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The role of natural killer cells in resistance to coccidiosis: investigations in a murine model

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SUMMARY

Natural killer (NK) activity, detected by the lysis of Yac-1 target cells, was examined in splenic and mesenteric lymph node (MLN) cells throughout the course of infection with *Eimeria vermiformis* in BALB/c and C57Bl/6 (B6) mice. These strains are, respectively, relatively resistant and susceptible to primary infections, which render them equally, and completely, resistant to challenge. Resting levels of NK activity were higher in B6 than in BALB/c, and B6 responded earlier in the course of infection than BALB/c, but splenic peak values were higher in BALB/c; the pattern of response in MLN cells was similar in both strains, but the peak was higher in BALB/c. At the time (7 days p.i.) of peak NK response in BALB/c mice there was, depending upon the choice of NK-resistant/lymphokine-activated killer (LAK)-sensitive target cells, either little ($P388D_1$), or no (P815) splenic LAK activity. Challenge of immunized BALB/c mice did not evoke a detectable NK response. Although the higher NK activity in BALB/c mice correlated with greater control of primary infection, depletion of NK activity (demonstrated in splenic cells) *in vivo* by treatment with anti-asialo GM1 antibodies did not greatly affect the course of infection. Furthermore, this treatment did not augment the exacerbation of infection produced by treatment with anti-interferon-gamma (IFN-γ) MoAb, indicating that, at least in this system, NK cells are not a fundamentally important source of this controlling cytokine of eimerian infections. The results suggest that NK cells may not greatly influence the outcome of coccidial infections.

Keywords coccidiosis *Eimeria vermiformis* immunity natural killer cells

INTRODUCTION

Natural killer (NK) and lymphokine-activated killer (LAK) activities have been implicated in limiting the growth of tumours, allograft rejection, graft-*versus*-host responses, the development of autoimmune diseases and immunoregulation; they are also stimulated in the initial phases of infection with a variety of microorganisms, and are thought to be involved in their control [1,2]. These antimicrobial effects could be mediated by a variety of mechanisms, including the secretion of immunoregulatory cytokines, the lysis of parasitized host cells and, *via* interactions with T cells, direct inhibition of the growth of microorganisms [3].

The evidence for the participation of NK/LAK cells in controlling infections with intracellular protozoa has been conflicting: for example, in malaria and babesiosis there have been claims both for [4] and against [5,6] such a role, and this has been ascribed to them in leishmaniasis [7] but not toxoplasmosis [8]. More recently, however, it has been shown that LAK cells can lyse host cells infected with *Toxoplasma gondii* [9], and that this parasite can activate NK cells to produce interferon-gamma (IFN-γ) [10], a cytokine able to control infection, both by exerting an antimicrobial effect and by regulating T cell development towards a protective Th1 response, e.g. in *Leishmania major* infections [11]. As evidence that NK/LAK cells have a role in resistance to intracellular protozoan infections is increasing, it seemed logical to examine their involvement in coccidiosis. This economically important disease occurs in a variety of domestic animals, and is caused by intracellular protozoa of the genus *Eimeria*. Invasion and development of the parasite occurs mainly in the epithelial cells of the intestinal mucosa. Not only are eimerian parasites of direct relevance to agriculture, particularly poultry production, but they also provide useful models of infection with related organisms, such as *Cryptosporidium* spp. [12], that are of clinical importance in humans. In avian coccidiosis there have been reports of changes in the NK activities of intestinal intraepithelial lymphocytes (I-IEL) and splenic cells in response to primary and secondary infection, and an increase in the numbers of I-IEL that express the asialo-GM1 antigen, after challenge, [13]. Additionally, with some, but not all, assays, NK activity in genetically different inbred strains of

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chickens [14] can be correlated with resistance/susceptibility to disease [15].

