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Detection of a novel 40 000 MW excretory *Toxoplasma gondii* antigen by murine Th1 clone which induces toxoplasmacidal activity when exposed to infected macrophages

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SUMMARY

To analyse target molecules of the CD4$^+$ T-cell response to toxoplasma infection, a panel of *Toxoplasma gondii*-specific murine CD4$^+$ T-cell clones has been established. Clone 3Tx15, belonging to the T helper 1 (Th1) subtype, abolished intracellular parasite growth when co-cultured with macrophages and live toxoplasma at a ratio of 2:2:1. This effect results from macrophage toxoplasmicidal activity induced upon parasite-dependent cellular interaction, an irrelevant Th1 clone failed in this three-party system. Clone 3Tx15 detects its corresponding antigen in the supernatant of infected cells and also reacts with a host cell-free preparation of *T. gondii*-excreted/secreted antigens. T-cell blot analysis of two-dimensionally separated toxoplasma lysate revealed a molecular weight of about 40 000 for the fractions stimulating clone 3Tx15. As checked in parallel enzyme-linked immunosorbent assay, the 40 000 MW T-cell antigen co-migrates with the excretory protein GRA4, the sole 40 000 MW *T. gondii* antigen hitherto known to be recognized by T lymphocytes. Nevertheless, neither recombinant GRA4 nor immunoaffinity-purified natural GRA4 was stimulatory for clone 3Tx15. Our findings thus demonstrate that Th1 clone 3Tx15 which induces toxoplasmicidal activity during antigenic interaction with infected macrophages defines a new 40 000 MW excretory *T. gondii* antigen.

INTRODUCTION

Infection with the intracellular protozoan parasite *Toxoplasma gondii* causes life-threatening disease in individuals with defective immunity such as fetuses, patients with acquired immune deficiency syndrome (AIDS) or cancer and transplant recipients whereas in immunocompetent humans toxoplasmosis is usually asymptomatic. Here, infection results in the persistence of encysted bradyzoites in various tissues, especially the brain, and leads to induction of lifelong protective immunity.

Immune resistance against *T. gondii* is cell-mediated. As shown in the mouse model, natural killer cells are critical for resistance during the early stage of primary infection[1] whereas adaptive immunity depends on T lymphocytes. Both the CD4$^+$ and the CD8$^+$ T-cell subsets are required for control of acute and chronic infection as shown by depletion experiments as well as by adoptive transfer of parasite-specific T cells.[2,3] Each of these cell types is capable of producing interferon-γ (IFN-γ), the pivotal mediator of protective immunity[4] which induces anti-toxoplasma activity in macrophages (Mϕ) and other cell types.[5,6] For the establishment of toxoplasma-specific immunity CD4$^+$ T cells are required, since their depletion during priming of mice with a low-virulent *T. gondii* strain prevents resistance against a subsequent lethal challenge.[7]

So far, little is known about the target antigens of the protective T-cell response. Five toxoplasma proteins – the major surface antigen SAG1, the rhoptry protein ROP2 and the dense granule antigens GRA1, GRA2 and GRA4 – have been shown to be recognized by T lymphocytes (for review see ref. 8). Remarkably, four of them are molecules released by the parasite. The present study describes mouse T-cell clone 3Tx15 detecting such an excretory *T. gondii* antigen. The prototypic T helper 1 (Th1) clone is shown to induce toxoplasmacidal activity during interaction with infected Mϕ. Detailed analyses of the corresponding target antigen provide evidence that this T-cell clone recognizes an as yet uncharacterized 40 000 MW excretory protein which is not identical with one of the T-cell-stimulatory toxoplasma antigens described so far.

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Abbreviations: ESA, excreted/secreted antigens; GRA, dense granule antigen; IFN-γ, interferon-γ; IL, interleukin; mAb, monoclonal antibody; Mϕ, macrophages; ROP, rhoptry protein; SAG, surface antigen; Th1, T helper 1; TLA, toxoplasma lysate antigen; TNF, tumour necrosis factor.

