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Absence of a role for natural killer cells in the control of acute infection by *Toxoplasma gondii* oocysts

H. P. A. HUGHES, L. H. KASPER,* JUNE LITTLE† & J. P. DUBEY‡ *Veterinary Research Laboratory, Montana State University, Bozeman, MT, *Department of Medicine and Microbiology, Dartmouth Medical School, Hanover, NH, †Immunex Corp., Seattle, WA and ‡USDA-ARS, Animal Parasitology Institute, Beltsville, MD*

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SUMMARY

The active phase of primary and challenge oral infections of *Toxoplasma gondii* was investigated with respect to natural killer (NK) activity against YAC-1 tumour cell targets *in vitro* and serum interferon (IFN) titres. Primary (non-lethal) oral infection of BALB/c mice with Me49 oocysts resulted in a rapid increase of serum IFN titres, followed by augmented NK activity. NK levels became depressed, rising again by 15 days after infection to normal levels, again preceded by elevated IFN titres. In challenge infections NK was not augmented and IFN titres rose only if a high dose of oocysts was given. IFN activity was pH2-labile in all cases and considered to be due to IFN-γ. Cold target inhibition studies indicated that *T. gondii* did not bind to NK cells. A bioassay for the effects of NK cells on *T. gondii* tachyzoites was developed and there was no evidence of killing *in vitro* by cells with NK function; *T. gondii* survived better when cultured with NK cells than when cultured alone. Studies using C57BL/6 *bg/bg*, *bg/+* and *+/+* mice showed that there was no difference in mean time to death after administration of a lethal ME49 oocyst infection by mouth. Cytotoxicity against YAC-1 in both spleen and mesenteric lymph node (MLN) cell populations was highly augmented in *bg/+* and *+/+*, but not in *bg/bg* mice. Genetic deficiency of NK activity had no effect on survival of mice after infection. Therefore NK has at best a minimal role to play in protection during the acute phase of *Toxoplasma* infection.

Keywords natural killer cell *Toxoplasma gondii* interferon infection

INTRODUCTION

Experimental infection of animals with some parasitic protozoa results in high levels of natural killer (NK) activity during acute stages of infection, with or without elevated serum interferon titres. However, there is some controversy regarding the significance of NK in controlling the initial stages of infection. NK-deficient beige (*bg/bg*) mice appear more susceptible to viscerotropic *Leishmania* infection (Kirkpatrick *et al.*, 1985) but there is no apparent correlation between mice with susceptibility phenotype and spontaneous or IFN-induced NK activity (Kirkpatrick & Farrell, 1984). Furthermore, no increased incidence of dermotropic *Leishmania* infection occurs in Chediak–Higashi patients, who have low (non-demonstrable) NK activity (Merino & Cruz, 1984). Likewise, although NK and IFN titres increase during acute *T. cruzi* infection, this response does not seem to contribute directly to protection of the infected animal (Quan *et al.*, 1983).

Correspondence: H. P. A. Hughes, Veterinary Research Laboratory, Department of Veterinary Science, College of Agriculture, Montana State University, Bozeman, Montana 59717, USA.

Protection against *T. gondii* is considered to be cell-mediated (reviewed by Hughes, 1985). Recently, considerable attention has focused on the immunological events associated with the acute stages of infection, and how these may regulate parasite proliferation. Parenteral inoculation of mice with *T. gondii* tachyzoites of the highly virulent RH strain causes significant augmentation of NK (Hauser, Sharma & Remington, 1982; Kamiyama & Hagiwara, 1982), followed by suppressed NK activity which gradually returns to normal levels (Kamiyama & Hagiwara, 1982).

IL-2 therapy with coincident augmentation of NK activity results in partial protection against a challenge with viable *T. gondii* (Sharma, Hofflin & Remington, 1985), and Hauser & Tsai (1986) have shown that NK cells from *T. gondii*-infected mice were cytotoxic for the parasite itself. All of these studies have used laboratory-adapted strains of *T. gondii* (such as the C56 or RH strains), given by parenteral inoculation. This may represent an unnatural infection administered by an unnatural route, so may be a poor model. In this report, we have studied the immunological events immediately following oral infection; the study suggests that NK cells have a minimal effect (if any) on the control of *T. gondii* proliferation following oral infection.

MATERIALS AND METHODS

Mice

Female, 6- to 8-week-old BALB/c and C57BL/6 mice were purchased from the Frederick Cancer Research Facility, Frederick, MD. C57BL/6 (*bg/bg*) and heterozygous C57BL/6 (bg/+) were purchased from Jackson Labs, Bar Harbor, ME.

