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Interaction Between Murine Natural Killer Cells and Trypanosomes of Different Species

JULIA W. ALBRIGHT,[1]* FRANK M. HATCHER,[2] AND JOSEPH F. ALBRIGHT[1]

Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809,[1] and Department of Microbiology, Meharry Medical College, Nashville, Tennessee 37208[2]

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The involvement of natural killer (NK) cells in the immunological resistance of mice to murine-specific *Trypanosoma musculi* was evaluated. Murine NK cells were found to be unable to kill or inhibit *T. musculi* or to protect recipients from infection. In addition, the ability of spleen cells from normal mice and from mice on day 3 of *T. musculi* infection, at the time of maximum NK augmentation, to kill *Trypanosoma cruzi* and *Trypanosoma lewisi* was evaluated. Spleen cells from normal mice displayed significant killing of both *T. cruzi* and *T. lewisi*. Furthermore, augmented spleen cells from *T. musculi*-infected mice were considerably more effective than normal spleen cells in killing both *T. cruzi* and *T. lewisi*. The activity of NK cells toward YAC-1 tumor target cells was inhibited in a dose-dependent fashion by either live *T. musculi* or extracts of *T. musculi*, but not by extracts of rat-specific *T. lewisi*. The results suggest that well-adapted protozoan parasites may be nonsusceptible to the natural cell-mediated resistance mechanisms of their hosts. Their nonsusceptibility could result from the ability to elaborate substances that either inactivate NK cells or block NK cell interaction with complementary sites on the parasite surface.

A subset of lymphocytes, termed natural killer (NK) cells, along with another, closely related set of lymphocytes known as naturally cytotoxic (NC) cells, expresses spontaneous cytotoxicity toward a variety of tumor cells (16, 26) as well as toward certain virus-infected (6, 19) and apparently normal target cells (7, 11, 22, 24, 27). In addition, evidence has been accumulating that NK cells may inhibit or kill various protozoan parasites (8, 12, 14, 15). This concept is appealing and has received some support from clinical investigations (23). To date, however, there has appeared only one report of direct effects of NK cells on a protozoan parasite (13) in which it was demonstrated that both the epimastigote (generative) and trypomastigote (mature) forms of *Trypanosoma cruzi* may be killed by murine NK cells.

In this report we present results concerning the ability of murine NK cells to kill the rodent trypanosomes *Trypanosoma musculi* and *Trypanosoma lewisi* and confirm the susceptibility of *T. cruzi* to murine NK cells. The rodent trypanosomes are highly host specific, *T. lewisi* for rats and *T. musculi* for mice. The rodent trypanosomes are extracellular parasites. In contrast, *T. cruzi* is not rigorously host specific and is a predominantly intracellular parasite.

After the experimental inoculation of any of a variety of strains of mice with *T. musculi*, there is a rapid elevation of NK activity in the spleen, bone marrow, peritoneal exudate, and peripheral blood (5). NK activity is maximal on days 3 and 4 after parasite inoculation and then declines rapidly to a plateau of subnormal activity that persists for at least 1 month. By day 3 after parasite inoculation, the elevation of NK activity above the normal level is three- to fourfold in the spleen and six- to eightfold in bone marrow and peritoneal fluid. As judged by several accepted criteria (adherence, treatment with anti-NK 1.2 serum and anti-Thy 1.2 serum, and adsorption to tumor cell monolayers), the elevated activity in *T. musculi*-infected mice is attributable to an increase in numbers of NK cells (3). We have studied, therefore, the interactions between trypanosomes and NK cells from both normal and 3-day-infected donor mice.

MATERIALS AND METHODS

Animals. Mice of the C3H/Anf Cum strain (Cumberland View Farms, Clinton, Tenn.) and of the C57BL/6J strain (Jackson Laboratory, Bar Harbor, Maine) were employed. The ages of the mice ranged from 3 to 6 months. The mice were maintained in a conventional animal facility and allowed free access to food and water.

Trypanosomes. The origin and maintenance of the stocks of *T. cruzi*, *T. lewisi*, and *T. musculi* employed in this investigation have been described previously (2, 4, 12). The trypanosomes were from mixed populations, i.e., not clones. For use in certain experiments, *T. musculi* was harvested from irradiated mice (600 rads) on day 6 after parasite inoculation. Such parasites lack surface-bound mouse proteins, as shown by their failure to bind fluorescein-labeled goat or rabbit antisera against mouse serum proteins or mouse immunoglobulins (unpublished data). Our procedure was patterned after that developed for *T. lewisi* (9).