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MATERIALS AND METHODS

Mice and reagents

Female (C57BL/10 × C3H/HeJ) F_1 mice originally supplied by the Zentralinstitut für Versuchstierforschung (Hannover, Germany) were used at the age of 3–6 months. Basic culture medium for all cells was Iscove's modified Dulbecco's medium from Gibco (Eggenstein, Germany) supplemented with 5% fetal calf serum (FCS; Gibco), 2 mM l-glutamine and 50 μM 2-mercaptoethanol. Reagents were purchased from Merck (Darmstadt, Germany) unless otherwise stated.

Toxoplasma gondii culture and antigen preparations

Mouse-virulent *T. gondii* strain BK was passaged in proliferating L929 fibroblasts, avirulent strain DX was grown in murine brain cell cultures. After leaving their host cells, extracellular toxoplasma were collected within the supernatant of 100 *g* centrifuged material and washed twice in phosphate-buffered saline (PBS; 600 *g*, 15 min).

Toxoplasma lysate (TLA) was prepared as aqueous extract from freeze-thawed BK toxoplasma.[9] To obtain excretory parasite products, murine brain cell cultures[9] were inoculated with a fourfold number of live or heat-killed DX toxoplasma. After 24 hr extracellular parasites were removed and fresh medium was added. At specified time-points the supernatant was collected, centrifuged at 10000 *g* and tested as a source of antigen. Excreted/secreted antigens (ESA) were prepared from BK toxoplasma under host cell-free conditions as described.[10] For Western blot analysis, ESA were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and electrophoretically transferred to nitrocellulose (30 min, 0·8 mA/cm² gel). After blocking, the nitrocellulose was incubated overnight with the following monoclonal antibodies (mAb):[11] T5 2B4 (anti-GRA1), T4 1F5 (anti-GRA2), T6 2H11 (anti-GRA3), and T9 4B9 (anti-GRA4). Binding of mAb was detected using peroxidase-conjugated anti-mouse IgG (Dianova, Hamburg, Germany) and 4-chloro-1-naphthol (Sigma, Deisenhofen, Germany).

GRA4 preparations

Truncated forms of GRA4 were expressed in *Escherichia coli* as glutathione S-transferase fusion proteins (amino acids according to the published[12] sequence): 1.S (24–106), S.1 (107–276), 10.1 (24–276), 8.2 (296–345), NC29 (24–276/296–345). Recombinant GRA4 fragments were affinity-purified using glutathione-sepharose beads (Sigma) as previously described.[13]

Natural GRA4 was immunoaffinity-purified from TLA using mAb Tx34.31[14] bound to protein G-Sepharose (Pharmacia, Freiburg, Germany). After loading the column with TLA and washing with PBS followed by 10 mM phosphate (pH 8), the antigen was eluted with 100 mM triethylamine (pH 11·5). Eluates were neutralized with 1 M phosphate (pH 6·8) and dialysed against PBS. The presence of GRA4 in the initial TLA preparation, in the flow-through and eluate was assessed by Western blot analysis using mAb Tx34.31.

T-cell clone and proliferation assay

Toxoplasma gondii-specific T-cell clone 3Tx15 has been established from TLA-primed lymph node T cells of (C57BL/10 × C3H/HeJ) F_1 mice. Clone 3Tx15 belongs to the Th1-subtype based on its $CD4^+$ αβ T-cell receptor-positive phenotype and its production of interleukin-2 (IL-2) (300 U/ml), IFN-γ (10^5 U/ml) and tumour necrosis factor (TNF) (3×10^4 U/ml) and failure to secrete IL-4 or IL-10 following mitogenic stimulation. The corresponding antigen was detected on mouse-virulent and avirulent *T. gondii* strains regardless of the parasite stage – tachyzoite or bradyzoite – tested.[8]

For proliferation assays, 3Tx15 cells were seeded at 2×10^4/well in flat-bottom microtitre plates containing 2×10^5/well irradiated (2000 rod) syngeneic spleen cells and antigen as indicated. After 2 days of incubation, test cultures were pulsed with 7·4 kBq/well [^{3}H]thymidine (Amersham-Buchler, Braunschweig, Germany) for 20 hr and then processed for liquid scintillation counting. Results are expressed as mean counts per minute (c.p.m.) ± SD of triplicate cultures.

