INFECTION AND IMMUNITY, July 1990, p. 2120–2126
0019-9567/90/072120-07$02.00/0
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Vol. 58, No. 7

Protection of Nude Rats against *Toxoplasma* Infection by Excreted-Secreted Antigen-Specific Helper T Cells

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Received 18 December 1989/Accepted 20 April 1990

In the present work we demonstrate the implication of excreted-secreted antigens in eliciting the protective cell-mediated immunity developed by rats toward *Toxoplasma gondii*. We first showed that 10^4 specific T cells from *T. gondii*-infected rats conferred to nude rats the ability to resist an infection by the highly virulent RH strain of *T. gondii*. In a second series of experiments, the role of excreted-secreted antigens in this protection was demonstrated. After the adoptive transfer to nude rats of various doses (10^3, 10^4, 10^5) of excreted-secreted antigen-specific helper T cells (propagated in vitro during one month), significant protection toward *T. gondii* was induced. Moreover, these cells were responsible for a specific antibody response in nude rats, which are normally unable to develop any specific humoral response. The specificity of these antibodies was directed toward different molecules with molecular masses of 104, 97, 57, 39, 30, 21, and 18 kilodaltons; some of these have been previously characterized as major excreted-secreted antigens.

Toxoplasmosis is a widespread infection caused by the intracellular protozoan parasite *Toxoplasma gondii*. All species of mammals, including humans, can be infected by this pathogen. It is generally a mild or inapparent infection in human populations, except in fetuses (25) and in immunosuppressed patients (21). In children and adults, *Toxoplasma* infection induces a protective immunity against reinfection. This resistance to toxoplasmosis seems to be mediated at least in part by cell-dependent immunity (18), but the interactions between the different cell populations in parasite killing or in inhibition of growth in vivo are not yet clearly defined.

Unlike other rodents such as mice and hamsters, rats are resistant to *T. gondii* infection (6). Therefore this experimental model has been less used in spite of some analogies with human toxoplasmosis (3). However, we have previously shown that genetically athymic rats (nude; *nu/nu*) did not survive an intraperitoneal infection with 10^3 tachyzoites of *T. gondii*. But when nude rats received lymph node cells they became resistant to the infection. This protection correlated with the level of toxoplasma-specific antibodies (28). This suggested that T cells played a major role in the resistance of euthymic rat against *Toxoplasma* infection and that they induced the production of antibodies that could be implicated in this protection.

The excreted-secreted antigens (ESA) from the tachyzoites also seem to play a crucial role in immunity against toxoplasmosis (8). Indeed, secretory antigens constitute the major part of the circulating antigens present in the plasma 24 h after the infection (14). T lymphocytes of patients are stimulated by these antigens (13), and genetically athymic Fischer rats are significantly protected by the passive transfer of sera from euthymic Fischer rats immunized with ESA (8). This strongly suggested that ESA induce a protective immunity and that antibodies could play a crucial role in the protection obtained in the rat model. There is evidence that, among the isotypes of immunoglobulins produced after immunization with ESA, immunoglobulin E (IgE) plays some role. Fischer rats infected or immunized with ESA showed a specific IgE antibody response that was quantifiable by radioimmunoassay (26). It has been suggested that IgE plays a role in antibody-dependent cellular cytotoxicity mechanisms involving platelets and eosinophils (26). Therefore, the nude rat seems to be an interesting model for the study of the mechanisms controlling in vivo *Toxoplasma* infection.

In the present report we underline the preponderant role played by T cells recovered from infected or ESA-immunized rats in immunity to *T. gondii* infection.

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MATERIALS AND METHODS

Parasites. *T. gondii* RH tachyzoites were obtained from Swiss mouse peritoneal fluids 3 days after mice were infected. The toxoplasma suspension was purified from remaining murine cells by filtration through 3-μm-pore-size polycarbonate membrane filters (Nuclepore Corp., Pleasanton, Calif.). For some experiments, the parasites harvested by this procedure were irradiated (100 Gy, 10 min) (Philips RT 100) and then stored in liquid nitrogen until use.

Animals. Inbred Fischer/Ico F344 rats (2 months old) and genetically athymic Fischer (nude; *nu/nu*) rats were maintained in the animal facilities of Pasteur Institute, Lille. For the passive transfer of ESA-specific T lymphocytes and the helper role of these cells, *nu/nu* Fischer rats were obtained from Harlan Olac (Oxon, England).

