INFECTION AND IMMUNITY, July 1983, p. 302–307
0019-9567/83/070302-06$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 41, No. 1

Enhanced Natural Killer Cell Activity in Experimental Murine Encephalitozoonosis

JERRY Y. NIEDERKORN,* JOAN K. BRIELAND,† AND ELIZABETH MAYHEW

Department of Ophthalmology and Division of Comparative Medicine, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Received 17 January 1983/Accepted 23 April 1983

Spleen cells from mice infected with the protozoan parasite *Encephalitozoon cuniculi* demonstrated enhanced in vitro cytolysis of YAC-1 lymphoma cells. Selective cell depletion experiments showed that the dominant cell population mediating cytolysis of YAC-1 tumor cells expressed the characteristic phenotype of murine natural killer (NK) cells because (i) pretreatment of spleen cells with anti-asialo GM 1 antiserum plus complement abolished the cytotoxic activity; (ii) augmented cytolysis was found in athymic nude mice; (iii) pretreatment of spleen cells with anti-Thy 1.2 plus complement did not affect the level of cytolysis; and (iv) nylon wool removal of adherent cells did not reduce the augmented cytolysis. The augmented cytolysis peaked 7 days after infection, gradually diminished, and finally returned to control levels by 21 days postinfection. The parasite-induced augmentation of NK cell activity was dose-dependent: inoculation of 10^7 parasites gave maximum enhancement, whereas 10^5 or 10^4 parasites had an insignificant effect on spontaneous NK cell cytolysis. The augmented NK cell cytotoxicity was dependent upon viable parasites; inoculation of killed parasites failed to stimulate a significant increase in spontaneous cytolysis. An active infectious process was an important component of this process. The peak of NK activity in euthymic mice was closely correlated with the active stage of infection, and reduction of NK cell activity coincided with recovery from infection. By contrast, athymic nude mice were unable to control *E. cuniculi* infections yet maintained persistently elevated NK responses. The present data, along with previous reports, indicate that infection with *E. cuniculi* evokes transient modulation of host immune functions.

Encephalitozoon cuniculi is an obligate, intracellular protozoan parasite that infects a wide variety of mammals (28). Clinical signs and death are rarely observed in laboratory animals, despite the high prevalance of infection in rabbits colonies (5, 21, 28).

The fact that encephalitozoonosis is asymptomatic and rarely fatal (28) suggests that host immune mechanisms control parasite multiplication in vivo. Moreover, treatment with an immunosuppressive agent, hydrocortisone, activates latent infections (3). The putative immunological resistance to encephalitozoonis is a T-cell-dependent process since hypothymic, nude mice develop fulminant, lethal infections after intraperitoneal injection of *E. cuniculi*, whereas similarly treated euthymic mice experience mild, asymptomatic infections (24).

Encephalitozoonosis has been shown to depress nonspecifically host responses to unrelated immunogens. In rabbits, infection leads to depressed humoral antibody responses to *Brucella abortus* immunogens (4). Infected C57BL/6 mice produce significantly lower humoral antibody titers to sheep erythrocyte immunogens and demonstrate reduced proliferative splenic responses to mitogens (24). Paradoxically, mice infected with *E. cuniculi* and challenged with various transplantable tumors have reduced tumor growth and prolonged survival times compared with those in uninfected controls (1). This curious, nonspecific resistance to tumor growth suggested the activation and participation of host anti-tumor processes reminiscent of natural killer (NK) cells.

It is now well established that many mammalian species possess lymphocytes that are able to lyse spontaneously a variety of tumor cells in vitro without prior host immunization (20). These cells have been termed NK cells (8) and represent a subpopulation of lymphocytes derived from bone marrow (8, 9). In other studies, murine NK cells have been shown to possess a

† Present address: College of Veterinary Medicine, University of Illinois, Urbana, IL 61801.

low density of Thy 1 antigens (14) and Fc receptors (13), as well as NK-specific differentiation antigens (7, 30). They are nonadherent and nonphagocytic and appear to lack surface immunoglobulin (23). Functional and morphological evidence indicates that NK cells are a heterogenous population of non-T and non-B lymphocytes (12). Augmentation of NK activity can be rapidly induced by in vivo treatment with a variety of biological and synthetic agents that evoke interferon production (6). Moreover, NK cell function can be elevated by in vitro treatment of spleen cells with interferon (25).