Toxoplasma gondii

Oocysts of the Me49 strain were obtained from feline faeces and used within 6 months (Christie, Pappas & Dubey, 1978). Preliminary titration of this strain in BALB/c mice showed that uniformly lethal infections occurred after oral or subcutaneous inoculation of 5×10^4 oocysts. Mice were infected as reported in the results section. RH strain tachyzoites of *T. gondii* were maintained as described (Hughes, Hudson & Fleck, 1986; Hughes *et al.*, 1984). C strain tachyzoites of *T. gondii* were maintained in cultured human fibroblast cells (Kasper, Currie & Bradley, 1985).

Interferon (IFN) assay

At various times after infection mice were bled from the retro-orbital plexus and the serum was separated and stored at −80°C. IFN was assayed and evaluated using a standard VSV plaque inhibition assay, murine L929 cells (ATCC CCL1) and murine. IFN-α to standardize the assay (Cerretti *et al.*, 1986). All serum samples were initially diluted 1:2 and then divided into two fractions. One was held at 4°C while the other was dialysed at pH 2 (using 0·1 M glycine–HCl). Following dialysis and re-adjustment to pH ≈7·4, each sample was tested in the VSV plaque inhibition assay.

Interleukin 2 (IL-2)

Human recombinant IL-2 was a gift from Immunex, Inc. (Seattle, WA). Animals were injected i.p. with five doses of 100 U IL-2 at days −1, +2, +4, +6 and +8 after an oral challenge with 5×10^4 Me49 oocysts (Sharma *et al.*, 1985).

Preparation of effector cells

Spleens and mesenteric lymph nodes (MLN) were dissected from mice, teased apart in serum-free RPMI (5 ml/three spleens) and single cell suspensions produced by forcing through an 18-gauge needle. Cells were layered onto an equal volume of 80% Mono-poly (Flow, McLean, VA) in RPMI-FBS and centrifuged (500 *g* for 30 min). Lymphocytes were harvested from the interface, washed in RPMI-FBS and resuspended to a final concentration of 10^7/ml. Plastic-adherent cells from spleen or MLN were removed by incubation of cell suspensions at 37°C in 5% CO_2 for 1–2 h in 25 cm^2 flasks. Nylon wool columns were also used to remove adherent cells (Julius, Simpson & Herzenberg, 1973). Non-adherent cells were then adjusted to a final concentration of 10^7/ml in RPMI-FBS and stored on ice until required. The viability of the cells was >95% as assessed by trypan blue exclusion.

Preparation of target cells

YAC-1 lymphoma and EL4 thymoma (ATCC) were maintained as stationary suspension cultures in RPMI-FBS. Cells (10^7) in RPMI were added to 200 to 300 μCi [^{51}Cr]Na_2CrO_4 (1 mCi/ml; New England Nuclear) and incubated for 90 min at 37°C (water bath), with frequent agitation. Cells were washed three times in RPMI-FBS and brought to a final concentration of 10^5/ml. Viability was >95%.

Tumoricidal cytotoxicity assay

A 4 h ^{51}Cr-release assay was used (Hauser *et al.*, 1982). Effector cells were combined with 10^4 labelled target cells in a total volume of 200 μl medium in triplicate wells of U-form microtitre trays for each effector:target (E:T) ratio tested and incubated for 4 h at 37°C. At the end of incubation, plates were centrifuged at 500 *g* for 10 min, and supernatants harvested using a supernatant harvesting system (Skatron, Sterling, VA). Radioactivity was measured with a gamma counter (Beckman Instruments, Fullerton, CA). Spontaneous release determined from triplicate wells containing labelled target cells and RPMI-FBS alone was between 5 and 10% of the total ct/min in all experiments. Maximum ct/min was the mean of ^{51}Cr from target cell suspensions after addition of detergents.