Antigen preparation. For soluble antigen (water-lysed antigens [S_2]), tachyzoites obtained from mouse peritoneal washings were filtered on 3-μm-pore-size polycarbonate membranes. The cells were washed twice in 0.1 M phosphate-buffered saline (pH 7.4), and the parasite pellet was adjusted to a concentration of 6×10^8 tachyzoites per ml in distilled water. The parasites were disrupted by sonication (four times per 1 min each), frozen, and submitted to five passages in an X press (LKB, Uppsala, Sweden). After centrifugation at 36,000 × *g* for 2 h at 4°C, the supernatant was used as the source of S_2 after the addition of 100 U of aprotinin per ml. The pellet containing the membrane antigens was solubilized by the addition of the detergent 3[(3-

cholamidopropyl)dimethyl ammonio]propansulfonic acid (Fluka, Buchs, Switzerland). ESA were prepared as described by Darcy et al. (8). Briefly, the parasites (1.33×10^8 tachyzoites per ml) were incubated for 3 h at 37°C in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated rat serum. The tachyzoite suspension was centrifuged at $1,000 \times g$ for 10 min, and the supernatant was filtered, concentrated, and stored at −20°C until use.

Immunization procedure. The ESA (equivalent of 10^8 tachyzoite excretory antigens) were mixed 1:1 with complete Freund adjuvant and injected into Fischer rats at the base of the tail by the method of Corradin et al. (7). After 3 weeks, the rats were injected with the same antigen without adjuvant. T lymphocytes were recovered 5 days after. The same procedure was used for immunization with bovine serum albumin (BSA).

Infection procedure. Fischer rats were intraperitoneally infected with 10^5 tachyzoites, and the T lymphocytes were recovered 30 days after the priming injection. For *nu*/*nu* rat infection, 10^5 or 10^4 parasites were injected by the same way; this dose was sufficient to kill all animals within 15 to 20 days (28).

Preparation of *T. gondii*- and ESA-specific T cells. Thirty days after the priming injection with 10^5 tachyzoites, inguinal and mesenteric lymph nodes were aseptically harvested, and cell suspensions were prepared. *T. gondii*-specific T lymphocytes were separated by the passage through a nylon wool column (16). For the preparation of ESA-specific T cells, 5 days after the second injection of ESA without adjuvant the T lymphocytes were isolated with the same method and maintained in culture at 37°C in 5% CO_2 atmosphere in RPMI 1640 with 10% fetal calf serum (GIBCO), interleukin-2, 2×10^6 irradiated (30 Gy) (Philips RT 100) thymic antigen-presenting cells and 5×10^4 irradiated parasites supplemented with 40 μg/ml of S_2 antigen in 24-well plates (Falcon 3047; Becton Dickinson Labware, Paramus, N.J.). After proliferation, T lymphocytes were used for adoptive transfer into genetically athymic rats.

Source of interleukin-2. Interleukin-2-containing medium was obtained by stimulation of murine EL4 tumor cells with phorbol myristate acetate. Interleukin-2 activity in the conditioned medium was assayed by testing for growth of concanavalin A-stimulated splenic cells.

T-cell proliferation assays. For T-cell proliferation, 5×10^5 specific T cells and 10^6 antigen-presenting cells were seeded in 96-well plates (Falcon 3072; Becton Dickinson) in triplicate in the presence of various dilutions of S_2 antigens or irradiated parasites, BSA (used as an irrelevant antigen), and concanavalin A (used as a positive control). The plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. Levels of DNA synthesis were determined by uptake of 18.5 kBq [^{3}H]thymidine (specific activity, 37 GBq/mmol; TMM-79A CEA, Gif-sur-Yvette, France) after a 18-h pulse on day 5. The amount of cell-incorporated radioisotope was determined by concentration on filter paper (Skatron, Lierbyen, Norway), and the radioisotope was counted in a liquid scintillation counter (LKB).

In vivo transfer of *T. gondii*- and ESA-specific T cells for protective assays. Recipient *nu*/*nu* Fischer rats were injected intravenously with 10^4 to 10^7 specific T lymphocytes or nonspecific T lymphocytes (BSA). One day later, the rats were infected with 10^5 tachyzoites. The same protocol was used for studying the protective role of ESA-specific T cells propagated in in vitro cultures for 3 weeks. These T cells (10^3, 10^4, or 10^5 cells) were adoptively transferred to Fischer nude rats 1 day before infection with 10^5 tachyzoites per rat. Control rats were injected with BSA-specific T lymphocytes. Before passive transfer experiments, the surface phenotype of the cells was analyzed by flow cytometry with monoclonal antibodies against T-cell subsets W3/13, W3/25, and OX8 and against B cells, OX12 (Seralab, Ltd., Crawley Down, U.K.).