In an attempt to clarify the position, we have examined NK and LAK (hitherto not investigated) activities in a murine model of coccidiosis, utilizing the marked variation in susceptibility to infection that is evident in different strains of mice [16–19], and an antibody (anti-asialo-GM1) capable of depleting NK activity *in vivo*. In mice with a 'resistant' phenotype, oocyst production in primary infections with several species of the parasite is greatly reduced in comparison with that seen in 'susceptible' mice [16,17,19]. Our investigations of infection with *Eimeria vermiformis* [18], a parasite of the small intestine, in resistant BALB, and susceptible C57Bl, mice have shown that asexual cycling in a primary infection is curtailed in the former [20] and that the difference between the strains in their immune responses to the parasite is largely temporal and not qualitative [21]; both strains become equally resistant to attempted reinfection. We have also shown that $CD4^+$ lymphocytes are effective in controlling primary infections, without the participation of an inflammatory response (reviewed in [22]), and that IFN-γ plays an important role in this process [23] and in resistance to infection with *E. pragensis* (parasitic in the large intestine) [24].

MATERIALS AND METHODS

Mice

Inbred female BALB/c and C57Bl/6J (B6) mice were obtained from Harlan Olac Ltd (Bicester, UK), for use at 7–8 weeks of age. They were kept in isolated premises, coccidia-free until inoculated with oocysts, as before [23].

Parasite

Eimeria vermiformis was originally obtained from K. S. Todd (University of Illinois), and has been maintained in this laboratory for approximately 10 years. Methods for its propagation and for measuring infection by determining the output of oocysts throughout patency in 24-h collections of faeces from individually caged mice have been described previously [18]; faeces were monitored until two consecutive samples were negative. Infections with *E. vermiformis* were induced by inoculating 10^3 sporulated oocysts into the oesophagus.

Reagents

Rabbit polyclonal anti-asialo-GM1 antibody (α-AGM1) was obtained from Wako Chemicals (Neussl, Germany). IgG1 rat anti-IFN-γ MoAb (α-IFN-γ MoAb), produced by Lee Biomolecular Research Inc., was obtained from Stratech Scientific Ltd (London, UK). Purified rat IgG and rabbit immunoglobulin (Sigma Chemical Co., Poole, UK) served as controls. Poly-inosinic poly-cytidilic acid (poly IC), used to augment NK activity, was obtained from Sigma.

The NK-susceptible Yac-1 lymphoma (ATCC no. T1B 160) and the NK-resistant, LAK-susceptible $P388D_1$ cell line (ATCC no. CCL 46) were obtained from Flow Labs (Rickmansworth, UK); the NK-resistant, LAK-susceptible cell line, P815 (ATCC no. T1B 64), came from ATCC (Rockville, MD). All these cell lines were cultured as stationary suspensions in the appropriate media (ATCC catalogue of cell lines and hybridomas, 6th edn, 1988).

Assays of NK and LAK activity

Spleens and mesenteric lymph nodes (MLN) were removed from the mice and treated individually, by rubbing the tissues through a nylon sieve with a syringe-plunger, to provide single-cell suspensions in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 50 μg/ml streptomycin. Erythrocytes were removed from the splenic cell suspensions by hypotonic 'flash' lysis. These 'effector' cells were then mixed with target cells in a standard 4-h chromium-release assay to determine their lytic potential. Suspensions of target cells, prepared from the maintenance cultures in log phase growth, were labelled by incubation with ^{51}Cr Na_2CrO_4 (Amersham International, Aylesbury, UK) (100 μCi/5 $\times$ 10^6 cells per 500 μl) for 1 h at 37°C, with frequent mixing. They were then washed three times, and, to minimize spontaneous release of label during the assay, incubated for a further 1 h, followed by two more washes. Effector cells were mixed with 10^4 labelled target cells in 200 μl medium in U-form microtitre plates (Nunc, GIBCO) to provide a range of ratios, each being set up in quadruplicate. Values for spontaneous and complete release of label were obtained from eight-well replicates of target cells alone, and target cells with Triton X100 (1·25%), respectively. Plates were incubated for 4 h at 37°C in 5% CO_2, after which they were centrifuged (500 *g* for 5 min) and the radioactivity in 100 μl of each supernate was measured (gamma counter; Packard Instruments, Caversham, UK). The per cent specific cytotoxic activity was calculated from:

$$\frac{\text{test ct/min} - \text{spontaneous ct/min}}{\text{maximum ct/min} - \text{spontaneous ct/min}} \times 100$$

Spontaneous release of label (in ct/min) usually varied between 5% and 15% of the maximum value; plates in which the spontaneous ct/min was >20% of the maximum value were rejected. The results of preliminary experiments with cells obtained from normal, untreated, and PBS- or poly IC-injected mice (see below) indicated that effector : target ratios of 200 : 1 to 25 : 1 were satisfactory, and these were used for the experiments on *E. vermiformis*-infected mice.

