND METHODS

Culture medium

RPMI 1640 (GIBCO, Courbevoie, France) containing 20 mM HEPES, 100 U penicillin/ml, and 100 μg streptomycin/ml was supplemented with 2 mM L-glutamine, 5×10^{-5} M mercaptoethanol and 1 mM sodium pyruvate and inactivated fetal calf serum (FCS) (Boehringer Mannheim, Meylan, France).

Parasites

RH strain tachyzoites were obtained from infected cell cultures according to the method of Betz (1968). Briefly, the tachyzoites recovered from the peritoneal fluids of infected mice were inoculated into a cellular monolayer of HEP 2 cells after 48–72 h of culture. The infection was performed in Dubelcco's medium without FCS. The supernatant containing the parasites was generally recovered on day 6 or 7 (after day 4 post-infection, the culture medium was changed every day).

Rats

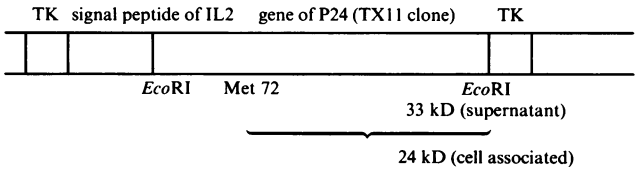
Male inbred Fisher/OCO F 344 rats and female genetically athymic Fisher rats were used throughout the experiment. The animals were obtained from Harlan Olac (Oxon, England).

Antigen preparation

Soluble antigens (S2) and membrane antigens (CHAPS extract) were prepared as described by Duquesne *et al.* (1990). Briefly, the tachyzoites were lysed in water. The supernatant contained the soluble antigens (S2) and the parasite pellet containing the membrane antigens was solubilized by the addition of 3 [(3-cholamidopropyl) dimethyl amino] propan sulphonic acid (Fluka, Buchs, Switzerland) (CHAPS).

Tachyzoite excreted/secreted products prepared as described (Darcy *et al.*, 1988) were used for the immunization of Fisher rats.

The P24 recombinant vaccinia virus: the *Eco*RI insert of TX11 cDNA (Cesbron-Delaw *et al.*, 1989) was cloned in frame with the sequences coding for the signal peptide and first eight amino acids of human interleukin-2 (IL-2), flanked by region of the vaccinia virus TK gene. Transfer into vaccinia virus was performed as described (Kieny *et al.*, 1984), generating the VVTG 2170 clone:



Infection and immunization

Inbred Fisher rats were infected by the intraperitoneal route with 10^6 parasites from cell cultures in and the inguinal and mesenteric lymph nodes were harvested aseptically every week. The T cells were tested against the different synthetic peptides.

Fisher rats were immunized at the base of the tail according to the method of Corradin, Etlinger & Chiller (1977) with ESA (equivalent of excretory antigens from 10^8 tachyzoites) mixed 1/1 with Freund's complete adjuvant (FCA). The second injection was performed without adjuvant after 2 weeks. T lymphocytes were recovered after 5 days. The same procedure was used for the immunization by bovine serum albumine (BSA).

For the preparation of peptide-specific T cells, Fisher rats were immunized at the base of the tail by the 170–193 peptide coupled to BSA as a carrier (50 μg) with FCA for the first injection and without carrier and with Freund's incomplete adjuvant (FIA) for the second and third injection at 1-week intervals.

The preparation of recombinant P24-specific T lymphocytes was performed by the injection of the P24 vaccinia virus construction (described above) according to two protocols: the first is the injection of two doses of 10^8 plaque-forming units of vaccinia virus at the base of the tail. With this procedure, only inguinal and periaortic lymph nodes were recovered. The second consisted in the injection of the same dose by intraperitoneal route. The mesenteric and periaortic lymph nodes and the spleen were recovered.

Selection of peptides

The determination of the primary structure of P24 by sequencing the gene (Cesbron-Delauw *et al*, 1989) allowed the prediction of exposed sequences according hydrophilicity criteria (Hopp & Woods, 1981) (Fig. 1). Prediction of α-amphipathic structures, presumably correlated to determinants recognized by T cells (Delisi & Berzofsky, 1985), was achieved by examining both helical propensity (Chou & Fasman, 1974) and hydrophobic moment (Eisenberg, Weiss & Terwilliger, 1984), calculated along the sequence for seven residue blocks, considering a periodicity of an α-helix (i.e. 3·6 residue per turn, 100° per residue). The occurrence of the linear pattern proposed by Rothbard & Taylor (1988) was also examined.