Trypanosome extract. Extracts of *T. lewisi* and *T. musculi* were prepared by repeated (six times), rapid freezing (dry ice-acetone bath) and thawing of suspensions of trypanosomes in phosphate-buffered (pH 7.2) salt solution. The supernatant collected after centrifugation (1,200 × *g* for 30 min) was considered an extract and stored in small portions at −70°C.

Spleen cell transfer. The spleens were removed from either normal or 3-day-infected donor mice and teased in RPMI 1640. The dispersed cells were washed by centrifugation and counted, and 5×10^7 cells (about one-third of a spleen) were injected intravenously into irradiated (500 rads) recipient mice. The latter were irradiated by exposure to a ^{60}Co gamma source at the rate of 96 rads/min about 3 h before the injection of donor spleen cells.

Assessment of NK activity. For certain experiments the appropriate target cells were YAC-1 tumor cells. Our use of

* Corresponding author.

these tumor cells for assessing NK activity by release of ^{51}Cr has been described elsewhere (3, 5) and is patterned after the conventional procedure. Evaluation of NK activity toward trypanosomes was performed microscopically. Spleen cells and freshly harvested bloodstream trypanosomes were mixed together in various ratios. These mixtures were cultured in six-well culture dishes (Costar; Microbiological Associates, Walkersville, Md.) for periods of up to 18 h. In some experiments a miniature version (12) of the culture procedure was adopted in which 96-well culture vessels were employed. At intervals, representative cultures were terminated and the number of viable trypanosomes was counted. Counting was performed under phase microscopy with the aid of thin hemacytometers. Viability was evaluated by evidence of even slight motility and lack of change in morphology, as judged by phase microscopy at 200× or 400× magnification.

RESULTS

Experiments were performed to determine whether or not spleen cells from normal or 3-day-infected donor mice were capable of inactivating or killing *T. musculi*. Both C3H and C57BL/6 spleen cells provided similar results. Target trypanosomes were collected from both normal and irradiated mice (the latter lacking surface-bound mouse proteins).

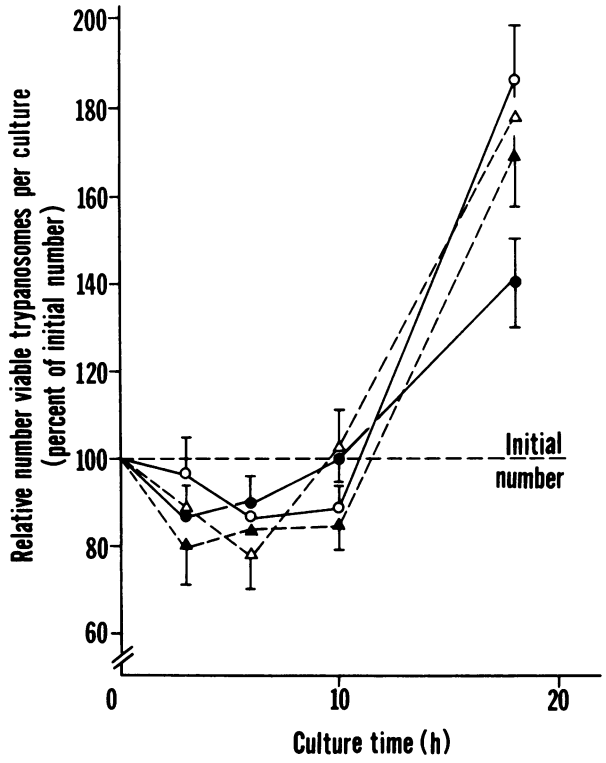

FIG. 1. Assessment of direct NK cytotoxicity of spleen cells from strain C57BL/6 normal mice or mice infected for 3 days (with *T. musculi*) on target trypanosomes. *T. musculi* was isolated from either unirradiated (solid lines) or irradiated (dashed lines) donor mice, mixed with spleen cells from either normal (open symbols) or 3-day-infected (closed symbols) donor mice, and cultured at a ratio of spleen cells to target trypanosomes of 100:1. Survival of trypanosomes was assessed by counting under a phase microscope. Each point represents the mean of 12 samples from four replicate experiments. Vertical bars are 1 standard error.