The % specific ^{51}Cr release was computed using the following formulae (Brooks & Flannery, 1980):

$$\%\ \text{release} = \frac{\text{ct/min in supernatant}}{\text{Maximum release from tumour cells}} \times 100$$

$$\text{cytotoxicity} = \frac{\%\ \text{effector cell release} - \%\ \text{spontaneous release}}{\text{Maximum} - \%\ \text{spontaneous release}}$$

Cold target inhibition

The ability of NK cells to bind to *T. gondii* RH strain tachyzoites was assessed by cold target inhibition. A standard microcytotoxicity assay was set up as described above, at an E:T ratio of 100:1, with the following modifications: Unlabelled ('cold') parasites were added to target cell suspensions so that the ratio of cold parasites to labelled ('hot') tumour targets was from 1:10 to 100:1. Control cultures consisted of adding cold YAC-1 targets to hot targets at the same cold:hot ratios. Cytotoxicity was assessed as described above, and the E:T ratio refers to effector:hot target ratio. Mean spontaneous release values used in this assay were derived from cultures of YAC-1 cells alone and YAC-1 cells with *Toxoplasma* at the different cold:hot ratios tested.

Bioassay of T. gondii susceptibility to NK

Effector cells from BALB/c mice were combined with 10^4 unlabelled RH strain tachyzoites in a total volume of 400 μl in triplicate tubes in RPMI-FBS or PBS with 20% newborn calf serum (NBCS), at an E:T ratio of 100:1. Control cultures were *T. gondii* cultured in the absence of effector cells. Following 4 h incubation (37°C/5% CO_2), cells were vigorously shaken and adjusted to 5×10^3 parasites/ml. Serial dilutions were then carried out, and syngeneic mice were injected with an estimated number of parasites ranging from 1000 to 1 in volumes of 200 to 300 μl. Previous titration of RH strain tachyzoites showed that a single parasite can kill a mouse (data not shown), thus the ability of NK to kill *T. gondii* could be assessed and compared with culturing *T. gondii* in the absence of effector cells. The functional capability of NK cells was assessed in parallel cultures, using ^{51}Cr labelled YAC-1 targets. In all cases, effector cells had normal or predicted NK activity against YAC-1 tumour targets.

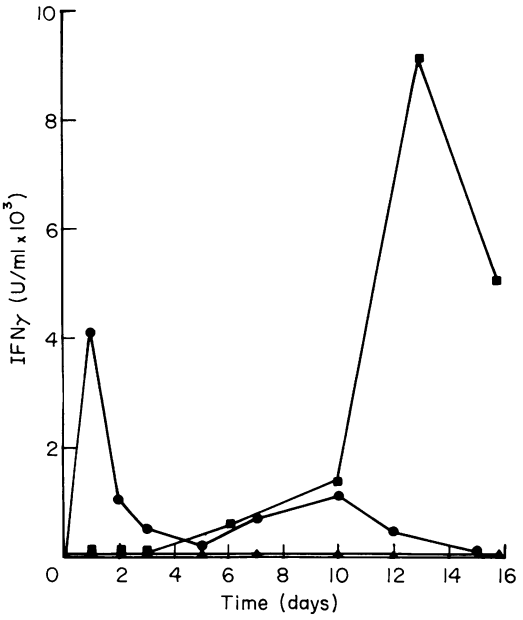

Fig. 1. Serum interferon (IFN) titres following primary and secondary infection with *T. gondii*. (●) BALB/c mice given 100 Me49 oocysts by mouth. Serum IFN peaked at days 1 and 10 after injection. Two experiments are shown. (▲) BALB/c mice were given a primary Me49 oocyst infection (100 oocysts) by mouth and orally challenged 25 days later with a further 100 oocysts. (■) BALB/c mice were given a primary infection (above) and orally challenged with 10,000 Me49 oocysts. IFN titres peaked at day 12 after challenge. All interferon activity was abrogated by pH 2 treatment. Each point represents a pool of five mice. s.e.m. was no greater than symbol size.

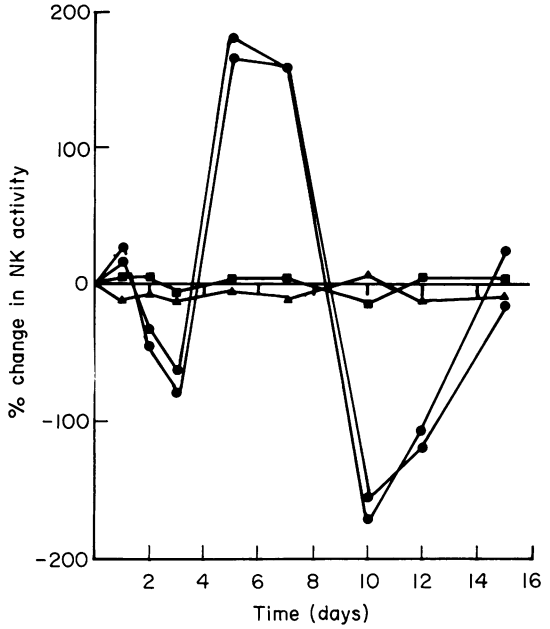

Fig. 2. Splenic natural killer (NK) activity during primary and secondary infection with *T. gondii*. (●) primary and (▲, ■) secondary infections in BALB/c mice were carried out as in Fig. 1. NK was only affected by primary infection. Activity peaked between day 5 and 7 after infection was suppressed by day 10, returning to normal levels by day 16 after infection. Each point represents a group of five mice. s.e.m. was no greater than symbol size.