Sequence numbering was according to the longest cDNA clone sequence described by Cesbron-Delauw *et al.* (1989). The open reading frame is likely to be initiated by Met-72. This precursor molecule is synthesized and could be then cleaved between 95 and 96 residues. Thus, the 64–79 peptide was present on the precursor molecule sequence, and was selected to control, if T cells are nevertheless in contact with this sequence during the infection. The four peptides (64–79, 170–193, 194–208 and 231–

Table 1. Amino acid sequences of the P24 peptides used in this study

Peptide	Sequence (Rothbard patterns are italicized)
64–79	CysSerLeuLysLysSerSer*LysMetValArg*ValSerAlaIleVal
88–109	CysLeuSerAlaGlyAlaTyrAlaAlaGluGlyGlyAspAsnGlnSerSerAlaValSerAspArg
170–193	ValGlu*GluValIleAsp*ThrMetLysSerMet GlnArgAspGluAspIlePheLeu*ArgAlaLeuAsn*Lys
194–208	Gly*GluThrValGluGluAlaIleGluAspValAlaGln*AlaGlu
231–250	AspGluMet*LysValIleAsp*AspValGlnGlnLeuGluLysAspLysGlnGlnLeuLys

250 sequences) contained potential α-amphipathic structures, and at least one linear Rothbard's linear pattern. The peptides synthesized (Table 1) have been chosen because their dual B and T cell epitope theoretical potentialities although in this study only the T cell reactivity they induced was tested.

Peptide synthesis

Peptides were synthesized by solid-phase method, according to the Boc-TFA scheme (Merrifield, 1963), on choloromethyl resins; side chain protection was as follows: Arg(tos), Cys(p-MeBzl), Asp(cHex), Glu(cHex), Ser and Thr(Bzl), Lys(ClBzl), Tyr(2,6-diClBzl). At the end of the synthesis, the peptide resins were cleaved and deprotected by high HF procedure. The crude peptide were purified by preparative HPLC on a 5m-300Å C18 RP column, eluted with a 0–50% isopropanol, 0·1% TFA gradient. Peptides were checked for homogeneity by thin layer chromatography and analytical RP-HPLC, and for identity by amino acid analysis after total acid hydrolysis.

Conjugation of peptides to the carrier protein

Peptides were conjugated to BSA with glutaraldehyde. The peptides (5 mmole) were dissolved in dimethyl formamide (500 ml) and 0·1 M sodium phosphate, pH 8 (1 ml), and introduced in a solution of BSA (4 mg) in 0·1 M sodium phosphate pH 8 (2 ml). A 2·5% glutaraldehyde solution (60 ml) was progressively added with continuous stirring. After 2 days, the resulting mixture was exhaustively dialysed against 0·1 M sodium phosphate, pH 7, NaCl 0·9%. The conjugates were sterilized by filtration on 0·22 μm filters (Sartorius, Göttingen, Germany). Mass ratio of peptide to carrier protein was determined from amino acid composition (Antoni & Presentini, 1989), and were 0·18 mg (64–79), 0·4 mg (231–250), 0·35 mg (194–208), 1·08 mg (88–109), 0·86 mg (170–193) peptide per mg carrier.

Proliferation assay

T lymphocytes isolated by passage through a nylon/wool column (Julius, Simpson & Herzenberg, 1973) were tested for their ability to proliferate in the presence of antigen (synthetic peptides, irradiated tachyzoites and BSA) by the measurement of thymidine incorporation. Assays were done in triplicate in flat-bottomed 96-well tissue culture plates (Falcon 3072). The cells were cultured at 37°C with 5% CO_2 in humidified air. On day 5, the cultures were pulsed with ^{3}H-thymidine (0·5 μCi/well) 18 h before harvesting by using a cell harvester (Skatron, Lierbyen, Norway). ^{3}H-thymidine incorporation was measured by using a liquid scintillation counter.

In vivo *transfer of 170–193 peptide-specific T cells*

One day before intraperitoneal infection with 10^3 tachyzoites collected from infected cell cultures, 10^4 170–193 peptide-specific T lymphocytes were injected intravenously (just after recovering) into each athymic rat. Control nude rats were injected intravenously with 10^4 BSA-specific T lymphocytes. Control rats were infected only intraperitoneally.