Dot blot immunoassay. *nu*/*nu* Fischer rats were used for studying the helper activity of ESA-specific T cells. Cells (10^6) recovered from Fischer rats immunized with ESA and cultured in vitro for 1 month were transferred into nude rats before infection with 10^4 parasites or immunization with ESA. For the detection of anti-*T. gondii* IgG, total antigens (6 μg) were spotted on strips of BA 85 nitrocellulose membrane (Schleicher & Schuell, Dassel, Federal Republic of Germany). After 1 h of saturation in 10 mM phosphate-buffered saline (pH 7.4) with 1.5% casein (E. Merck AG, Darmstadt, Federal Republic of Germany), serum diluted 1:50 was added for 2 h. Preparations were washed in phosphate-buffered saline with 1.5% casein, and the strips were incubated with an anti-rat IgG serum labeled with peroxidase (Miles Laboratories, Inc., Naperville, Ill.). After 2 h, a mixture of 30 mg of HRP color (Bio-Rad Laboratories, Richmond, Calif.), 10 ml of cold methanol, 40 ml of phosphate-buffered saline, and 30 μl of 30% H_2O_2 was added. The reaction was stopped with distilled water. The intensity of the spots was measured with a light-reflecting densitometer (Gretag, Regensdorf, Switzerland) and expressed in optical density units. The *T. gondii*-specific IgE antibodies were measured by a double sandwich technique with a rabbit anti-rat IgE serum diluted 1:2,000 (MIAB, Uppsala, Sweden) revealed with a 1:200-diluted anti-rabbit peroxidase-labeled serum (Diagnostics Pasteur, Marnes-la-Coquette, France).

Electrophoresis and immunoblotting. Total toxoplasma antigens S_2 added with 3[(3-cholamidopropyl)dimethyl ammonio]propanesulfonic acid extract were separated by electrophoresis in 13% polyacrylamide gels with the discontinuous buffer system of Laemmli (19) in unreduced conditions. Proteins were transferred from sodium dodecyl sulfate-polyacrylamide gels to 0.45-μm-pore-size nitrocellulose paper (Schleicher & Schuell) by electrophoresis at 200 mA for 3 h. Briefly, strips were then incubated with various serum samples (1:100 diluted), and the fixed antibodies were revealed with an anti-rat peroxidase-labeled serum (Miles Laboratories).

RESULTS

Reactivity and transfer of specific T cells from infected rats. T cells from infected animals recovered 30 days after infection reacted against S_2 antigens (Table 1). Thus, the T lymphocytes were able to recognize the cytoplasmic antigens released after parasite lysis and to proliferate in in vitro experiments. In the S_2 antigen extract, surface proteins were not included. For this reason, another method of in vitro stimulation was performed. The reactivity of parasite-specific T cells was tested against two different doses of irradiated parasites (10^3 and 10^4 per well) added with a constant concentration of soluble antigens (40 μg/ml). Moreover, an additive effect of the S_2 antigens (cytoplasmic antigens) and of the irradiated tachyzoites, also containing the membrane antigens, clearly appeared. Controls showed that neither S_2 nor irradiated parasites were able to induce a proliferative response in nonimmune T cells.

The in vivo study of a protective role for T cells was carried out. Parasite-specific T cells (10^4 to 10^7 cells per rat)

TABLE 1. In vitro proliferation of infected Fischer rat lymph node cells stimulated by S_2 antigen and irradiated tachyzoite preparations

Antigen source	[^{3}H]thymidine incorporation (cpm ± SD[a])	SI[b]
S_2 (40 μg/ml)	8,000 ± 1,000	6.4
Irradiated parasites (10^3 parasites per well)	5,250 ± 3,000	4.2
(10^4 parasites per well)	9,500 ± 1,250	7.6
Irradiated parasites + S_2 (40 μg/ml) (10^3 parasites per well)	21,500 ± 1,500	17.2
(10^4 parasites per well)	18,000 ± 5,000	14.4
Concanavalin A (5 μg/ml)	35,200 ± 2,200	28
BSA (40 μg/ml)	2,600 ± 1,520	2
Medium	1,250 ± 400	1