In vivo *induction of NK cell activity*

To establish the assay, provide a positive control in test systems, and to confirm the efficacy of α-AGM1 in depleting NK cells, poly IC was used as an inducer of NK cell activity [25]. It was injected (15 μg/g body weight in 500 μl PBS pH 7·2) intraperitoneally 18 h before the mice were killed for the assays, a standard protocol that was shown to be effective in preliminary experiments.

In vivo *depletion of NK cells and/or IFN-γ*

To deplete NK cells *in vivo*, three injections, each of 200 μg of α-AGM1 in 200 μl PBS, were given intravenously. The first injection was 2 h before the mice were inoculated with oocysts of *E. vermiformis*, and the second and third on days 4 and 8 after inoculation (p.i.), respectively. To neutralize endogenous IFN-γ, 50 U of α-IFN-γ MoAb in 200 μl PBS were injected intravenously 2 h before the initiation of infection with *E. vermiformis*. This dose, determined from previous titration [23], was half of the minimum amount capable of exerting a maximum exacerbating effect on infection (measured by the production of oocysts in the faeces) in BALB/c mice. Where both antibodies were used they were given as a mixture in 200 μl

PBS. Controls received equivalent amounts of rabbit immunoglobulin or rat IgG or PBS.

Design of experiments

In an initial experiment the splenic cells of uninfected mice that had been injected with poly IC were used to standardize the NK cell assay. The assay was then used to determine the effect of infection with *E. vermiformis* on NK cell activity in 'resistant' BALB/c and 'susceptible' B6 mice. LAK cell activity was investigated in infected BALB/c. Mice of this strain were also used in an examination of the effects of depletion of $AGM1^+$ cells, either alone, or in combination with neutralization of IFN-γ, on the course of infection with *E. vermiformis*. The numbers of mice in different groups are given in the tables and figures in Results; Student's *t*-test was used in the statistical analyses.

RESULTS

NK cell activity during the course of infection with E. vermiformis *in 'resistant' BALB/c and 'susceptible' B6 mice*

NK cell activity was assayed in the spleen and MLN, with Yac-1 cells as targets before, and at intervals during, the course of infection with *E. vermiformis* in BALB/c and B6 mice. The results are presented in Fig. 1 (effector : target = 100 : 1). Far more activity was detected in splenic than in MLN cells. In the spleen, the resting level was higher in B6 mice than in BALB/c, and infection of B6 resulted in a significant increase in activity from day 3 until day 9, after which (days 11 and 13) there was a return to the resting value. In contrast, the increase was not seen in BALB/c until day 5, but the peak level was higher ($P < 0{\cdot}001$) than in the B6 mice (day 9). On day 13 the value in BALB/c was similar to that of B6 mice.

In the MLNC suspensions there was no detectable NK activity in uninfected mice, but there was a response to infection in both strains. As with the spleen cells, NK activity attained a lower peak ($P < 0{\cdot}002$) in B6 than in BALB/c mice. In other respects, although NK activity was detected earlier in B6, the pattern was similar in the two strains, with significant increases from the resting levels on days 5, 7 and 9, peaking on day 7.