For the determination of the helper role of these T cells, the same protocol was used except that 10^6 170–193 peptide-specific T lymphocytes (*in vitro* propagated for 4 weeks) were injected before infection and the athymic rats were bled every week.

Western blotting of SDS-PAGE

SDS–PAGE was carried out on slab gels according to Laemmli (1970). Gels were composed of a 5% acrylamide stacking gel and a homogeneous 13% acrylamide separating gel. Total toxoplasma antigen (S2-CHAPS extract) was separated by electrophoresis and transferred from the separating gel to a nitrocellulose membrane (Schleicher & Schüll, BA 45, Germany) by using procedures based on those of Towbin, Staehelin & Gordon (1979).

T. gondii antigens specific IgG from athymic rats was then revealed by incubation with peroxidase labeled anti-rat IgG (Miles Laboratories, Naperville, IL) and stained by addition of the enzyme substrate.

RESULTS

Determination of P24 antigen-T cell epitopes in infected rats

To investigate the kinetics of the fine cellular specificities recognized during the course of *T. gondii* infection, the T cell response towards P24 antigen-derived synthetic peptides has been carried out in rats. Evidence of P24 peptide-specific response of lymphocytes was determined by incubating nylon/wool-separated inguinal and mesenteric T lymphocytes of infected Fisher rats. These lymph nodes were harvested on day 0, 7, 21, 35, 42 or 49 after infection with 10^6 tachyzoites by the intraperitoneal route (Fig. 2). For this study, the peptides were coupled to BSA. Indeed, non conjugated P24 peptides did not induce proliferative response of T lymphocytes in our experimental conditions (data not shown). Figure 2 shows the stimulation of T cells from infected rats at a concentration of 10 μg/ml of antigens.

The kinetics of P24 peptide-specific response show that the peptides recognized by T lymphocytes during the course of infection varied. At day 0, T lymphocytes were not stimulated by the different peptides or by BSA as a control. The main T cell-stimulating peptides were the 88–109 (J21), 170–193 (J21; J35)

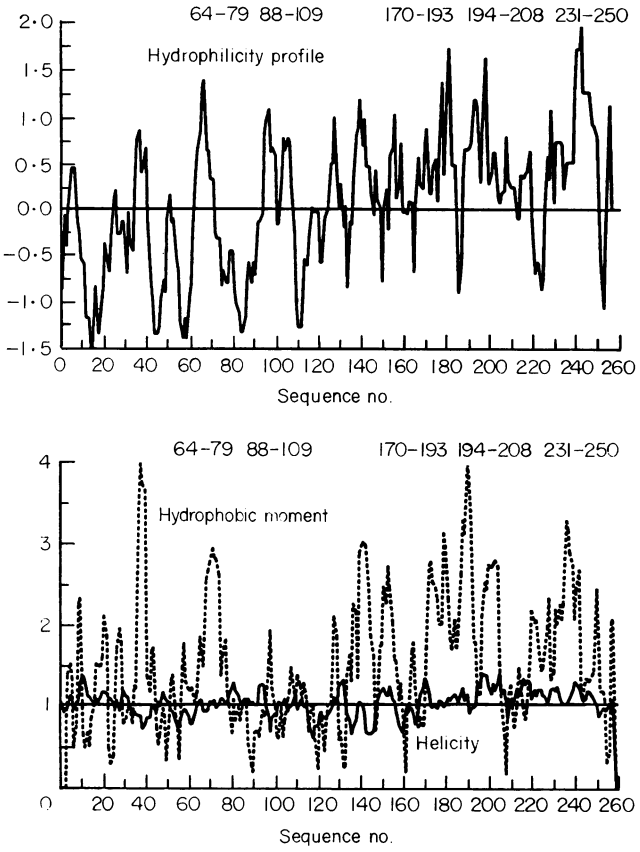


Fig. 1. Analysis of the P24 antigen sequence according to the accessibility criteria (Hopp & Woods, 1981).