TABLE 1. Comparison of the susceptibility of trypanosomes of different species to the cytotoxicity of spleen cells from mice with normal and augmented NK activity

Donor of spleen cells[a]	E/T[b]	% Destruction[c] of trypanosomes at risk		
		T. cruzi	*T. lewisi*	*T. musculi*
Normal	10:1	5.9	4.1	0.0
	50:1	9.4	11.8	0.0
T. musculi infected	10:1	18.1	0.0	0.0
	50:1	30.3	16.7	0.0

[a] C57BL/6 female mice, 10 to 16 weeks old, were either normal or inoculated 3 days previously with 5×10^5 blood forms of *T. musculi*. Spleen cells were pooled from three similarly treated mice.

[b] Spleen cells (2×10^5 or 1×10^6) were cultured with 2×10^4 target trypanosomes for 6 h. E/T, effector cells/target cells.

[c] Percent destruction of cultured parasites was determined by counting viable trypanosomes. The results are the means of duplicate cultures from one of three experiments.

Mixtures were prepared with various ratios of effector spleen cells to target trypanosomes, ranging from 100:1 to 20:1. The results of this set of experiments are depicted in Fig. 1. There appeared to be a slight decline in the proportion of surviving parasites over the first 3 h of culture, followed by a stable plateau of some 3 to 7 h duration, and finally a rapid rise in the number of trypanosomes, reflecting their propensity for growth in vitro under these conditions (1). There was no difference in the survival of trypanosomes from either irradiated or unirradiated donor mice. Nor was there any difference in parasite survival in the presence of spleen cells from normal and infected donors, even though the latter were almost three times more active in killing a standard YAC-1 tumor target cell (5).

The susceptibility of the three species of trypanosomes to killing by murine NK cells was assessed in a series of experiments in which the different trypanosomes were incubated concurrently with portions of the same preparation of spleen cells. The latter were obtained both from normal mice and mice sacrificed on day 3 of a *T. musculi* infection. The results of a representative experiment are shown in Table 1. Both *T. cruzi* and *T. lewisi* were attacked by mouse spleen cells. The proportion of trypanosomes killed increased with an increase in the ratio of spleen cells to target trypanosomes. Furthermore, the augmented activity of spleen cells obtained from mice on day 3 of *T. musculi* infection was evident; in the case of target *T. cruzi* there was a 3-fold increase in cytotoxicity in comparison with spleen cells from normal mice and a 1.5-fold increase in the case of target *T. lewisi*. The results with respect to *T. musculi* were in sharp contrast. Spleen cells from normal or 3-day-infected mice did not attack *T. musculi*.

It has been demonstrated that substances elaborated by *T. musculi* are potent inhibitors of humoral immune responses (2, 4). It has not been determined whether *T. musculi* elaborates substances capable of inhibiting NK activity. To test this possibility, live, freshly isolated *T. musculi* was added to mixtures composed of NK-containing spleen cells and target YAC-1 tumor cells. Graded numbers of trypanosomes were added to mixtures prepared with either normal spleen cells or with spleen cells from 3-day-infected donors. The proportion of the ^{51}Cr label, affixed to YAC-1 cells, that was released during a 4-h incubation period was assessed as an index of target cell lysis (Table 2). At a concentration of 4

TABLE 2. Inhibition of murine NK activity by live *T. musculi*

Source of spleen cells[a]	No. of trypanosomes per culture	% YAC-1 cell lysis[b]
Normal	0	23.4 (± 2.2)
	4×10^5	14.6 (± 1.8)
	8×10^5	12.0 (± 1.9)
	4×10^6	6.9 (± 1.8)
	4×10^7	0
Infected	0	49.0 (± 4.8)
	4×10^5	43.7 (± 5.7)
	8×10^5	36.1 (± 5.3)
	4×10^6	25.9 (± 5.0)
	4×10^7	0

[a] Spleen cells from normal or 3-day-infected C57BL/6 mice.
[b] Effector to target cell ratio, 200:1. Numbers in parentheses are 1 standard error of the means of five to six replicate samples. The experiment was repeated several times with comparable results.

$\times 10^7$ trypanosomes per 1 ml of culture the lysis of target YAC-1 cells was completely inhibited. Lower numbers of trypanosomes caused less inhibition in a dose-dependent fashion. Toxicity or overcrowding of the trypanosome-containing cultures was evaluated by counting viable spleen cells upon termination of the cultures. The cell recovery from cultures containing 4×10^7 trypanosomes was slightly less than in the case of all other cultures, suggesting overcrowding in these cultures. The recovery of viable cells from all other trypanosome-containing cultures was quite similar to the controls lacking trypanosomes.