Determination of T cell-dependent anti-toxoplasma effect

Thioglycollate-elicited peritoneal exudate Mϕ (2×10^4/well) were co-cultured with BK toxoplasma (10^3–10^5/well) and 3Tx15 T cells (2×10^4/well) or supernatant (diluted 1:10) of mitogen-stimulated 3Tx15 T cells or medium. As control, the insulin-specific Th1 clone M4[15] which produces similar cytokine activities was used. Cultures were incubated for 3 days, for the last 24 hr [^{3}H]uracil (Amersham-Buchler) was added (37 kBq/well). The amount of [^{3}H]uracil uptake, directly corresponding to the growth of parasites,[16] was measured by liquid scintillation counting. Results are given as mean c.p.m. ± SD of triplicate cultures.

For microscopic analysis, 2×10^5 Mϕ were allowed to adhere on coverslips for 2 hr and were then infected with 8×10^5 toxoplasma (strain DX). After 6 hr of co-culture, extracellular parasites were removed. One sample was fixed and stained to determine the initial percentage of infected Mϕ. Other samples were incubated for 72 hr with additonal 3Tx15 T cells (2×10^5) or without. Immunostaining of Mϕ was performed with anti-CD11b mAb M1/70.15, biotinylated anti-rat IgG (both from Dianova) and the Vectastain ABC kit plus 3,3'-diaminobenzidine (Boehringer Ingelheim Bioproducts, Germany). Parasites were stained using rabbit anti-*T. gondii* antiserum (BioGenex, San Ramon, CA) and dichlorotriazinyl-amino-fluorescein-conjugated anti-rabbit IgG (Dianova). The percentage of infected Mϕ and the number of intracellular toxoplasma are given as mean values ± SD of two independent experiments.

T-cell blot analysis

Following two-dimensional PAGE, separated TLA was transferred into soluble fractions as described by Gulle *et al.*[17] using an electroelution device (Biometra, Göttingen, Germany). The gel was equilibrated (2 × 10 min in 5 mM Tris) and proteins were eluted (30 min, 0·8 mA/cm² gel) into holes of the masterplate each filled with 100 μl 5 mM Tris. Eluates were transferred into 96-well microtitre plates, sterilized with ultraviolet light and tested for antigen activity in T-cell proliferation assay with clone 3Tx15 and in enzyme-linked immunosorbent assay (ELISA) with anti-GRA4 mAb T9 4B9. Here, eluates were used as solid-phase antigen. Binding of the anti-GRA4 mAb was detected using peroxidase-conjugated anti-mouse IgG (Dianova) and *o*-phenylene-diamine (Sigma).

Statistics

The Mann–Whitney *U*-test was performed and $P=0{\cdot}05$ was considered as significant.

RESULTS

Th1 clone 3Tx15 induces toxoplasmacidal activity when exposed to infected Mϕ

Based on their Th1 phenotype 3Tx15 cells were expected to mediate antiparasitic activity. This was tested in direct co-culture with peritoneal exudate Mϕ and viable toxoplasma. Such a three-party system has been developed to assess the antiparasitic capacity of T-cell clones taking into account their individual antigen sensitivity and cytokine production. In host Mϕ alone, toxoplasma proliferated well as monitored via [^{3}H]uracil uptake (Fig. 1a). In the presence of additional 3Tx15 T cells, toxoplasma growth was reduced to background level at parasite : Mϕ ratios $\leq 1:2$. Even with five parasites/Mϕ a 87% inhibition compared to the T cell-free reference culture was observed. The antiparasitic effect proved to depend on antigenic interaction between Mϕ and T cells, since in controls containing the insulin-specific Th1 clone M4 the parasite multiplication was unaffected (Fig. 1b). When infected Mϕ were incubated with the supernatant of mitogen-activated 3Tx15 T cells, the inhibition of toxoplasma proliferation was just as efficient as in the presence of the T-cell clone (Fig. 1a). This suggests that T cell-derived cytokines induce inhibition of parasite multiplication. The T-cell supernatant did not unspecifically impair toxoplasma multiplication as checked by using human endothelium cells as host cells (data not shown).