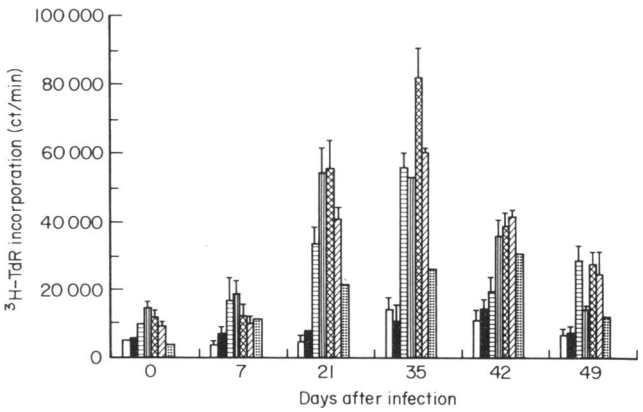


Fig. 2. Proliferation of T lymphocytes from infected Fisher rats after *in vitro* stimulation with the five P24-derived synthetic peptides coupled to BSA, at days 0, 7, 21, 35, 42 and 49. □, Medium; ■, BSA; ▤, 67–79–BSA; ■, 88–109–BSA; ▩, 170–193–BSA; ■, 194–208–BSA; ▨, 231–250–BSA.

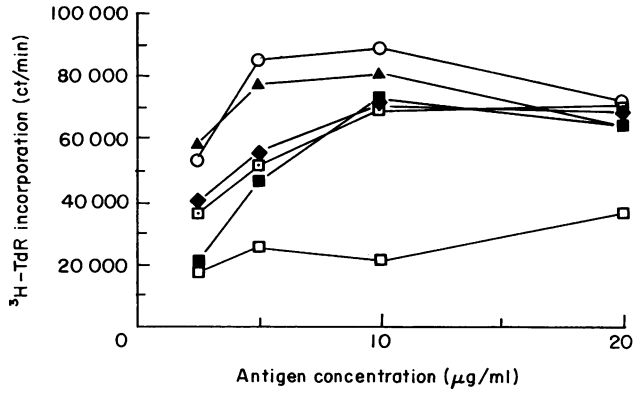


Fig. 3. *In vitro* proliferation of ESA-immunized Fisher rat lymph nodecells restimulated by various concentrations of the five P24-derived synthetic peptides: ⊡, 64–79; ◆, 88–109; ▲, 170–193; ○, 194–208; ■, 231–250, linked to a carrier protein (BSA) (□).

and the 194–208 (J35; J42) peptides. BSA alone never stimulated the T lymphocytes of infected rats (these first results showed that the form of P24 presented by the parasite can prime specific T lymphocytes during the infection of rats). The 64–79 peptide also induced a T cell proliferation, suggesting that the P24 precursor molecule is in contact with the immune system before its possible processing.

Stimulation of ESA-specific T lymphocytes with P24 antigen-derived peptides

As the P23 antigen is present in excreted/secreted products, the response of ESA-specific T cells against the P24 synthetic peptides was studied. ESA-specific T cells were recovered from ESA-immunized Fisher rats five days after the second injection. Our results indicate (Fig. 3, representative of three experiments) that most of the P24 peptides were able to induce the *in vitro* proliferation of ESA-specific T cells. Nevertheless, among the five peptides, 170–193 and 194–208 induced a higher response. In contrast, a weaker proliferation was obtained with the other peptides of the P24 molecule (64–79, 88–109 and 231–250). In addition, no proliferation was observed with the carrier protein (BSA).

Determination of the T cell epitopes of P24 in Fisher rats immunized with P24 recombinant vaccinia virus (VVTG 2170)

The immunization of Fisher rats with the VVTG 2170 construction (P24 have the identical amino acid sequence as that in *Toxoplasma* strain used for infections) had been undertaken to determine the T cell epitopes of this molecule with the help of the five synthetic peptides. With the two immunization procedures (at the base of the tail, Fig. 4; and by intraperitoneal route, Fig. 5), a greater *in vitro* proliferation was obtained with the 88–109 and 194–208 peptides. In addition, when VVTG 2170 was injected by intraperitoneal route, spleen cells and lymph node cells were stimulated with the same peptides.

Preparation of 170–193 peptide-specific T cells and in vivo *transfer of these cells into nude rats*

The choice of the preparation of 170–193 peptide-specific T cells was considered for two reasons: this peptide contains T cell epitopes in view of the results presented in the previous section either when T cells were recovered from infected rats or from rats immunized with ESA; and the monoclonal anti-P24 antibody prepared in our laboratory (TG17-43) only recognizes the peptide 170–193, demonstrating that a B cell epitope is also