Crude extracts of *T. musculi* and *T. lewisi*, prepared by freezing and thawing, were tested for their ability to inhibit NK lysis of YAC-1 target cells. Graded concentrations of extract were added to mixtures of cells at the beginning of the 4-h incubation period. For this series of experiments spleen cells were collected from both normal C3H and C57BL/6 mice as well as from mice on day 3 of *T. musculi* infection. The inhibitory potency of these extracts is described in Table 3. A concentration of extract corresponding to 3.8×10^7 ($\log_{10}$ 7.6) *T. musculi* per culture reduced the NK activity of a normal spleen cell suspension to about one-third of the uninhibited activity. Similarly, the same concentration of *T. musculi* extract reduced the NK activity of cell suspensions from 3-day-infected mice to about 12% of the uninhibited level. Lower concentrations of extract were less inhibitory in a graded fashion. In contrast, similar concentrations of extract from *T. lewisi* were only slightly, if at all, inhibitory. The extracts of *T. lewisi* thus served as excellent control preparations.

T. musculi infections are established by proliferation of the reproductive, epimastigote forms of the parasite. By day 6 or so after parasite inoculation, most of the reproductive forms have disappeared, giving rise to the mature, nondividing, trypomastigote forms. Because this is the form in which virtually all of the parasites exist when immunological cure of the infection is initiated, it was important to determine whether or not NK cells might play a significant role in the elimination of infection.

Mice of both the C3H and C57BL/6 strains were inoculated with trypanosomes. On day 6 of infection these mice were irradiated (500 rads) and then injected with spleen cells from syngeneic donors; the latter were sacrificed on day 3 of infection at the time of the maximum level of NK activity. Recipient mice were provided with 5×10^7 donor spleen cells, a number that has been shown to transfer NK resistance to metastasis of tumor cells (10). Subsequently, the course of infection in the recipient animals was followed in parallel with the infection in control mice that were provided with normal spleen cells from uninfected donors. The course of parasitemia in these animals is illustrated in Fig. 2. It is apparent that there was no difference in the magnitude or duration of infection in mice that received either normal spleen cells or those which had substantially elevated NK activity. Control mice that were irradiated but not provided with spleen cells died with high parasitemia within 12 days after irradiation and infection.

DISCUSSION

We have found no evidence that murine NK cells are able to kill murine-specific *T. musculi*; nor, it appears, do NK cells play a major role in the cure of an infection. Even in the presence of spleen cells displaying heightened NK activity toward the conventional YAC-1 target cells, the trypanosomes displayed the typical growth profile that is characteristic of *T. musculi* cultured in vitro (1). In contrast, both *T. lewisi* and *T. cruzi* were killed spontaneously by mouse spleen cells, the magnitude of killing being greater in the case of augmented spleen cells from *T. musculi*-infected mice. This confirms earlier work with *T. cruzi* (12–14) in which formal proof of NK cytotoxicity was provided. One obvious explanation of the apparent resistance of *T. musculi* to NK cells is the effect on NK cells of the inhibitors which the parasites elaborate. The deliberate contamination of cultures of NK cells and target YAC-1 cells with live *T. musculi* showed how effective this inhibition may be. The number of contaminating trypanosomes required to inhibit by 50% the activity of NK cells in normal and in 3-day-infected spleen cell preparations was approximately 8×10^5 and 4×10^6, respectively. Extracts of *T. musucli* also inhibited NK

TABLE 3. Effects of extract of rodent trypanosomes on murine (strain C57BL/6) NK cell activity

Source of spleen cells[a]	Source of extract (trypanosome species)	Amt of extract per culture ($\log_{10}$ trypanosome equivalents)[b]	% of uninhibited YAC-1 cell lysis (mean ± SD)[c]
Normal		None	100
	T. musculi	6.6	79.3 (± 7.0)
	T. musculi	7.0	51.0 (± 10.5)
	T. musculi	7.6	33.7 (± 12.8)
	T. lewisi	7.0	80.7 (± 16.5)
	T. lewisi	7.6	92.0 (± 11.3)
Infected		None	100
	T. musculi	6.0	92.7 (± 3.7)
	T. musculi	6.6	86.7 (± 2.5)
	T. musculi	7.0	62.7 (± 8.0)
	T. musculi	7.6	11.8 (± 8.3)
	T. lewisi	6.6	90.0 (± 4.2)
	T. lewisi	7.0	79.3 (± 5.7)
	T. lewisi	7.6	77.0 (± 4.0)

[a] Mice were either normal or in day 3 of *T. musculi* infection; similar results were obtained with both C3H and C57BL/6 mice.
[b] Number of parasites corresponding to the dilution and volume of extract added per culture.
[c] Means and standard deviation from three replicate experiments and a total of eight to nine cultures per point; each experiment was performed with a different extract preparation.