To differentiate whether clone 3Tx15 induces toxoplasmastasis or killing of intracellular parasites, co-cultures of Mϕ, toxoplasma and T cells were analysed microscopically. After a 6-hr incubation, 5·8% of the Mϕ were infected with one toxoplasma each (Table 1). 72 hr later the number of infected Mϕ had increased up to 17·3% with 11·2 toxoplasma per infected Mϕ, whereas in the presence of clone 3Tx15 the percentage of infected Mϕ had significantly decreased to 2% ($P=0{\cdot}05$). Thus, by interacting with infected Mϕ Th1 clone 3Tx15 induces killing of *T. gondii*.

Table 1. 3Tx15-induced killing of *T. gondii* by Mϕ

Time postinfection* (hr)	Presence of 3Tx15 T cells in Mϕ-toxoplasma co-culture	% infected Mϕ†	No. toxoplasma/ infected Mϕ‡
6	–	5·8±1·3	1·0±0·0
6 + 72	–	17·3±2·4	11·2±1·8
6 + 72	+	2·0±0·3	1·6±0·4

* Mϕ (2×10^5) were infected with 8×10^5 toxoplasma (strain DX) for 6 hr. After removal of extracellular toxoplasma, one culture was fixed, the others were incubated for further 72 hr in the absence or presence of 2×10^5 3Tx15 T cells.

†‡ As determined by immunostaining of Mϕ and toxoplasma. Values are the means ± SD of two experiments.

Clone 3Tx15 detects excretory toxoplasma antigen in the supernatant of infected brain cells

As a relevant source of parasite antigens, medium conditioned by toxoplasma-infected murine brain cells was tested against clone 3Tx15. As shown in Fig. 2, 3Tx15 T cells were stimulated by such supernatant. Antigen activity was detectable from day 4 on, only when viable parasites had been used for inoculation. Control cultures containing heat-killed toxoplasma which were phagocytosed by host cells did not provide the T cell-stimulatory antigen nor did untreated cultures. Heat treatment does not destroy the antigen as checked by using killed parasites in a T-cell proliferation assay (not shown).

Reactivity of 3Tx15 T cells for excretory parasite products was confirmed with a preparation of ESA obtained under host

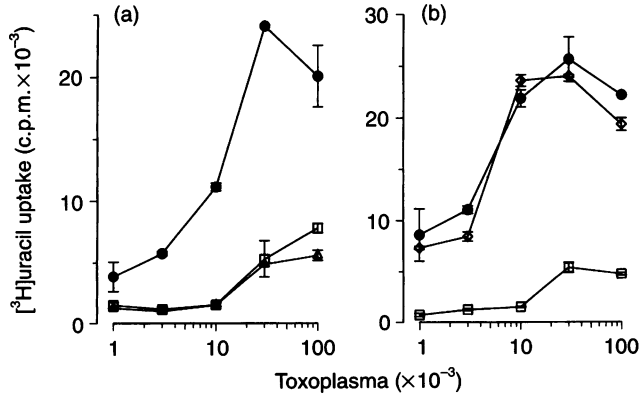

Figure 1. 3Tx15-dependent inhibition of toxoplasma growth in Mϕ. Peritoneal exudate Mϕ (2×10^4/well) were incubated for 3 days with toxoplasma (titrated from 10^3 to 10^5/well) of strain BK (●). (a) Parallel test cultures additionally contained 2×10^4/well 3Tx15 T cells (□) or 1:10 diluted supernatant of mitogen-stimulated 3Tx15 cells (△). (b) To a part of test cultures 2×10^4/well T cells of clone 3Tx15 (□) or M4 (◇) had been added. Toxoplasma growth was measured via uptake of [^{3}H]uracil during the last 24 hr. Results are expressed as mean c.p.m. ± SD of triplicate test cultures. [^{3}H]uracil uptake in controls lacking toxoplasma or host cells was ≤ 1200 c.p.m.