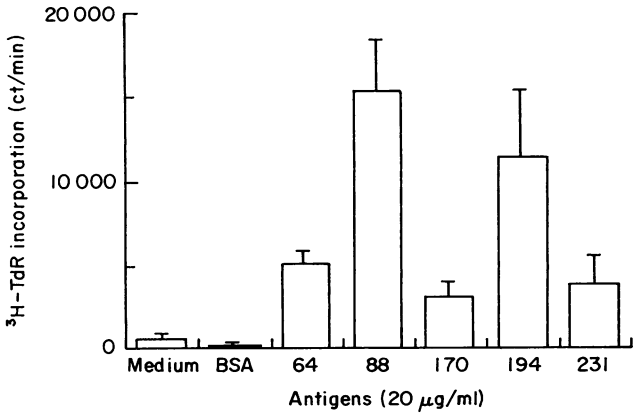


Fig. 4. Activation of lymph node cells from Fisher rats immunized with the P24 recombinant vaccinia virus after incubation with the synthetic peptides coupled to BSA. The irrelevant antigen used in these experiments was BSA.

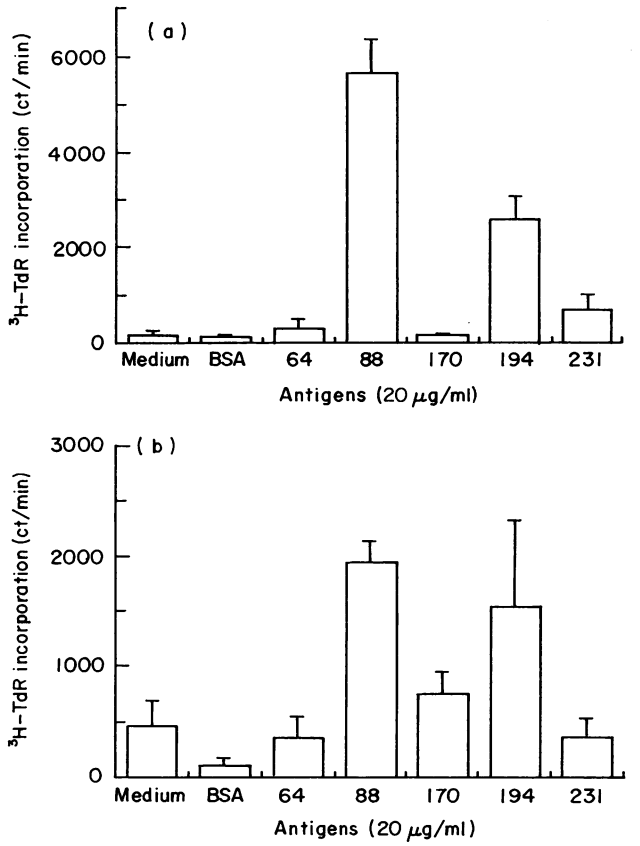


Fig. 5. *In vitro* proliferative response of spleen cells (a) and lymph node cells (b) from Fisher rats immunized with the P24 recombinant vaccinia virus after stimulation with the synthetic peptides. Proliferations were obtained with the dose of 50 μg of antigen per ml.

present. In the rat model, no antibody response against the P23 antigen during infection or after ESA or 170–193 peptide immunizations had been shown. However, the immunization with this peptide can prime T lymphocytes (Fig. 6). Indeed these T cells proliferated with either the 170–193 peptide conjugated