RESULTS

BALB/c mice were inoculated orally with 100 oocysts and killed at days 1, 2, 3, 5, 7, 10, 12 and 15 after inoculation. A dose of 100 oocysts was chosen for primary infections as it was not lethal for any strain of mouse used, and previous studies had shown that it induced solid immunity measured by (a) antibody response, (b) lymphocyte transformation and (c) protection against lethal challenge (Hughes, unpublished). Serum interferon (IFN) levels rose rapidly to >4000 IRU/ml on day 1 falling to normal levels by day 5. Following another smaller rise, levels had returned to those of control animals by day 15 (Fig. 1). NK levels were augmented on day 5 and day 7, suppressed by day 10, returning to normal levels by day 15 (Fig. 2). Augmentation of NK or recovery from suppression occurred 4 to 5 days after both peaks in IFN activity (cf Figs 1, 2). All detectable IFN activity was abrogated by treatment at pH 2·0.

BALB/c mice were given a primary oral inoculation (100 oocysts), followed by a low (100 oocysts) or high dose (10,000 oocysts) oral challenge 25 days later. The high dose challenge was lethal in naive, sex- and age-matched mice, whereas the low dose was not (data not shown). Interferon and NK levels were largely unaffected in both groups of challenged mice, with a rise in IFN titres only occurring in those given a high dose challenge at day 10 to day 16 (Figs 1 & 2).

Cold target inhibition experiments were carried out to assess whether *T. gondii* could inhibit NK cytolysis of YAC-1 cells by binding to cell surface receptors on splenic lymphocytes with NK function. Inhibition experiments were carried out using

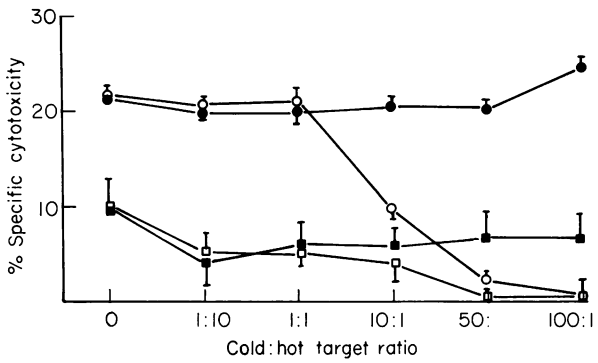

Fig. 3. Cold target inhibition of YAC-1 cytolysis by NK cells. Percoll-enriched spleen cell populations were incubated with labelled ('hot') YAC-1 tumour targets. Cold (unlabelled) YAC-1 (○, □) or *T. gondii* (●, ■) were added at cold to hot ratios from 0 (no cold targets) to 100:1. Cytotoxicity was assessed at E: (hot) T ratios of 100:1. The addition of cold YAC-1 (●, ■) at a cold:hot target ratio of 10:1 resulted in above 50% inhibition of cytolysis. At 50:1 cytolysis was almost totally impaired. At 100:1 no cytolysis of hot targets could be detected. The additon of cold *T. gondii* (●, ■) had no effect on YAC-1 cytolysis, though a significant rise in ^{51}Cr release could be observed at cold:hot ratios of 100:1. (○, ●) RPMI 1640 was used as the culture medium. (□, ■) PBS was used as the culture medium; NK cytotoxicity was severely depressed, though the same trend is apparent when RPMI 1640 was used. Error bars represent s.e.m. This experiment was repeated with similar results. In all cases, there was no increase in cytotoxicity against EL4 cells ($P > 0.05$) compared with control levels (2% ± 0·3).