[a] Each value represents the mean ± standard deviation of triplicate samples from one representative experiment.
[b] SI, Stimulation index (ratio between counts per minute from stimulated lymphocytes and counts per minute from lymphocytes incubated in the culture medium).

from infected Fischer rats were transferred to *nu/nu* Fischer rats before infection with 10^5 tachyzoites of the virulent RH strain of *T. gondii*. Previous experiments had shown (28) that the passive transfer of 10^6 or more nonspecific T cells to nude rats was sufficient to induce a significant increase in survival. For this reason, in these experiments we reduced to 10^4 per rat the number of specific T cells transferred to evaluate their protective role in comparison with rats receiving 10^4 BSA-specific T cells. The survival of rats that did not receive T cells or that received BSA-specific T cells did not exceed 30 days after infection, whereas the transfer of T cells recovered from infected Fischer rats (the phenotype of which is 97% W3/13$^+$, 68% W3/25$^+$, 35% OX8$^+$, and 5% OX12$^+$) led to a significant increase of the survival from 30 to 50 days and more (Fig. 1). These experiments suggested that only 10^4 *T. gondii*-specific T cells conferred to nude rats the ability to resist the infection for a longer time than did 10^4 T cells specific for an irrelevant antigen.

Reactivity and transfer of specific helper T lymphocytes from rats immunized with ESA. Because of the protective role previously demonstrated in the nude rat model for ESA-specific antibodies, ESA-specific T lymphocytes were produced. A T lymphocyte-enriched population was prepared; the proliferative response of these cells to antigenic stimulation and their protective effect after adoptive transfer were tested. ESA-specific T cells proliferated in a dose-dependent manner either with S_2 antigen (Table 2) or with irradiated tachyzoites associated with a constant dose of S_2 (40 μg/ml) (Table 3). Nevertheless, it clearly appeared that the reactivity of ESA-specific T lymphocytes was essentially if not totally due to the antigens present in the S_2 antigenic fraction. Indeed the proliferative response of these cells with only irradiated tachyzoites was very low. After 1 month in culture, T-cell lines contained 90% W3/13$^+$, 88% W3/25$^+$, 13% OX8$^+$, and undetectable OX12$^+$ cells. This demonstrated that lymph node cells maintained in long-term cultures were strongly enriched in T cells expressing the helper marker (W3/25) in rats. Among rats that had received 10^4 or 10^5 ESA-specific T cells (Fig. 2A and B), the majority (70%) were completely protected against toxoplasma infection, since they were still alive 70 days after the initial infection. Moreover, as few as 10^3 ESA-specific helper T cells were sufficient to extend the survival of nude rats compared with rats that received BSA-specific T cells (Fig. 2C). The rats that received 10^5 T cells were reinfected on day 60 with 10^5 parasites. All animals survived after the challenge, suggesting that the transfer of ESA-specific T cells conferred to nude rats a protective immunity against reinfection.

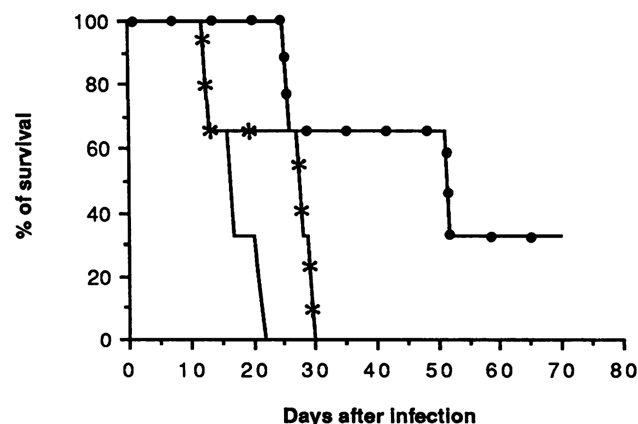

FIG. 1. Transfer of T lymphocytes from infected Fischer rats (●) and from BSA-immunized Fischer rats (*) in nude rats. Control rats (—) were not transferred. All animals were infected with 10^5 tachyzoites of the RH strain.