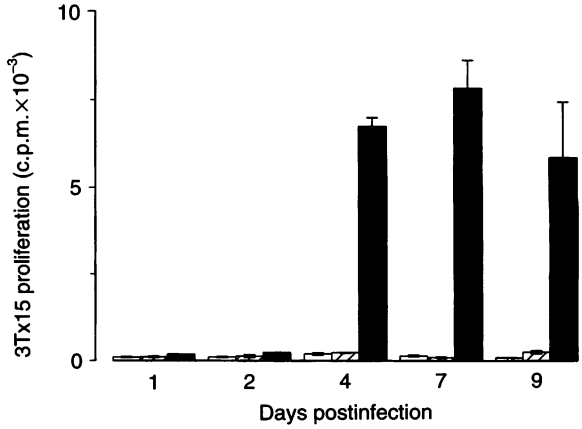

Figure 2. Reactivity of clone 3Tx15 to supernatant of toxoplasma-infected brain cell culture. Murine brain cells had been incubated with heat-killed (hatched bars) or live (black bars) toxoplasma (strain DX) or remained untreated (open bars). Supernatant was collected at the time-points indicated and was tested as antigen in subsequent T-cell proliferation assay with clone 3Tx15. Results are given as mean c.p.m. ± SD representing the T-cell response to two independently produced supernatants.

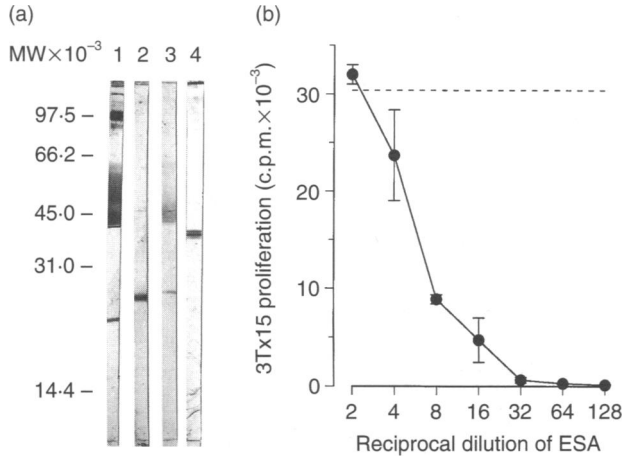

Figure 3. Proliferative response of 3Tx15 T cells to *T. gondii* excreted/secreted antigens. (a) Western blot analysis of ESA probed with mAb against GRA molecules. Lane 1, T5 2B4 (anti-GRA1); lane 2, T4 1F5 (anti-GRA2); lane 3, T6 2H11 (anti-GRA3); and lane 4, T9 4B9 (anti-GRA4). Apparent molecular weights ($\times 10^{-3}$) of standard proteins are given. Smear above 40 000 MW in lane 1 and lane 3 is due to cross-reactivity of the respective antibody preparations with FCS contained in the ESA preparation. (b) Titrated ESA (●) were tested as antigen in T-cell proliferation assay with clone 3Tx15. Results are given as mean c.p.m. ± SD of triplicate test cultures. The dashed line indicates proliferation in control culture stimulated with an optimal dose of TLA (1 μg/ml), proliferation without antigen was 130 c.p.m.

cell-free conditions. The presence of reference excretory antigens, namely GRA proteins, was controlled by Western blot analysis. GRA1, GRA2, GRA3, and GRA4 were detected (Fig. 3a). This ESA preparation proved to stimulate 3Tx15 T cells to a proliferative response as high as induced with an optimal dose of TLA (Fig. 3b).