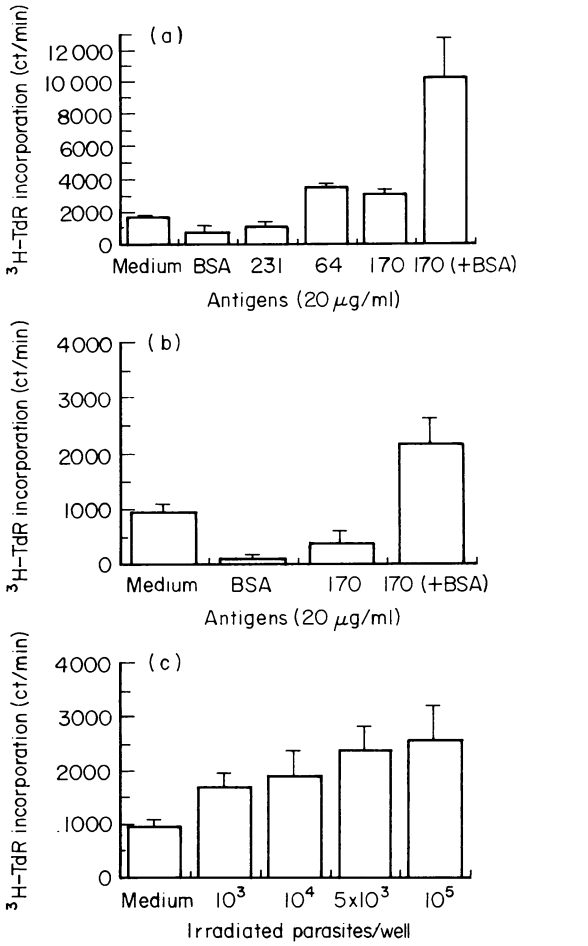


Fig. 6. Activation of lymph node T cells from Fisher rats immunized with the 170–193 peptide after incubation with the 170–193, 231–250 and 64–79 synthetic peptides coupled to BSA and with the non-conjugated 170–193 peptide (a). In another experiment, T lymphocytes were restimulated with either irradiated parasites (c) or with the 170–193 peptide conjugated or not to BSA (b).

to BSA (Figure 6a,b) or irradiated tachyzoites (representing the native antigen, Fig. 6c) but neither in the presence of the same peptide not coupled, nor with the carrier protein (BSA). The 170–193 peptide-specific T cells are not restimulated by other P24 peptides such as the 64–79 and 231–250 peptides (Fig. 6a). Moreover, BSA-specific T cells were not stimulated by irradiated tachyzoites discording the possible mitogenic effect of the parasites.

In order to study the protective effect of 170–193 peptide-specific T lymphocytes, a passive transfer of 10^4 T cells (just after recovery) into athymic rats was performed 1 day before their infection with 10^3 tachyzoites. Fifty per cent of the rats having received 170–193-specific T cells were totally protected (Fig. 7) even after a second challenge infection with 10^3 tachyzoites (data not shown). The athymic rats protected by the 170–193 peptide-specific T cells were bled and the IgG response of the sera was analysed by immunoblotting (Fig. 8). One of the rats showed an antibody response against the P30 antigen(s) complex demonstrating that the T cell epitope present in the 170–193 peptide elicits *in vivo* functional T cells. In contrast, the other

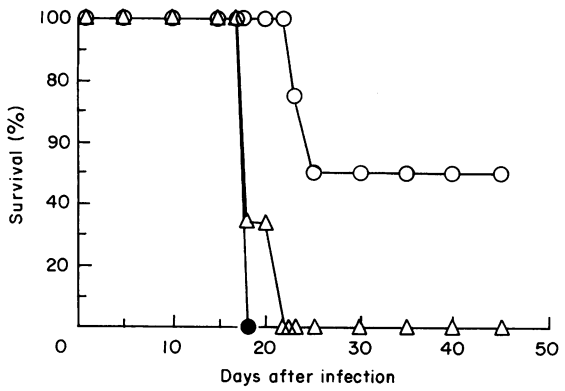


Fig. 7. Adoptive transfer of 10^4 T lymphocytes from 170–193 peptide (○) and BSA-immunized (△) Fisher rats into athymic rats. Control rats (●) were not transferred. In each group there were eight animals which were infected with 10^3 tachyzoites of the RH strain.