TABLE 2. In vitro proliferative response of ESA-immunized Fischer rat inguinal lymph node T cells stimulated with various concentrations of S_2 antigen

Antigen source	Concn (μg/ml)	[^{3}H]thymidine incorporation (cpm ± SD[a])	SI[b]
S_2	80	35,480 ± 2,813	7.3
	40	19,621 ± 1,604	4
	20	12,985 ± 2,148	2.7
	10	9,390 ± 3,357	2
Concanavalin A	5	51,240 ± 4,230	10.5
BSA	40	6,855 ± 1,620	1.4
Medium		4,873 ± 997	1

[a] Each value represents the mean ± standard deviation of triplicate samples from one representative experiment.
[b] SI, Stimulation index (see Table 1, footnote b).

TABLE 3. In vitro proliferative response of ESA-immunized Fischer rat inguinal lymph node T cells stimulated by S2 antigen and irradiated tachyzoite preparations

Antigen source	[^{3}H]thymidine incorporation (cpm ± SD[a])	SI[b]
S_2 (40 μg/ml)	13,080 ± 2,443	2.7
Irradiated parasites (10^5 parasites per well)	6,680 ± 160	1.4
(10^4 parasites per well)	5,025 ± 100	1
(10^3 parasites per well)	3,150 ± 500	0.6
Irradiated parasites + S_2 (40 μg/ml) (10^5 parasites per well)	15,800 ± 3,400	3.3
(10^4 parasites per well)	21,300 ± 1,500	4.4
(5×10^3 parasites per well)	17,800 ± 660	3.7
(10^3 parasites per well)	13,750 ± 2,700	2.8
Concanavalin A (5 μg/ml)	39,940 ± 1,430	8.3
BSA (40 μg/ml)	5,745 ± 860	1.2
Medium	4,800 ± 1,160	1

[a] Each value represents the mean ± standard deviation of triplicate samples from one representative experiment.
[b] SI, Stimulation index (see Table 1, footnote *b*).

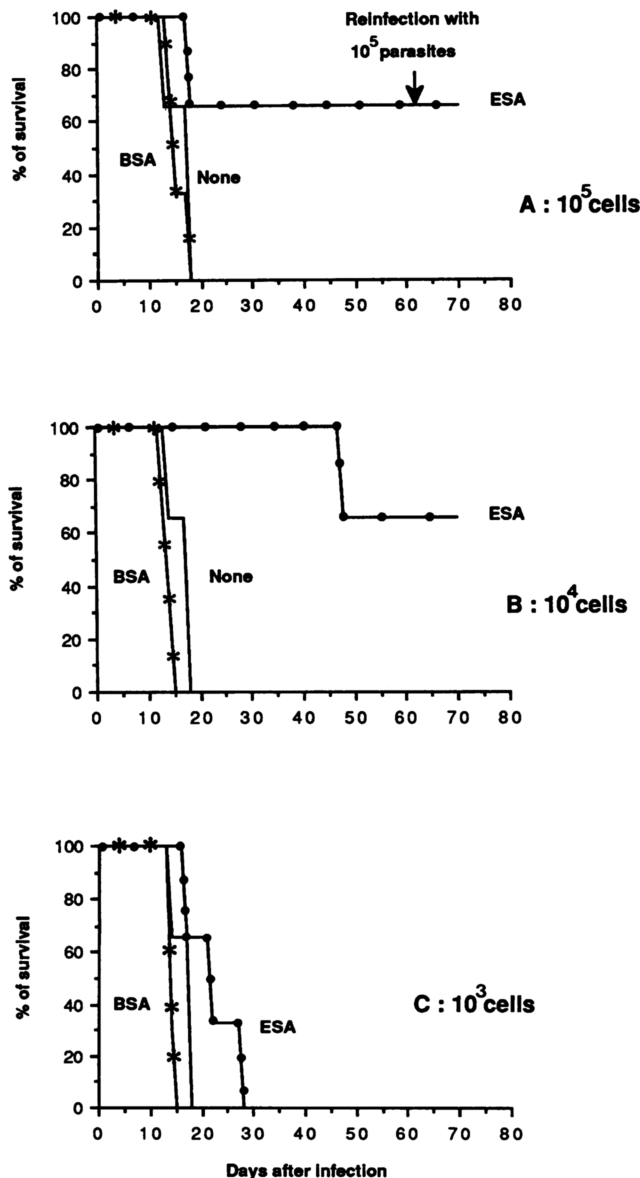

FIG. 2. Adoptive transfer of ESA-specific (●) and BSA-specific (*) helper T lymphocytes. The nude rats received 10^5 (A), 10^4 (B), or 10^3 (C) T cells and were challenged with 10^5 tachyzoites. Control nude rats (-) were only infected with the same number of parasites. In panel A, nude rats that received ESA-specific T cells were reinfected on day 60 with 10^5 tachyzoites. The observed differences in panel C are significant according to the Wilcoxon statistic test (control versus ESA treatment, $P < 0.05$; BSA versus ESA treatment, $P < 0.05$).