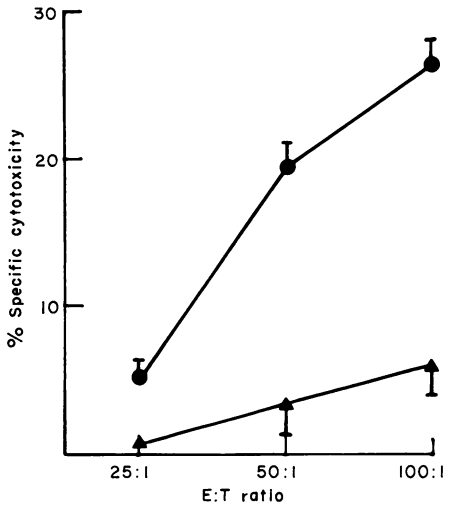

Fig. 4. *T. gondii* tachyzoites induce ^{51}Cr release from labelled YAC-1 cells. (●) Percoll enriched splenocytes (effector cells); YAC-1 targets. (▲) *T. gondii* (RH strain) tachyzoites (effector cells); YAC-1 targets. Detectable ^{51}Cr release observed at 50:1 and 100:1, presumably due to invasion of cells by *T. gondii* during culture. Error bars represent s.e.m. of the mean of three experiments.

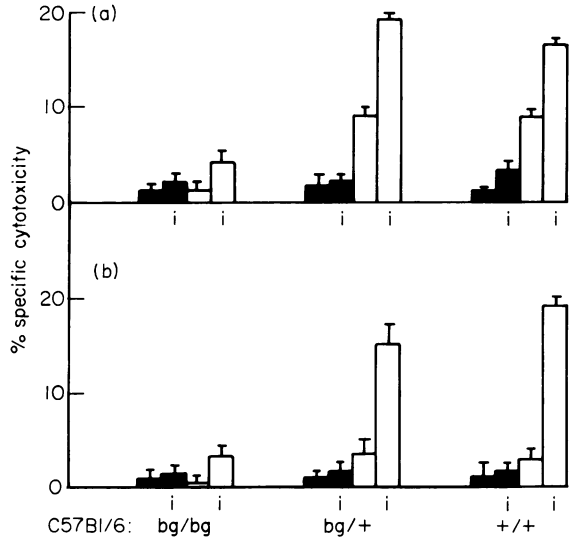

Fig. 5. NK cytotoxicity of YAC-1 tumour targets in spleen and mesenteric lymph node (MLN) of C57BL/6 *bg/bg*, *bg/+* and +/+ mice. NK cells were Percoll-enriched 5 days after an oral challenge of 100 Me49 oocysts, when NK levels were at their highest (Fig. 2), and compared to age and sex-matched controls. i = infected. Solid bars: cytotoxicity of EL4; open bars: cytotoxicity of YAC-1. Infection augmented NK cytotoxicity of YAC-1 in cultures of both spleen and MLN of *bg/+* and +/+ mice ($P < 0.01$). *Bg/bg* NK cytotoxicity of YAC-1 targets was not significantly higher than EL4 cytotoxicity ($P > 0.05$), and was lower than control (*bg/+* or +/+) NK levels.

Table 1. Effect of the addition of NK cells to *T. gondii* cultured *in vitro*

	No. mice dead/no. inoculated										
Dose*	1024	512	256	128	64	32	16	8	4	2	1
E:T 100	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5	1/5
E:T 0†	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	1/5	0/5	0/5

*No. of parasites inoculated.
†Parasites cultured in the absence of effector cells.
Cultures carried out in RPMI-FBS.

Table 2. Effect of IL-2 and the beige mutation on *T. gondii* infection

Parasite strain	IL 2†	Mean time to death*					
		bg/bg	*P*‡	*bg/+*	*P*	+/+	*P*
Me49§ (5)¶	+	9	>0·05	8	>0·05	9	>0·05
Me49 (5)	−	8	>0·05	8	>0·05	8	-
C (10)	−	7	>0·05	7	0·05	7	-

*Rounded to nearest day.
†Mice given IL-2 as described in Materials and Methods (+) or left untreated (−).
‡Significance of difference between *bg/bg*, *bg/+* or +/+ mean time to death compared with +/+ untreated mice.
§7500 Me49 oocysts (oral) or 5×10^6 C strain tachyzoites i.p.
¶No. mice/group.

both PBS (Hauser & Tsai, 1986) and RPMI media. Effector cells were prepared from BALB/c mice orally infected with 100 Me49 oocysts 5 days before the assay, when NK levels were at their peak (Fig. 2). When unlabelled (cold) YAC-1 targets were incorporated into the cytotoxic assay, lysis of ^{51}Cr-labelled (hot) targets was inhibited by about 50% at cold:hot targets ratios of 10:1, and was almost at basal levels at ratios of 50:1 (Fig. 3). When unlabelled *T. gondii* (RH) tachyzoites were used as cold targets no inhibition of YAC-1 lysis occurred, and a small but significant increase in ^{51}Cr release occurred at cold (*T. gondii*) : hot (YAC-1) target ratios of 100:1. When PBS was used as the culture medium rather than RPMI, NK lysis of tumour targets in a standard cytotoxicity assay was much reduced (cold:hot target ratio 0, Fig. 3). However, regardless of whether PBS or RPMI was used, cold YAC-1 cells inhibited lysis, whereas RH strain tachyzoites did not.