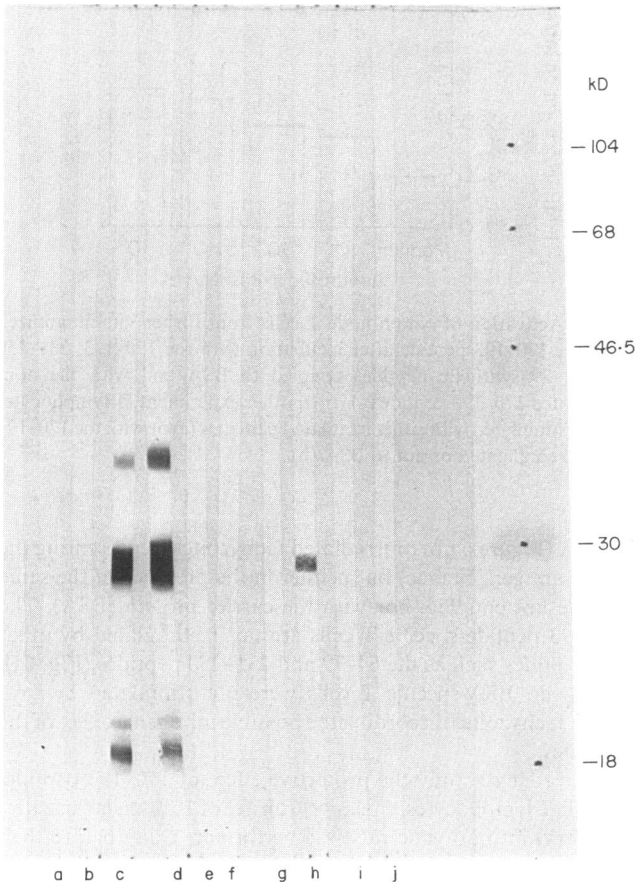


Fig. 8. Western blot analysis of Toxoplasma antigens. SDS-PAGE analysis (13% acrylamide) in non-reducing conditions and blotting developed by sera from: lane a, uninfected Fisher rats; lane b, uninfected Nu/Nu Fisher rats; lanes c, d, ESA-immunized Fisher rats; lanes e, f, 170–193 peptide-immunized Fisher rats; lanes g, h, reconstituted by 10^4 170–193 peptide-specific T cells and infected athymic Fisher rats; lane i, reconstituted by 10^4 BSA-specific T cells and infected athymic Fisher rat; and lane j, by the monoclonal antibody TG 17-43.

rats did not present any IgG response suggesting that these antibodies were not necessary in the protection conferred.

Helper role of 170–193 peptide-specific T cell lines maintained in in vitro *culture*

T cells from 170–193 peptide- or from BSA-immunized rats were cultured *in vitro* for 1 month in the presence of irradiated tachyzoites or BSA and of irradiated antigen presenting cells. In order to analyse the ability of the 170–193 peptide-specific T lymphocytes to induce an antibody response, athymic rats were adoptively transferred with 10^6 T cells from BSA- or 170–193 peptide-immunized Fisher rats 1 day before their infection with 10^3 tachyzoites. Every week, the animals were bled and their sera were tested by dot-immunoassay, immunoblotting with total antigen extract and immunoprecipitation with methionine ^{35}S-labelled ESA. None of these three methods allowed the demonstration of an antibody response against *Toxoplasma* antigen either in the sera of rats having received 170–193 peptide-specific T cells or BSA-specific T cells. These results suggest that the T lymphocytes induced by the immunization with this peptide are not able to help an IgG response in the nude rat model.

DISCUSSION

The role of tachyzoite ESA in the protective immunity against *Toxoplasma* infection has been clearly demonstrated in our laboratory. Darcy *et al.* (1988) have shown that the passive transfer of sera from ESA-immunized euthymic Fisher rats to genetically athymic rats, infected by lethal doses of the highly virulent RH strain of *T. gondii*, conferred a significant level of protection. In addition, T cell lines specific for excreted/secreted products can protect susceptible nude rats adoptively against a challenge infection with *T. gondii*. The reconstituted infected nude rats developed an antibody response able to recognize *Toxoplasma* antigens (Duquesne *et al.*, 1990).

In the strategy for cloning protective antigens of *T. gondii*, based on the hypothesis that the definitive protection observed in natural infection is due to the presence of encysted bradyzoites in host tissues, a 24-kD secreted molecule present both in tachyzoites and bradyzoites has been cloned (Cesbron-Delauw *et al.*, 1989). In view of the results indicating the crucial role of ESA in immunity against toxoplasmosis (Darcy *et al.*, 1988; Ridel *et al.*, 1988; Duquesne *et al.*, 1990), further investigations concerning the immunogenicity of this protein have been undertaken.

The purpose of our study was to consider the recognition of synthetic peptides, derived from the primary structure of P24, by T lymphocytes educated against the native P24 molecule, either after immunization with ESA or P24 recombinant vaccinia virus, or during *T. gondii* infection.

By analysing this series of results, it appeared that T lymphocytes from infected rats or from rats immunized with ESA can be restimulated by the synthetic peptides. During the rat infection, the T cell response dependent upon the different synthetic peptides varied. At the beginning of infection, infected rat T cells recognized the 88–109 peptide epitope. In contrast, at day 35 of infection, an optimal *in vitro* proliferation can be observed with the 170–193 peptide. From day 42 after infection, the whole cellular response decreased.