Helper activity of T cells from donor Fischer rats immunized with ESA. To analyze the helper activity of ESA-specific T cells, 10^6 cells recovered from Fischer rats immunized with ESA and cultured in vitro for 1 month were transferred into nude rats before infection with 10^4 tachyzoites (Fig. 3A) or immunization with ESA (Fig. 3B). The sera of the rats that received BSA-specific T cells did not contain *T. gondii*-specific IgG. In contrast, rats that received ESA-specific helper T lymphocytes and that were infected with tachyzoites produced an important amount of specific IgG (Fig. 3A). Among Fischer nude rats immunized with ESA (corresponding to the excretion of 10^8 parasites) 1 day after the adoptive transfer with 10^6 ESA-specific T helper cells (Fig. 3B), no difference in tachyzoite-specific IgG production was observed between the animals that received ESA-specific T lymphocytes and those that had received BSA-specific T lymphocytes. Previous experiments had shown that a significant amount of specific IgE is produced during rat toxoplasmosis (26) and suggested that this IgE response was involved in the protective immunity. For this reason, dot blot immunoassays were used to determine the amount of IgE in nude rats that received T cells and then were infected with tachyzoites or immunized with ESA. In both conditions, nude rats that received ESA-specific helper T cells produced a significant quantity of seric tachyzoite-specific IgE (Fig. 3B), whereas control rats did not. These results clearly showed that the T cells from Fischer rats immunized with ESA, maintained in in vitro culture for several weeks, remained functional in vivo and were able to help the production of *T. gondii*-specific antibodies in nude rats normally unable by themselves to develop a specific humoral response.

Characterization of the antigens recognized by sera from *nu/nu* rats that received ESA-specific T cells. The sera from

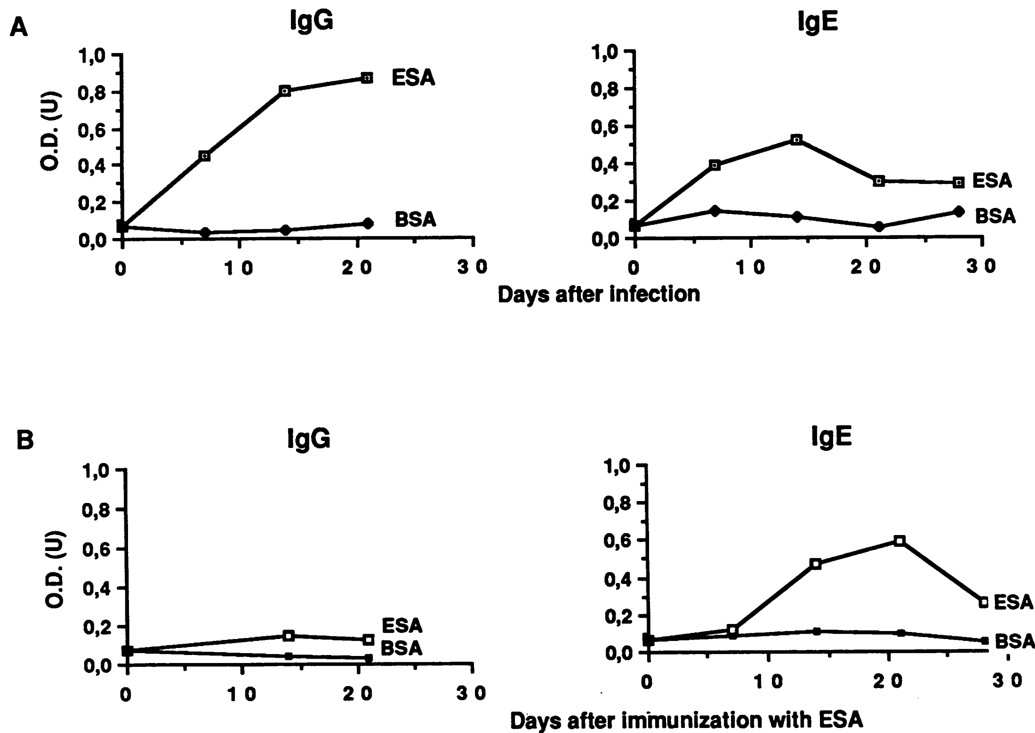

FIG. 3. Determination of *T. gondii* antigen-specific antibodies (IgG and IgE) in sera of nude rats that received BSA- or ESA-specific T cells before infection (A) or before immunization with ESA (B).