When RH strain tachyzoites were used as effector cells in a standard assay, at E:T ratios of 50:1 and 100:1, significant release of ^{51}Cr from YAC-1 targets occurred (Fig. 4). *Toxoplasma gondii* could also be observed inside YAC-1 target cells and this cell line can successfully propagate *T. gondii* (Hughes, unpublished). *Toxoplasma gondii* infection of YAC-1 cells did not significantly affect NK cytolysis (Fig. 3), except at high parasite:cell ratios. Likewise, parasitism of NK cells did not affect cytolysis of YAC-1 targets (below).

Previous studies (Hauser & Tsai, 1986) have suggested that NK may have a direct role in control of infection. We chose to investigate this further *in vitro* and *in vivo* using mice infected with the Me49 or C strain of *T. gondii*. Enriched NK cells from mice infected 5 days previously with Me49 oocysts were incubated with *T. gondii* (RH strain) or YAC-1 for 4 h.

Cytotoxicity of YAC-1 targets was measured by ^{51}Cr release and was at levels similar to those described above (i.e. between 180 and 200% of uninfected mice, Fig. 2). Initial studies assessing the effects of inoculation in RPMI-FBS compared with PBS indicated that *T. gondii* survived better in RPMI-FBS than in PBS ($P < 0.05$), and that NK cytotoxicity was greatly reduced when PBS was used as the culture medium compared with RPMI-FBS ($P < 0.001$) (data not shown). Experiments were then performed to assess the effects that co-culture of NK cells and *T. gondii* on parasite viability, assessed by infectivity of mice. The results indicate that when cells were not added to cultures, RH tachyzoite viability decreased with an LD_{50} between 8 and 4 parasites/mouse. When splenic effector cells were added at 100:1, the LD_{50} was between 2 and 1 parasite/mouse (Table 1).

Human recombinant IL-2 (rIL-2) was administered i.p. to C57BL/6 *bg/bg*, *bg/+*, and *+/+* mice as described previously (Sharma *et al.*, 1985). The results presented in Table 2 illustrate that neither rIL-2 treatment nor the beige mutation had any effect on the outcome of *T. gondii* infections. Furthermore, when 'less virulent' C strain tachyzoites were administered parenterally (i.p.) to *bg/bg*, *bg/+*, or *+/+* mice, there was no difference in the mean time to death among the three groups (Table 2). NK augmentation in beige and heterozygous mice was studied at 5 days after oral inoculation (Me49 oocysts), when splenic NK levels peaked (Fig. 2). In *bg/+* or *+/+* mice, there was significant augmentation of NK. Although some NK activity could be detected in *bg/bg* mice after inoculation, levels never rose above 4%, and were lower than control (uninfected) animals in all cases. Augmentation occurred in both spleen and MLN populations; cytotoxicity against EL4 targets was between 1 and 4% (Fig. 5).

DISCUSSION

Active infection of BALB/c mice with *T. gondii* results in enhanced NK cytotoxicity for YAC-1 target cells (Hauser *et al.*, 1982). Further studies have shown that NK activity is then suppressed, gradually returning to normal levels after 6 weeks of infection. No correlation between IFN titres and NK augmentation was noticed (Kamiyama & Hagiwara, 1982). These studies led to further work in which IL-2 administration was shown to have a partially protective effect against lethal parenteral infection (Sharma *et al.*, 1985) and a report stating that NK cells were cytotoxic for *T. gondii* (Hauser & Tsai, 1986).