When the cellular responsiveness generated by ESA immunization was examined, the 170–193 and 194–208 epitope containing peptides allowed a better proliferative response than the other synthetic peptides. When the P24 antigen is presented with the vaccinia virus to the immune system, T lymphocytes elicited by the subcutaneous or intraperitoneal immunizations mainly recognized the 88–109 and 194–208 peptides.

In these experimental conditions, lymph node or spleen cells are not able to be stimulated by the synthetic peptides when they are not linked to a carrier protein (BSA) while BSA by itself never induced a non specific T cell proliferative response. Moreover, the clue that this technical approach allows effectively the identification of potent T cell epitopes is confirmed by the fact that 170–193 peptide-specific T cells were restimulated by irradiated tachyzoites. One possible explanation for this observation could be that the conjugation stabilizes the peptide conformation or reduces their processing by the antigen presenting cells. In addition, it can be observed that the cellular responsiveness generated against the P24 molecule is different during the course of infection and after immunization with ESA or P24 recombinant vaccinia virus. Different routes of injection and modes of antigen presentation to the immune system can induce various T cell responses. The role of antigen-presenting cells and the molecular environment of the presented antigen are probably two crucial parameters in the induction of the immune response.

After the infection with *T. gondii* or ESA immunization, the highest lymph node cells proliferative response was obtained after stimulation with the 170–193 epitope containing peptide. A T cell line specific for this peptide has been established *in vitro* and its *in vivo* functional role has been studied after adoptive transfer of these cells into recipient nude rats, 1 day before infection. The transfer of 10^4 T lymphocytes (just after recovering) induced a significant protection of the nude rats. Fifty per cent of the rats having received 170–193 peptide-specific T lymphocytes were totally protected even after a second challenge infection. These 170–193 peptide-specific T cells can thus be involved in the protective mechanisms, evidencing the functional relevance of this epitope. Among these mechanisms, antibodies do not seem to be essential in the conferred protection since only one of the surviving rats showed significant IgG antibody response against the P30 antigen(s) complex. When the helper role of these cells was studied by adoptive transfer of 10^6 T lymphocytes *in vitro* cultured with irradiated tachyzoites, for 1 month, no antibody response could be observed after the infection of athymic rats. These data confirmed previous observations showing that in Fisher rats no antibody response against the native antigen or against the recombinant P24 molecule can be obtained after infection or immunizations with ESA or with the 170–193 peptide. These T lymphocytes specific for the 170–193 epitope containing peptide are probably not directly involved in the antibody production. Nevertheless, in our study only the IgG response has been followed. We cannot exclude that other isotypes such as IgE or IgA are produced and that these antibodies are involved in the protective immunity (Ridel *et al.*, 1988; Godard *et al.*, 1990). However, the main activity of these cells would be their involvement in antibody-independent cellular effector mechanisms. The 170–193 peptide-specific T lymphocytes could act through the production of lymphokines notably interferon-gamma (INF-γ) which activates cells of monocytes lineage as described by several investigators (Black, Catteral & Remington, 1987; Murray *et al.*, 1987). Another hypothesis is the induction of cytotoxic lymphocytes (CTL). Recently, Khan, Smith & Kaster (1988) have described T lymphocytes exhibing the Thy $1,2^+$, lyt $2,3^+$ phenotype that are directly cytotoxic against extracellular parasites. Therefore, the T lymphocytes elicited after immunization with the 170–193 peptide could either be directly cytotoxic for the parasites or, more likely, could help for the generation of CTL through the production of IL-2. The exact role of the T lymphocytes specific for the peptide 170–193 in the resistance of athymic rats infected with *T. gondii* remains to be defined.

This study is the first determination of T immunodominant epitopes of an ESA in toxoplasmosis. The ability to predict peptidic sequences eliciting T cell reactivity allows a potential application of synthetic immunogens as vaccines. Indeed, T cell immunity is necessary not only for a cellular immune defence against pathogens, but also to help an antibody response and cytotoxic T cell activity. Therefore, any vaccine should have not only sites that elicit antibodies, but also sites that elicit T cells. We have reported the presence in the P24 antigen of T immunodominant epitopes which probably preferentially induce a cellular mediated immunity. Interestingly, the 170–193 peptide also contains a B cell epitope since a monoclonal anti-P24 antibody (TG 17–43) recognized this peptide (H. Charif H, personal communication). The study of other protective antigens in toxoplasmosis is now in progress in our laboratory. The characterization of T and B immunodominant epitopes in these molecules could favour the development of a synthetic vaccine that would optimize an effective immune response.

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