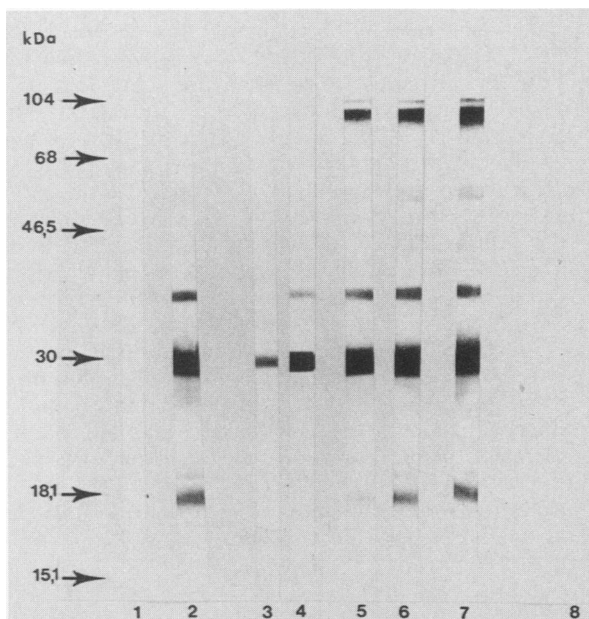

FIG. 4. Western blot analysis of toxoplasma antigens. Sodium dodecyl sulfate–13% polyacrylamide gel electrophoresis in unreduced conditions and blotting developed by sera from the following (lanes): 1, uninfected *nu/nu* Fischer rat; 2, ESA-immunized Fischer rat; 3 through 7, *nu/nu* Fischer rats that received 10^6 ESA-specific T cells on days 7, 14, 21, 28, and 35, respectively, before infection; 8, *nu/nu* Fischer rat that received 10^6 BSA-specific T cells 21 days before infection.

nude rats infected after adoptive transfer of ESA-specific helper T cells recognized several antigens of the total tachyzoite extract (Fig. 4). These antibodies progressively appeared during the course of infection. Seven days after infection, these nude rats had antibodies to 30-kilodalton (kDa) antigens (lane 3). This large band at 30 kDa likely corresponds to both P30, the major surface protein, and to the major 28.5-kDa ESA. Indeed, blots with anti-P30 and anti-28.5 monoclonal antibodies showed the same migration for the corresponding antigens in nonreduced condition. In addition, antibodies to a 39-kDa antigen (lane 4) and to 104-, 97-, 57-, 21-, and 18-kDa antigens (lanes 5, 6, and 7) were present in the sera of rats as soon as 21 days postinfection. In contrast, rat serum reconstituted with BSA-specific T lymphocytes (lane 8) or normal rat serum (lane 1) did not recognize any *T. gondii* antigens. The majority of these antigens were recognized by sera from ESA-immunized Fischer rats (lane 2), except the 104- and 97-kDa antigens, but the immunized rats were bled before 21 days of infection. These results confirmed those mentioned above and demonstrated that the adoptive transfer of ESA-specific helper T lymphocytes to the nude rats was able to induce production of antibodies to the major tachyzoite ESA such as the 97-, 57-, 39-, 28.5-, and 21-kDa antigens described previously (8).

DISCUSSION

The data reported here show that a T-cell line specific for tachyzoite ESA can protect susceptible nude rats against a challenge infection with *T. gondii*. Moreover, the infected nude rats that received these T cells developed an antibody response that was able to recognize toxoplasma antigens. In our laboratory, we had previously shown that the intravenous injection of lymph node cells from uninfected rats

induced the resistance of athymic Fischer rats against toxoplasma infection. In addition, this protection correlated with a specific antibody production. These preliminary results suggested that the T-cell participation was essential for the resistance to acute *T. gondii* infection in vivo (28). In these experiments, at least 10^6 T cells injected into rats were necessary to confer such protection. In this report, we demonstrate that the transfer of T cells from infected Fischer rats can confer to athymic rats resistance against lethal infection. With only 10^4 *T. gondii*-specific T lymphocytes, we obtained a significant increase in survival in comparison with rats that received T cells that were specific for an irrelevant antigen (BSA). These data can be related to the work of Pavia (24) in guinea pigs, but the protective effect of transferred T cells in his experiments was determined by reduction of dissemination of *T. gondii* parasites.