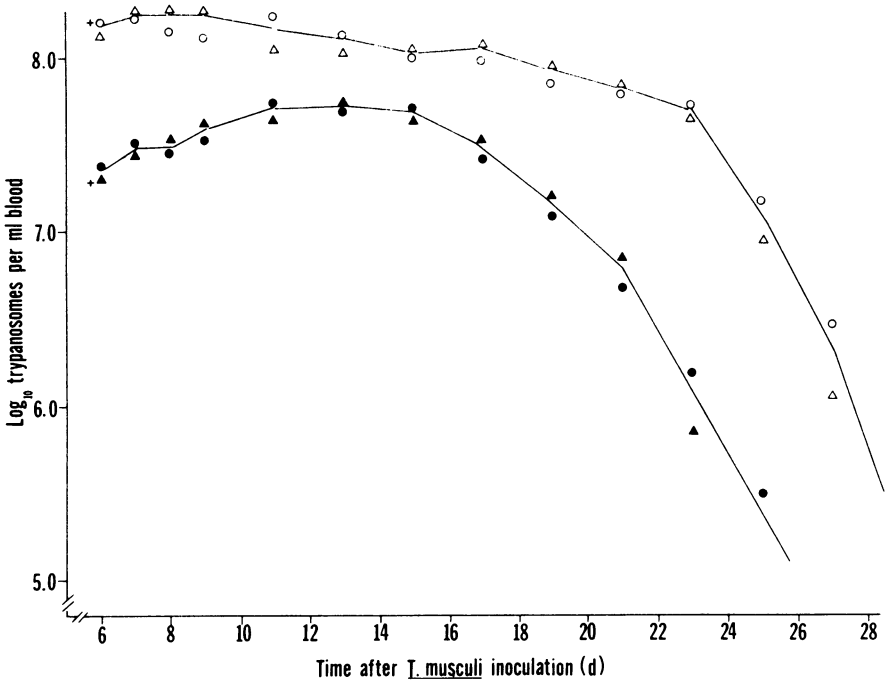

FIG. 2. Course of parasitemia in mice infused with spleen cells from either normal or 3-day-infected, syngeneic donors. Recipient mice were in day 6 of *T. musculi* infection; after exposure to 500 rads of gamma radiation they were injected with 5×10^7 spleen cells. The upper curve represents the course of parasitemia in C3H mice injected with spleen cells from either normal (△) or 3-day-infected (○) C3H donors. The lower curve represents C57BL/6 mice injected with spleen cells from either normal (▲) or 3-day-infected (●) C57BL/6 donors. Each point is the mean of samples from six to eight mice.

activity, whereas extracts of *T. lewisi* did not. To inhibit by 50% the activity of NK cells from 3-day-infected donors, an amount of *T. musculi* extract equivalent to 10^7 (approximately) trypanosomes per ml was required. Assuming that the inhibitory substances are the same in both cases, the extract contained far less of the inhibitors than was produced by the live trypanosomes during the culture period. Thus, it is possible that live trypanosomes may produce NK inhibitory substances continuously.

The nature of the inhibitory substances is unknown at present. They may be substances which interfere with the metabolism of NK cells or possibly of cells generally. It should be instructive to determine whether or not the same substances act in the same way to inhibit both humoral immunity (2, 4) and NK cell activity. The inhibitory substances may act by binding to or blocking sites on target cells which are recognized by NK cells. Target structures recognized by NK cells have not been well defined, although recently transferrin receptors have become prime candidates (28). Other candidates include the neutral glycolipid asialo-GM2 (18) and various glycoproteins that have been prepared from tumors (25).

The explanation of the insensitivity of *T. musculi* to murine NK cells does not involve a possible coat of host mouse serum proteins, in particular, immunoglobulins. This was shown by comparing the sensitivity of *T. musculi* harvested from unirradiated and irradiated mice, the latter being devoid of surface-bound mouse proteins (unpublished data). The importance of eliminating this possibility lies in part in the demonstrations that immune complexes may inhibit NK target cell interactions (20).