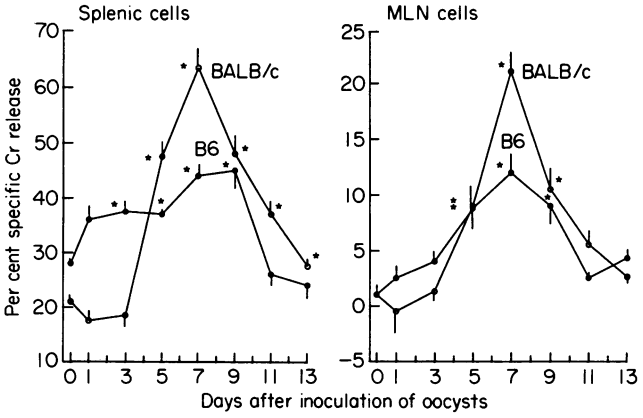

Fig. 1. Natural killer (NK) cell activity in suspensions of splenic and mesenteric lymph node (MLN) cells during the course of infection with *Eimeria vermiformis* (10^3 oocysts inoculated on day 0). Yac-1 cells were used as targets (effector : target = 100 : 1). The values are means for groups of 5–10 mice; within the strain, * indicates that they differ significantly ($P < 0{\cdot}0001$ to $< 0{\cdot}03$) from the resting level. The values obtained from mice killed 18 h after the injection of poly-inosinic poly-cytidilic acid (poly IC), assayed in the same experiment, were 59% and 7% for spleen and MLN, respectively, in BALB/c, and 54% and 5% for B6.

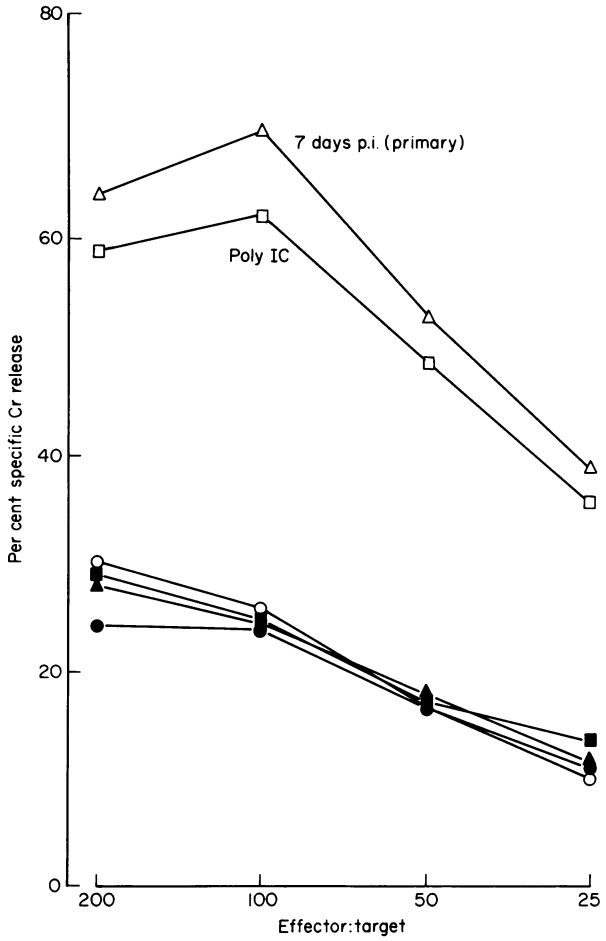

Fig. 2. Natural killer (NK) cell activity in suspensions of splenic cells of BALB/c mice, assayed with Yac-1 cells as targets (in differing ratios of effector : target). Mice had been primed with 10^3 oocysts of *Eimeria vermiformis* and were challenged with 10^3 oocysts 30 days later. Spleens were obtained from control untreated (○) mice, primed unchallenged (▲) mice, and those killed 1 (●) or 3 (■) days after challenge; also examined were mice killed on day 7 post infection with *E. vermiformis* (△), or 18 h after treatment with poly-inosinic poly-cytidilic acid (poly IC) (□). Values are means obtained from groups of five mice.

In both splenic and MLN cell suspensions the peak values in infected mice were equal to, or greater than, those measured 18 h after the injection of poly IC, which were (% specific Cr release): BALB/c, spleen 58·7 ± 3·5, MLN 7·0 ± 1·4; B6, spleen 54·3 ± 2·4, MLN 5·3 ± 0·8.