The 40 000 MW antigen stimulating 3Tx15 T cells co-migrates with the excretory *T. gondii* antigen GRA4

To further characterize the antigen recognized by clone 3Tx15, its molecular weight was estimated in T-cell blot analysis. Toxoplasma lysate was separated by two-dimensional PAGE as shown in Fig. 4 (a), eluted into 528 soluble fractions and subsequently tested in T-cell proliferation assay. Clone 3Tx15 detected peak antigenic activity in fractions of about 40 000 MW (Fig. 4b) therefore the antigen was named p40. Recently, it has been demonstrated that the 40 000 MW fraction in toxoplasma lysate exhibits an above-average stimulatory activity for $CD4^+$ T lymphocytes from chronically infected mice.[18] Since T cell-stimulatory activity has been reported for the 40 000–41 000 MW excretory antigen GRA4,[19] eluates were tested in parallel ELISA for the presence of GRA4. The anti-GRA4 mAb reacted with the fractions stimulating clone 3Tx15 (Fig. 4c, dark bars). However, the levels of antibody and T-cell reaction to some of these eluates greatly differed. Furthermore, strong GRA4 activity was detected in additional fractions (Fig. 4c, light bars). The comparison of T-cell and antibody reactivity revealed only partial congruency between the p40 and GRA4 activity patterns. If the T-cell clone and the mAb recognize the same molecule, the epitope for the mAb must be additionally expressed on other forms of the protein which are not recognized by clone 3Tx15.