When animals were inoculated orally with oocysts, essentially the same basic pattern of augmentation and suppression was apparent compared with previously published work (Hauser *et al.*, 1982; Kamiyama & Hagiwara, 1982). However, there were two important differences. Firstly, the time scale was much reduced; NK levels returned to normal levels by 15 days instead of approximately 6 weeks after inoculation. Secondly, there appeared to be a correlation between NK and IFN titres. Both the initial augmentation of NK and recovery from suppressed levels were preceded by significant increases in serum IFN titres. Although others have described elevated IFN-α titres 24 h after inoculation (Omata *et al.*, 1984), all detectable IFN activity was pH2 labile in the present study, and tentatively identified as IFN-γ (Georgiades & Johnson, 1981). As there was no evidence of NK augmentation in challenge infections, NK fluctuations appear to be related solely to the acute stages of a primary oral infection.

Since enhanced NK activity during experimental toxoplasmosis was first described, considerable attention has focused on elucidating any role that NK cells may directly have on the control of infection and the parasite itself. *In vivo* administration of IL-2 has helped protect animals against a lethal parenteral challenge (Sharma *et al.*, 1984). Of the cellular events following administration of IL-2 and challenge, NK levels alone seemed to be affected. That IL-2 augments NK and cytotoxic T lymphocyte activity *in vitro* and *in vivo* has been well established (Riccardi, Vose & Herberman, 1983; Hefeneider *et al.*, 1983). In our hands, IL-2 administration had no effect on survival of mice challenged orally (Me49) or i.p. (C strain). Whether or not CTL have a role to play in the control of *T. gondii* infection has not been adequately investigated, though the hypothesis that they do not has been proposed (Handman, Chester & Remington, 1980). There are no firm data either way.

In a series of cold target inhibition experiments, we found that *T. gondii* could not inhibit lysis of NK sensitive YAC-1 cells at the cold:hot ratios tested. This indicates that there are no binding sites for NK cells on the parasite surface or that the parasite is so (relatively) small, its presence does not affect NK cytolysis. Our results would suggest that NK cells cannot bind to *T. gondii* as (1) there was no inhibition of NK cytolysis at C:H ratios of even 100:1 (Fig. 3). This ratio is 10 times that required for detectable inhibition by cold YAC-1 targets and at high C (*Toxoplasma*): H ratios, active penetration of labelled YAC-1 cells occurred, resulting in release of ^{51}Cr and a false impression of augmented cytolysis (Figs 3 & 4). In order to confirm the apparent inability of NK cells to kill *T. gondii*, we developed a sensitive bioassay which showed that *T. gondii* survives as well or better when cultured with NK cells in either PBS or RPMI. We are presently investigating the growth of *T. gondii* in purified human LGL with NK phenotype and function. There is clear evidence that *T. gondii* tachyzoites not only survive, but can also replicate in LGL (Hughes *et al.*, unpublished).

Studies were carried out using NK deficient (bg/bg) mice. Neither the hereditary absence of NK cells nor the administration of IL-2 affected the survival of *bg/bg* and control animals (Table 2). Any augmentation of NK that occurred could not be related to survival. NK augmentation was significant in both *bg/+* and *+/+* mice in both spleen and MLN. As MLN has little resting NK activity (Mowat *et al.*, 1983), the augmentation noticed in the present study may be due to recruitment of other cells or counteraction of a suppressor pathway (Hauser, Sharma & Remington, 1983).

Previous work on NK activity with respect to *Toxoplasma* infection has concentrated on attempting to define a role for NK in the control of infection. We conclude that *T. gondii* tachyzoites do not inhibit NK cytotoxicity against YAC-1, that there is no evidence for *in vitro* killing of *T. gondii* by NK using a bioassay and that NK deficient animals die of acute toxoplasmosis at the same rate as normal animals.

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REFERENCES

BROOKS, C.G., & FLANNERY, G.R. (1980) Quantitative studies of natural immunity to solid tumours in rats. Persistence of natural immunity throughout reproductive life, and absence of suppressor cells in infant rats. *Immunol.* **39,** 187.

CERRETTI, D.P., MCKEREGHAN, K., LARSEN, A., COSMAN, D., GILLIS, C. & BAKER, P. (1986) Cloning, sequence and expression of bovine interferon-γ. *J. Immunol.* **136,** 4561.

CHRISTIE, E., PAPPAS, P.W. & DUBEY, J.P. (1978) Ultrastructure of excystment of *Toxoplasma gondii* oocysts. *J. Protozool.* **25,** 438.

GEORGIADES, J.A. & JOHNSON, H.M. (1981) Partial purification and characterization of mouse immune interferon. *Meth. Enzymol.* **78,** 545.