The response to a challenge inoculum of oocysts, of mice that had been immunized by previous infection, was examined only in BALB/c mice (both strains of mice being equally resistant to reinfection). Values for spleen cells taken 1 or 3 days after challenge are shown in Fig. 2, together with data (obtained contemporaneously) from groups of mice primed but unchallenged, or killed on day 7 after a primary infection, and

Table 1. Natural, and lymphokine-activated, killer activity in splenic cells from BALB/c mice infected with *Eimeria vermiformis*

		Mean specific ^{51}Cr-release (%)*					
		NK/LAK-sensitive cells		NK-resistant/LAK-sensitive cells			
		Yac-1		$P388D_1$		P815	
Experiment	Treatment of mice†	200:1‡	100:1	200:1	100:1	200:1	100:1
1	None	$22{\cdot}8^a$	$17{\cdot}4^a$	$-1{\cdot}9^a$	$-1{\cdot}0^a$		
	7 days p.i. *E. vermiformis*	$64{\cdot}7^b$	$63{\cdot}0^b$	$11{\cdot}6^b$	$7{\cdot}9^b$		
	11 days p.i. *E. vermiformis*	$42{\cdot}0^c$	$34{\cdot}7^c$	$1{\cdot}1^a$	$0{\cdot}7^a$		
	Poly IC	$69{\cdot}1^b$	$59{\cdot}6^b$	$18{\cdot}8^b$	$15{\cdot}9^b$		
2	None	$38{\cdot}0^a$	$31{\cdot}6^a$	$0{\cdot}0^a$	$0{\cdot}0^a$	0·1	−0·4
	7 days p.i. *E. vermiformis*	$61{\cdot}7^b$	$64{\cdot}7^b$	$2{\cdot}9^b$	$4{\cdot}6^b$	−0·4	−0·4
	Poly IC	$61{\cdot}0^b$	$58{\cdot}3^b$	$5{\cdot}2^c$	$4{\cdot}6^b$	−1·6	−0·3

* Values are means±s.e.m. obtained from three individual spleens (experiment 1) or from four pools, each made up of two spleens, i.e. a total of eight mice (experiment 2). Within experiments and within columns, values with different superscript letters differ significantly from each other ($P < 0{\cdot}05$). All s.e.m. were < 10% of the mean values presented.

† Mice were inoculated with 10^3 sporulated oocysts of *E. vermiformis* and killed 7 or 11 days later. Positive controls were injected with poly-inosinic poly-cytidilic acid (poly IC) (15 μg/g body weight in 500 μl PBS, intraperitoneally) 18 h before sampling.

‡ Ratio of effector : target cells.

positive (poly IC-injected) and negative (untreated, normal) controls. The high NK activity of splenic cell suspensions from 7 day post-primary infected mice was confirmed, but there was no response to the challenge inoculum. Similar results were obtained with MLNC (data not shown).

LAK cell activity in BALB/c mice infected with E. vermiformis

Suspensions of splenic cells from BALB/c mice, prepared on days 7 and 11 p.i. with *E.vermiformis*, were examined for LAK, as opposed to NK, activity by comparing the results obtained with different target cells. The Yac-1 line is sensitive to both NK and LAK activity, whereas the $P388D_1$ and P815 cells are NK-resistant and LAK-sensitive. The data, obtained with splenic cells from two experiments (Table 1), show that the levels of activity with Yac-1 cells were as anticipated, and those recorded with the NK-resistant, LAK-sensitive cells were comparatively low. Only when $P388D_1$ cells were cultured with spleen cells taken on day 7 p.i. was there significant lysis.

No activity was detectable in MLNC taken on day 7 p.i. and tested against the NK-resistant, $P388D_1$ cell line at an effector : target ratio of 200 : 1 (data not shown).

Course of infection with E. vermiformis *in mice treated with anti-asialo GM1 antibody*

The effectiveness of treatment with α-AGM1 in depleting NK cell activity was confirmed by the results of a preliminary experiment with splenic cells, shown in Table 2. The results of two further experiments, designed to examine the effects of depleting mice of cells capable of exerting NK activity on the course of infection with *E. vermiformis*, are given in Table 3.

Table 2. Natural killer cell activity in splenic cells of poly-inosinic poly-cytidilic acid (poly IC)-injected, anti-asialo GM1 antibody-treated, BALB/c mice