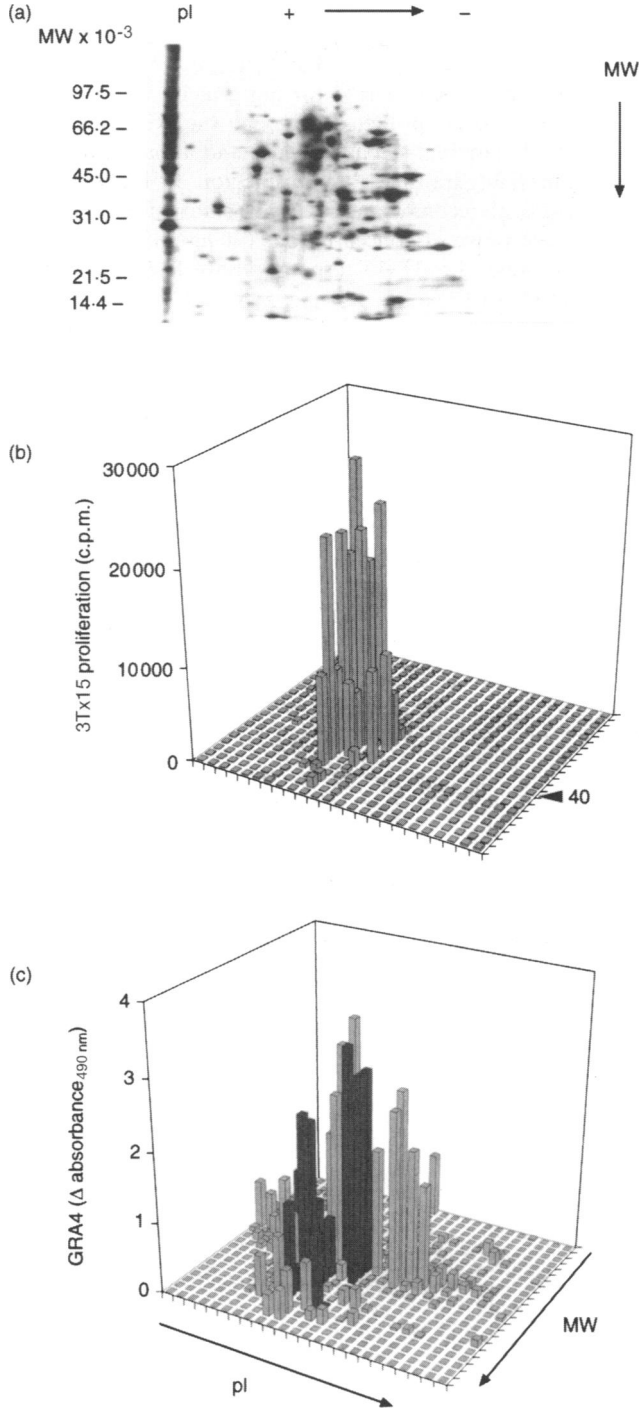

Figure 4. Partial congruency of the reactivities of anti-GRA4 mAb and T cell clone 3Tx15 for fractions of two-dimensionally separated TLA. (a) Coomassie Blue staining of TLA separated first by isoelectric focusing according to pI and then by SDS–PAGE according to molecular weight (MW). Numbers on the left indicate apparent MW ($\times 10^{-3}$) of standard proteins. Proteins separated in a parallel gel were electroeluted into 528 fractions and then tested in T-cell proliferation assay with clone 3Tx15 (b) and by ELISA with anti-GRA4 mAb T9 4B9 (c). Each bar represents one eluate. In control cultures 3Tx15 proliferation was 24 527 c.p.m. with 0·3 μg/ml TLA and 106 c.p.m. without antigen. In ELISA, background absorbance without antigen (0·3) was subtracted from each value. Dark bars indicate fractions stimulatory for 3Tx15 T cells.

p40 is not identical with GRA4

To clarify the relation of GRA4 and p40, several recombinant GRA4 fragments were tested for antigenicity towards clone 3Tx15. The fusion proteins represent the whole molecule except the N-terminal signal sequence and an internal hydrophobic, probably transmembranous, region. In T-cell proliferation assays, all recombinant GRA4 preparations were titrated over a wide range of concentrations (30 μg/ml to 30 pg/ml), but none induced a 3Tx15 response above background level (data not shown).

The possibility that clone 3Tx15 recognizes a GRA4 epitope not expressed by the recombinant fragments was checked by using immunoaffinity-purified natural GRA4 as antigen. By control Western blot analysis GRA4 was detected in the initial TLA preparation (Fig. 5 a, lane 1) and in the fraction eluted from the immunosorbent column (Fig. 5 a, lane 3). As shown in Fig. 5 a, lane 2, TLA was completely depleted of GRA4 by the procedure. When tested for p40 activity in T-cell proliferation assay (Fig. 5b), such GRA4-depleted TLA induced optimal 3Tx15 proliferation, whereas the eluate was not stimulatory. This failure was not due to toxicity of the eluted fraction as controlled by adding an optimal dose of TLA to test cultures incubated with the eluate. Taken together, these experiments confirm that p40 recognized by T-cell clone 3Tx15 is not identical with GRA4.

DISCUSSION

Previous studies have shown that *T. gondii*-specific $CD4^+$ T cells can secrete IFN-γ and that T cell-derived IFN-γ induces antitoxoplasma effector mechanisms in infected Mϕ.[20,21] In those experiments, Mϕ had to be preactivated at least 48 hr prior to infection thus raising the question whether toxoplasma-specific Th1 cells present can rescue Mϕ from fatal infection. In this study, evidence is given for the first time that direct interaction of *T. gondii*-specific Th1 cells and infected Mϕ results in killing of intracellular toxoplasma. Since all components are simultaneously co-cultured, the present three-party system rather reflects the *in vivo* situation and provides an appropriate criterion for the antiparasite capability of T-cell clones by taking their individual antigen reactivity or cytokine production into account. The toxoplasmacidal effect induced depends on the antigen-directed interaction between T cells and infected Mϕ and therefore speaks against a bystander role of Th1 cells. Implication for a T-cell receptor-independent generation of antitoxoplasma activity can be derived from the release of TNF-α and IL-12 by which peritoneal exudate Mϕ respond to toxoplasma infection[22] and could influence IFN-γ production of adjacent $CD4^+$ T cells. Besides, human $CD4^+$ T-cell clones have been shown to be cytotoxic for infected host cells[23] or tumour targets.[24] We have no evidence that clone 3Tx15 exhibits such cytolytic activity. By contrast, the finding that the supernatant of activated 3Tx15 T cells can substitute their presence in the three-party system confirms the classical role of Th1-mediated help.