HANDMAN, E., CHESTER, P.M. & REMINGTON, J.S. (1980) Delayed hypersensitivity to *Toxoplasma* and unrelated antigens in *Toxoplasma*-injected mice: induction and elicitation of delayed-type hypersensitivity by antigen pulsed macrophages. *Infect. Immun.* **28,** 524.

HAUSER, W.E., SHARMA, S.D. & REMINGTON, J.S. (1982) Natural killer cells induced by acute and chronic *Toxoplasma* infection. *Cell. Immunol.* **69,** 330.

HAUSER, W.E., SHARMA, S.D. & REMINGTON, J.S. (1983) Augmentation of NK cell activity by soluble and particulate fractions of *Toxoplasma gondii*. *J. Immunol.* **131,** 458.

HAUSER, W.E. & TSAI, V. (1986) Acute *Toxoplasma* infection of mice induces spleen NK cells that are cytotoxic for *T. gondii in vitro*. *J. Immunol.* **136,** 313.

HEFENEIDER, S.H., CONLON, P.J., HENNEY, C.S. & GILLIS, S. (1983) *In vivo* interleukin 2 administration augments the generation of alloreactive cytotytic T lymphocytes and resident natural killer cells. *J. Immunol.* **130,** 222.

HUGHES, H.P.A. (1985) Toxoplasmosis: the need for improved diagnostic techniques and accurate risk assessment. In *Parasite Antigens in Protection, Diagnosis and Escape* (ed: RME Parkhouse). *Current Topics in Microbiology and Immunology*, Vol. 120, p. 105. Springer-Verlag, Berlin.

HUGHES, H.P.A., CONNELLY, C.A., STRANGEWAYS, J.E.M. & HUDSON, L. (1984) Antigen specific lymphocyte transformation induced by secreted antigens from *Toxoplasma gondii*. *Clin. exp. Immunol.* **58,** 539.

HUGHES, H.P.A., HUDSON, L. & FLECK, D.G. (1986) *In vitro* culture of *Toxoplasma gondii* in primary and established cell lines. *Int. J. Parasitol.* **16,** 317.

JULIUS, M.H., SIMPSON, E. & HERZENBERG, L.A. (1973) A rapid method for isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.

KAMIYAMA, T. & HAGIWARA, T. (1982) Augmented followed by suppressed levels of natural cell mediated cytotoxicity in mice infected with *Toxoplasma gondii*. *Infect. Immun.* **36,** 628.

KASPER, L.H., CURRIE, K.M. & BRADLEY, M.S. (1985) An unexpected response to vaccination with a purified major membrane tachyzoite antigen (P30) of *Toxoplasma gondii*. *J. Immunol.* **134,** 3426.

KIRKPATRICK, C.E. & FARRELL, J.P. (1984) Splenic natural killer-cell activity in mice injected with *Leishmani donovani*. *Cell Immunol.* **85,** 201.

KIRKPATRICK, C.E., FARRELL, J.P., WARNER, J.F. & DENNERT, G. (1985) Participation of natural killer cells in the recovery of mice from visceral leishmaniasis. *Cell. Immunol.* **92,** 163.

MERINO, F. & CRUZ, I. (1984) Natural killer activity in experimental cutaneous leishmaniasis. *Int. Arch. All. App. Immunol.* **73,** 347.

MOWAT, A. MCI., TAIT, R.C., MACKENZIE, S., DAVIES, M.D.J. & PARROTT, D.M.V. (1983) Analysis of natural killer effector and suppressor activity by intraepithelial lymphocytes from mouse small intestine. *Clin. exp. Immunol.* **52,** 191.

OMATA, Y., SAKURAI, H., IZUMO, M., SAITO, A. & SUZUKI, N. (1984) Interferon production in non-immune and immune mice after Toxoplasma infection. *Jap. J. Vet. Sci.* **46,** 155.

QUAN, P-C., RAGER-ZISMAN, B., WITTNER, B., TANOWITZ, M. & LAWRENCE, H. (1983) Interferon and natural killer cells in murine Chagas' disease. *J. Parasitol.* **69,** 1164.

RICCARDI, C., VOSE, B.M. & HERBERMAN, R.B. (1983) Modulation of IL-2-dependent growth of mouse NK cells by interferon and T lymphocytes. *J. Immunol.* **130,** 228.

SHARMA, S.D., HOFFLIN, J.M. & REMINGTON, J.S. (1985) *In vivo* recombinant interleukin 2 administration enhances survival against a lethal challenge with *Toxoplasma gondii*. *J. Immunol.* **135,** 4160.