	Mean specific ^{51}Cr-release (%)*			
	Effector : target (Yac-1) ratio			
Treatment of mice†	200:1	100:1	50:1	25:1
α-AGM1 + poly IC	$0{\cdot}8 \pm 0{\cdot}9^a$	$0{\cdot}2 \pm 0{\cdot}5^a$	$0{\cdot}7 \pm 1{\cdot}2^a$	$0{\cdot}0 \pm 0{\cdot}8^a$
Rabbit IgG + poly IC	$55{\cdot}5 \pm 3{\cdot}5^b$	$47{\cdot}1 \pm 3{\cdot}4^b$	$33{\cdot}6 \pm 2{\cdot}8^b$	$20{\cdot}9 \pm 1{\cdot}4^b$
Poly IC	$61{\cdot}0 \pm 5{\cdot}1^b$	$53{\cdot}7 \pm 4{\cdot}5^b$	$39{\cdot}2 \pm 3{\cdot}0^b$	$28{\cdot}0 \pm 3{\cdot}5^b$
None	$22{\cdot}2 \pm 1{\cdot}3^c$	$21{\cdot}8 \pm 2{\cdot}4^c$	$14{\cdot}8 \pm 0{\cdot}6^c$	$11{\cdot}6 \pm 1{\cdot}1^c$

* Values are means ± s.e.m. obtained from three individual spleens; within columns, those that have different superscript letters differ significantly from each other ($P < 0{\cdot}05$).

† Three injections, each of 200 μg in 200 μl of PBS, of α-AGM1 or rabbit IgG were given intravenously 4 days apart. Poly IC (15 μg/g body weight in 500 μl) was given intraperitoneally at the time of the third injection of antibody, and all the mice were killed for assay 18 h later.

Table 3. Course of infection with *Eimeria vermiformis* in BALB/c mice treated with anti-asialo GM1 antibody and/or anti-IFN-γ MoAb

Experiment	Treatment of mice†	Infection with *E. vermiformis**	
		Numbers of oocysts produced (10^6)	Duration of patency (days)
1	α-asialo GM1	6·9 ± 0·4	5·2 ± 0·3
	Rabbit immunoglobulin	6·0 ± 0·8	5·5 ± 0·3
	None	7·0 ± 1·4	5·5 ± 0·2
2	α-asialo GM1 and α-IFN-γ MoAb	12·0 ± 1·4[a]	6·9 ± 0·2[a]
	α-asialo GM1	5·0 ± 1·0[b]	6·0 ± 0·2[b]
	α-IFN-γ MoAb	10·7 ± 1·6[a]	6·0 ± 0·2[b]
	Rabbit immunoglobulin and rat immunoglobulin	4·1 ± 0·8[b]	5·0 ± 0·0[c]
	PBS	4·4 ± 0·5[b]	5·2 ± 0·1[c]

*Values are means ± s.e.m. for groups of eight mice and refer to the total numbers of oocysts passed during patency. In experiment 1 the values for the groups did not differ significantly; in experiment 2, within columns, values with different superscript letters differ significantly from each other ($P < 0·05$).

† Anti-asialo GM1 treatment consisted of three injections, each of 200 μg antibody in 0·2 ml, given 2 h before, and on days 4 and 8 after, the inoculation of oocysts; the relevant control groups received equivalent treatment with rabbit immunoglobulin. Anti-IFN-γ MoAb was given as a single injection of 50 neutralizing units in 0·2 ml, 2 h before the inoculation of oocysts, and the control mice received 0·8 μg of rat immunoglobulin. In experiment 2, where treatments were combined, the doses were given as a mixture, and a control group, injected with diluent (PBS), according to the same schedule as the α-asialo GM1 treatment, was included. Infections with *E. vermiformis* were induced by inoculating 10^3 sporulated oocysts into the oesophagus.

The data show that treatment with α-AGM1 antibody did not cause an enhanced replication of the parasite, although the duration of patency was increased in one of the experiments. A possible contribution of asialo-GM1-bearing cells to resistance, mediated by the secretion of IFN-γ, was investigated by combining α-AGM1 treatment with neutralization of endogenous IFN-γ. To ensure maximum sensitivity, the dose of α-IFN-γ MoAb was selected to be suboptimal in its effect on the course of infection with *E. vermiformis*. The results (Table 3, experiment 2) show the expected effect of α-IFN-γ MoAb in increasing the replication of *E. vermiformis* and the duration of patency. Combining the IFN-γ neutralizing antibody with the α-AGM1 treatment did not cause a further increase in oocyst production, although there was an extension of patency.