In our experiments, the nude rats could be partially or totally protected, and their survival was proportional to the number of *T. gondii*-specific T cells transferred. In healthy humans, toxoplasma infection induces a life-long protective immunity against reinfection (10, 11). This suggests that during the first infection an efficient stimulation of the immune response occurs. The same situation was observed in our experimental model. The infected nude rats that had received *T. gondii* antigen-specific T cells were protected against a second infection with the lethal strain of *T. gondii*.

Three studies (15, 30, 32) attributed a preponderant role for cytoplasmic and surface antigens of tachyzoites, the proliferative forms present during acute toxoplasmosis, in producing immunity. The present study is based upon a different approach (2), in which we postulated a major role played by tachyzoite ESA in the immune response. Darcy et al. (8) have clearly demonstrated that the passive transfer of sera from ESA-immunized euthymic Fischer rats to genetically athymic rats infected with lethal doses of the highly virulent RH strain of *T. gondii* confers a significant level of protection. Thus the humoral immune response appeared to play an important role in the protective immunity of *T. gondii*-infected rats.

The adoptive transfer of T cells led to the production of specific antibodies. It is now established that in rat toxoplasmosis, antibody induced mechanisms of antibody-dependent cellular cytotoxicity involve eosinophils or platelets as effector cells (26). Moreover, antibodies would lyse extracellular toxoplasmas in the presence of complementlike accessory factors (27, 29). The antibodies in serum from nude rats that had received ESA-specific T cells and that were protected against infection were analyzed. It appeared that those antibodies had the same antigenic specificity as antibodies from ESA-immunized Fischer rat serum, which induced significant protection when passively transferred to nude rats (8). It would be hazardous to attribute the protection to the antibody production alone. Indeed, numerous data demonstrate that cell-mediated immunity is a major component of the host defense mechanisms against toxoplasmosis (9, 20) and that immune lymphocyte products are involved. When lymphocytes from animals chronically infected with *T. gondii* were incubated in the presence of specific antigen, they produced and released into the culture medium a variety of biologically active substances, such as macrophage migration inhibitory factor (12), interleukin-2 (1), gamma interferon (31), and toxoplasma mediator (5). For this reason, we supposed that the ESA-specific helper T cells transferred could participate by means of production of lymphokines in the elimination of parasites by activating macrophage oxidative metabolisms and antiprotozoal activity (22). Lymphokines like gamma interferon can activate macrophages for the elimination of intracellular parasites (23). Gamma interferon appeared to be the major mediator of resistance against *T. gondii*. Indeed, mice treated with a monoclonal antibody against gamma interferon died with acute toxoplasmosis, whereas control mice developed immunity and survived (33). Studies on lymphokine production by ESA-specific T cells and their role in the protective immunity of nude rats are presently underway.

A recent study demonstrated in the murine model that T lymphocytes from mice immunized with a temperature-sensitive mutant strain of *T. gondii*, Ts4, protect normal recipient mice against death caused by the moderately virulent C56 strain (34). The phenotype Lyt-2^+ and Lyt-1^+ L3T4^+ T cells were responsible for resistance against toxoplasmosis, but the Lyt-2^+ T lymphocytes also could be involved. In addition, a recent report (17) demonstrated the induction of cytotoxic T cells by the major *T. gondii* membrane antigen P30. Since an antibody response toward P30 was observed in our model, the protection of nude rats could be due at least partly to P30-specific T cells with the phenotype OX8^+, which represent about 15% of the T-lymphocyte populations after in vitro culture.

Since we show herein that ESA-specific antibodies and T cells induced protection, it is now important to find out which among the panel of ESA induce the major T-cell response and which are the main targets of the protective antibodies. The cloning of an ESA was recently carried out in our laboratory (4), and we are now studying the potential of these ESA for vaccines and characterizing the major T- and B-cell epitopes by using synthetic peptides.

ACKNOWLEDGMENTS

We thank H. Caron for the production of parasites and I. Wolowczuk for useful advice and discussions.

This work was supported by the Institut National de la Santé et de la Recherche Médicale U167-Centre National de la Recherche Scientifique 624.

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