There are now two reports dealing with direct investigations of the role of NK cells in trypanosome infections. The earlier report (13) demonstrated directed killing of *T. cruzi*. The present report confirms the cytotoxicity toward *T. cruzi* and extends this finding to include cytotoxicity toward *T. lewisi*. In contrast, we found no evidence of NK cytotoxicity toward *T. musculi*. The failure of NK-augmented spleen cells to affect the course of an existing *T. musculi* infection is in agreement with the results of a recent investigation (29) in which no role for NK cells in the resolution of murine infections with *Babesia microti* and *Plasmodium vinckei petteri* could be demonstrated.

One obvious explanation of the contrasting results obtained with *T. cruzi* and *T. musculi* is the fact that the parasites differ markedly in their habitats; *T. cruzi* thrives intracellularly, whereas *T. musculi* is an extracellular parasite. It is not clear at the moment in what way this residential difference might matter. Another explanation for the difference in the way in which murine NK cells perceive the parasites lies in the fact that *T. musculi* is the natural murine parasite, well adapted, content with inducing relatively innocuous pathological changes in the host (17). In contrast, *T. lewisi* is not normally parasitic for mice, and *T. cruzi* affects a fairly wide range of hosts. The process of *T. musculi* adaptation to the host may well have involved loss of surface molecules that can be recognized by the host's NK cells, or possibly, acquisition of the ability to elaborate substances capable of blocking NK recognition of parasite surface structures. The latter explanation would account for the striking difference between *T. musculi* and rat-specific *T. lewisi* vis-a-vis inhibition of murine NK activity. Our findings may serve as another reminder of the importance of studying parasites in their natural hosts to learn about the

fundamental nature of parasitism (21; J. F. Albright and J. W. Albright, Contemp. Top. Immunobiol., in press).