DISCUSSION

Infection with *E. vermiformis* induced an increase in the NK cell activity of cell suspensions prepared from the spleens and MLN of BALB/c and B6 mice. The pattern of response was similar to that reported for mice that had been orally infected with *T. gondii* [8], but differed from the earlier one generally found in most other protozoan infections in mice, e.g. with *Plasmodium* spp. [26], *Babesia microti* [6], *Trypanosoma musculi* [27] and *L. donovani* [7]. This difference may be associated with the site of development of the parasites, since parenteral inoculation with *T. gondii* also results in an early increase in NK cell activities [28,29]. Not seen with *E. vermiformis* infection was a reduction below normal values, reported to follow the peak in some other protozoan infections [6,26–29]. In chickens infected with *E. acervulina* or *E. maxima*, the major characteristic of the NK response in splenic and I-IEL cells to a primary infection appears to be a lowering of the normal values in the first week, followed by a return to normal, or slightly elevated, levels [13]. The administration of a secondary inoculum of *E. maxima*, however, resulted in an increased activity in splenic and I-IEL cells, on days 1 and 3 after challenge. This is in contrast to our results, and those for mice orally inoculated with *T. gondii* [8], where there was no response to a secondary inoculum. This difference is surprising in view of the resemblances in the host–parasite relationship evident with *E. maxima* and *E. vermiformis*. In both cases a primary infection of small magnitude induces complete resistance to reinfection, and this is expressed within 2–3 days after the challenge inoculum. It could be argued that such a response is likely to be highly localized, and therefore evident only in cells in close proximity to the site of entry of the sporozoites, but in the case of *E. maxima* a response in the spleen was reported [13].

Peak NK activity was detected at a time in infection when there is an intense cellular immune response [21] and considerable release of cytokines [30], yet LAK activity appeared to be comparatively low, and evident with only one of the two lines of NK-resistant target cells used. The apparently low activity of LAK cells may well reflect the use of inappropriate target cells, as has been shown for *T. gondii*-infected mice [9].

Mice of the BALB background are more resistant to primary infection than C57Bl mice, and were found to respond with higher levels of NK activity in both the spleen and MLN; in the case of the spleen, the peak in NK activity occurred earlier, correlating with other (lymphocyte-mediated) measures of responsiveness to infection [21]. Resting levels were, however, higher in B6 than in BALB/c mice, and the initial response of B6 was earlier. Thus, there is no clear-cut correlation between resistance to infection and NK activity, which is similar to findings reported for chickens [13].

Further indication that NK cell activity is not a major

component of the expression of resistance to *E. vermiformis* is provided by the failure of treatment with α-AGM1 antibody to influence the course of infection (Table 3). This applied when the antibody was used alone, and in conjunction with sub-optimal neutralization of IFN-γ, the latter result indicating that NK cells were not a vital source of this cytokine, known to be active in defence against *Eimeria* spp. [23,24]. There are several recent reports of similar failure of abrogation of NK cell activity alone to exacerbate infection with a variety of infectious organisms [31], including the coccidium *Cryptosporidium parvum* [32]. Additionally, NK cells seem to be an unlikely source of the IFN-γ that regulates early visceral leishmaniasis in the mouse [33], although they are thought to contribute to the control of listeriosis [34] and cryptosporidiosis [35] by this means. The outcome of our experiments is, therefore, consistent with data relating to other intracellular protozoa. Evidence that α-AGM1 antibody depleted NK cell activity in our experiments was obtained from assays on splenic cells, and the possibility has to be considered that NK cells at the site of infection, i.e. those present in the gut, might not have been affected to the same extent. For example, it has been reported that similar treatment, whilst depleting circulating NK cells, had a lesser effect on cells in the uterine epithelium [36]. However, the results of other experiments (this laboratory, unpublished) have also suggested a relatively minor role for NK cells in controlling eimerian infections: young *bg/bg* (beige, NK-defective) mice produced significantly fewer oocysts of *E. vermiformis* than B6, but this difference was not evident in older mice.

Although the results reported here indicate that the activity of NK cells is not necessary for the expression of resistance to this eimerian infection of the small intestine, it is likely that, in the intact animal, it contributes to it, more probably by immunoregulation rather than cytolysis. Activity was undoubtedly induced by infection, and the consequent release of cytokines would have influenced the network of immune responses, both innate and acquired.

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