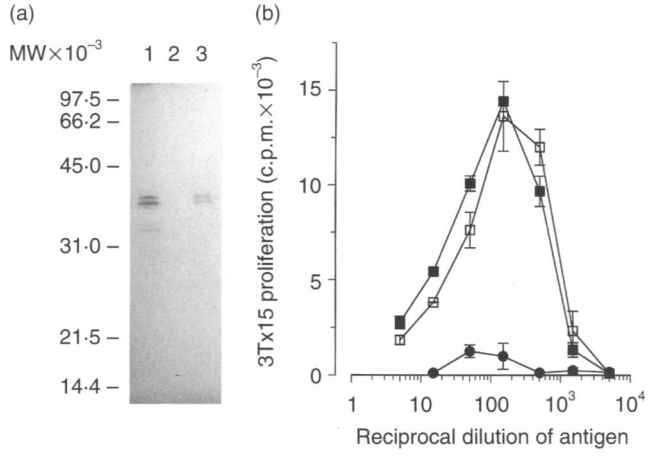

Figure 5. Proliferative response of clone 3Tx15 to immunoaffinity-purified natural GRA4 and GRA4-depleted TLA. (a) Western blot of samples from the purification procedure probed with anti-GRA4 mAb Tx34.31. Lane 1, TLA; lane 2, flow-through; lane 3, eluate. The apparent molecular weights ($\times 10^{-3}$) of standard proteins are given. (b) TLA (□), GRA4-depleted TLA (■) and affinity-purified GRA4 (●), diluted as indicated, were tested for antigenic activity against clone 3Tx15 in T-cell proliferation assay. Results are given as mean c.p.m. ± SD of triplicate test cultures. In controls containing purified GRA4 plus 1 μg/ml TLA 3Tx15 proliferation was ≥17430 c.p.m. from a dilution of 1:50–1:5000. Without antigen [^{3}H]thymidine incorporation was 178 c.p.m.

Using Th1 clone 3Tx15 as tool, we define a new 40000 MW excretory *T. gondii* antigen which differs from the T cell-stimulatory toxoplasma molecules hitherto described: p30/SAG1,[25] p54/ROP2,[26] p23/GRA1[27] and p28/GRA2.[28] Furthermore, to our knowledge p40 is not identical to one of the toxoplasma antigens of about 40000 MW: Since p40 is an excretory toxoplasma product, it is unlikely to be identical to the the 43000 MW surface molecule SAG3.[29] Acccording to its migration pattern in two-dimensional PAGE, p40 has a more basic pI (approximately 7) than the 42000 MW rhoptry protein ROP6 (pI 4·7).[11] Different to the microneme antigen MIC3, a 90000 MW heterodimer of two disulphide-linked 38000 MW proteins (pI 6·7 and 6·75, respectively),[30] p40 retains its molecular weight when separated under non-reducing conditions (not shown). The dense granule protein GRA4, an ESA of 40000–41000 MW under both reducing and non-reducing conditions,[12] co-migrates with p40 in two-dimensional PAGE. Nevertheless, p40 is not identical with GRA4 since neither recombinant nor natural GRA4 exhibited antigen activity for clone 3Tx15, whereas GRA4-depleted TLA did. T cell-stimulatory activity has been reported for GRA4 by using 40000 MW proteins electroeluted from TLA.[19] Although the presence of GRA4 in the eluate has been confirmed, a stimulatory activity for T lymphocytes from infected mice,[18] might, at least in part, be attributable to p40, which must have been co-eluted.

A relevant biological characteristic of p40 is provided by the finding that the antigen represents a toxoplasmic ESA released following infection of brain cells *in vitro*. Although secretion of toxoplasma proteins by the intact host cell has not yet been proven, the first step, namely the entry of toxoplasma molecules from the parasitophorous vacuole into the host cell cytoplasm has been demonstrated.[31] Besides, free toxoplasma are a probable source of the p40 detected in the brain cell supernatant since isolated toxoplasma can secrete p40 and extracellular parasites were observed from day 3 postinoculation. Toxoplasma excretory antigens are supposed to be important in mediating protection against the parasite. Since

IFN-γ supports maintenance of the *T. gondii* cyst stage in cultured astroglia[32] and Mϕ,[33] patrolling ESA-reactive Th1 cells are presumed to prevent reactivation of latent chronic toxoplasmosis. Within the brain, occasional cyst rupture and transiently extracellular parasites will make the target antigens accessible to such T cells. Based on its immunofunctional profile and antigen specificity, clone 3Tx15 represents this type of T cell.

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