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Albright, J. W., and J. F. Albright.** 1978. Growth of *Trypanosoma musculi* in cultures of murine spleen cells and analysis of the requirements for supportive spleen cells. Infect. Immun. **22**:343–349.
2. **Albright, J. W., and J. F. Albright.** 1980. Trypanosome-mediated suppression of murine humoral immunity independent of typical suppressor cells. J. Immunol. **124**:2481–2484.
3. **Albright, J. W., and J. F. Albright.** 1983. Age-associated impairment of murine natural killer activity. Proc. Natl. Acad. Sci. U.S.A. **80**:6371–6375.
4. **Albright, J. W., J. F. Albright, and D. G. Dusanic.** 1978. Mechanisms of trypanosome-mediated suppression of humoral immunity in mice. Proc. Natl. Acad. Sci. U.S.A. **75**:3923–3927.
5. **Albright, J. W., K. Y. Huang, and J. F. Albright.** 1983. Natural killer activity in mice infected with *Trypanosoma musculi*. Infect. Immun. **40**:869–875.
6. **Bloom, B. R., N. Minato, A. Neighbour, L. Reid, and D. Marcus.** 1980. Interferon and NK cells in resistance to persistently virus-infected cells and tumors, p. 505–524. *In* R. B. Herberman (ed.), Natural cell-mediated immunity against tumors. Academic Press, Inc., New York.
7. **Cudkowicz, G., and P. S. Hochman.** 1979. Do natural killer cells engage in regulated reactions against self to ensure homeostasis? Immunol. Rev. **44**:13–41.
8. **Eugui, E. M., and A. C. Allison.** 1980. Differences in susceptibility of various mouse strains to haemoprotozoan infections: possible correlation with natural killer activity. Parasite Immunol. **2**:277–292.
9. **Giannini, S. H., and P. A. D'Alesandro.** 1982. Trypanostatic activity of rat IgG purified from the surface coat of *Trypanosoma lewisi*. J. Parasitol. **68**:765–773.
10. **Hanna, N., and R. C. Burton.** 1981. Definitive evidence that natural killer (NK) cells inhibit experimental tumor metastasis *in vivo*. J. Immunol. **127**:1754–1758.
11. **Hansson, M., K. Kärre, R. Kiessling, J. Roder, B. Anderson, and P. Häyry.** 1979. Natural NK-cell targets in the mouse thymus: characteristics of the sensitive cell population. J. Immunol. **123**:765–771.
12. **Hatcher, F. M., and R. E. Kuhn.** 1981. Spontaneous lytic activity against allogeneic tumor cells and depression of specific cytotoxic responses in mice infected with *Trypanosoma cruzi*. J. Immunol. **126**:2436–2442.
13. **Hatcher, F. M., and R. E. Kuhn.** 1982. Destruction of *Trypanosoma cruzi* by natural killer cells. Science **218**:295–296.
14. **Hatcher, F. M., R. E. Kuhn, M. C. Cerrone, and R. C. Burton.** 1981. Increased natural killer cell activity in experimental American trypanosomiasis. J. Immunol. **127**:1126–1130.
15. **Hauser, W. E., Jr., S. D. Sharma and J. S. Remington.** 1982. Natural killer cells induced by acute and chronic toxoplasma infection. Cell. Immunol. **69**:330–346.
16. **Herberman, R. B., and H. T. Holden.** 1978. Natural cell-mediated immunity. Adv. Cancer Res. **27**:305–337.
17. **Hirokawa, K., Y. Eishi, J. W. Albright, and J. F. Albright.** 1981. Histopathological and immunocytochemical studies of *Trypanosoma musculi* infection of mice. Infect. Immun. **34**:1008–1017.
18. **Kiessling, R., and H. Wigzell.** 1981. Surveillance of primitive cells by natural killer cells. Curr. Top. Microbiol. Immunol. **92**:107–123.
19. **Lopez, C.** 1980. Genetic resistance to herpes virus infections: role of natural killer cells, p. 253–265. *In* E. Skamene, P. A. L. Kongshavn, and M. Landy (ed.), Genetic control of natural resistance to infection and malignancy. Academic Press, Inc., New York.
20. **Merrill, J. E., S. Golub, M. Jondal, F. Lanefeldt, and B. Fredholm.** 1982. Presence of FcR for IgG and IgM on human NK cells: the role of immune complexes in the regulation of NK cell cytotoxicity, p. 631–637. *In* R. B. Herberman (ed.), NK cells and other natural effector cells. Academic Press, Inc., New York.
21. **Mitchell, G. F.** 1979. Responses to infection with metazoan and protozoan parasites in mice. Adv. Immunol. **28**:451–511.
22. **Nunn, M. E., R. B. Herberman, and H. T. Holden.** 1977. Natural cell-mediated cytotoxicity in mice against non-lymphoid tumor cells and some normal cells. Int. J. Cancer **20**:381–387.
23. **Ojo-Amaize, E. A., L. S. Salimonu, A. I. O. Williams, O. A. O. Akinwolere, R. Shabo, G. V. Alm, and H. Wigzell.** 1981. Positive correlation between degree of parasitemia, interferon titers, and natural killer cell activity in *Plasmodium falciparum*-infected children. J. Immunol. **127**:2296–2300.
24. **Riccardi, C., A. Santoni, T. Barlozzari, and R. B. Herberman.** 1981. *In vivo* reactivity of mouse natural killer (NK) cells against normal bone marrow cells. Cell. Immunol. **60**:136–143.
25. **Roder, J. C., L. Ährlund-Richter, and M. Jondal.** 1979. Target-effector interaction in the human and murine natural killer system: specificity and xenogeneic reactivity of the solubilized natural killer-target structure complex and its loss in a somatic cell hybrid. J. Exp. Med. **150**:471–481.
26. **Stutman, O., P. Dien, R. Wisun, G. A. Pecoraro, and E. C. Lattime.** 1980. Natural cytotoxic (NC) cells against solid tumors in mice: some target cell characteristics and blocking of cytotoxicity by D-mannose, p. 949–961. *In* R. B. Herberman (ed.), Natural cell-mediated immunity against tumors. Academic Press, Inc., New York.
27. **Timonen, T., and E. Saksela.** 1977. Human natural cell-mediated cytotoxicity against fetal fibroblasts. I. General characteristics of the cytotoxic activity. Cell. Immunol. **33**:340–349.
28. **Vodinelich, L., R. Sutherland, C. Schneider, R. Newman, and M. Greaves.** 1983. Receptor for transferrin may be a "target" structure for natural killer cells. Proc. Natl. Acad. Sci. U.S.A. **80**:835–839.
29. **Wood, P. R., and I. A. Clark.** 1982. Apparent irrelevance of NK cells to resolution of infections with *Babesia microti* and *Plasmodium vinckei petteri* in mice. Parasite Immunol. **4**:319–327.