In the present study, we explored the effect of experimental encephalitozoonosis on murine NK cell activity in vitro. Two pieces of evidence led us to believe that encephalitozoonosis might stimulate NK cell activity: (i) mice infected with *E. cuniculi* demonstrate increased resistance to transplanted tumors (1); and (ii) tissue-cultured rabbit kidney cells elaborate an interferon-like molecule after in vitro infection with *E. cuniculi* (2).

In the present study, we demonstrate that experimental infection with *E. cuniculi* results in a transient augmentation of splenic NK cell activity. The augmented NK cell function was found to be T cell independent, since infected hypothymic nude mice demonstrated markedly enhanced splenic NK cell expression.

MATERIALS AND METHODS

Mice. Female C57BL/6 (*H-2^b^*), BALB/c (*H-2^d^*), and the beige (*bg/bg*) mutant of C57BL/6 mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Nude (*nu/nu*) BALB/c mice were purchased from Harlan-Sprague/Dawley, Inc., Indianapolis, Ind. All mice were 4 to 8 weeks of age when used as experimental subjects. Mice were age-matched in each experiment.

Infection with *E. cuniculi*. Parasites were cultured in rabbit fibroblasts (27) and harvested by centrifugation ($500 \times g$ for 10 min) of infected tissue culture supernatants. Mice were infected intraperitoneally (i.p.) with 10^7 parasites suspended in 1.0 ml of sterile Hanks balanced salt solution (HBSS).

Tumor cells. YAC-1 lymphoma (A/Sn origin) cultures were a gift from Michael Bennett (University of Texas Health Science Center at Dallas) and were cultured in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer (Sigma Chemical Co., St. Louis, Mo.), 10% heat-inactivated fetal calf serum (GIBCO), penicillin (100 μ/ml), and streptomycin (100 μg/ml) (complete medium). Tumor cells were transferred into fresh, complete medium 24 h before use in ^{51}Cr release assays (NK assays).

NK cell assay. The methodology for NK cell cytotoxicity assays has been described previously (10). Individual experiments were performed by using cells pooled from three to four mice. Tumor target cells were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass.) and placed into wells of Microtest II plates (Falcon Labware, Division of Becton, Dickinson & Co., Oxnard, Calif.) with various numbers of fresh spleen cells suspended in 0.1 ml of complete medium. Generally, each spleen cell suspension was plated at three different effector-to-target cell ratios ranging from 200:1 to 50:1. Spontaneous release of ^{51}Cr was determined by counting supernatants from culture wells containing only tumor cells and medium. Total ^{51}Cr release was determined by lysing tumor target cells with Hematall stromatolytic reagent (Fisher Scientific Co., Fair Lawn, N.J.).

Calculation of percent lysis was performed by using the formula % ^{51}Cr release = [(test counts per minute − spontaneous release counts per minute)/(total counts per minute − spontaneous release counts per minute)] × 100.

Nylon wool column fractionation spleen cells. Spleen cell suspensions were selectively depleted of B cells and adherent macrophages by nylon wool column fractionation (18). Briefly, spleen cell suspensions were treated by hypotonic shock to remove erythrocytes. Cells were washed in complete medium, applied to nylon wool columns, and incubated for 1 h at 37°C. The nonadherent cell populations were eluted with warm (37°C) complete medium, washed twice, resuspended in complete medium, and used as effector cells in the standard NK assay.

Anti-Thy 1.2 and complement treatment. Spleen cell suspensions were selectively depleted of T lymphocytes by treatment with a mouse monoclonal immunoglobulin M (IgM) anti-Thy 1.2 reagent (New England Nuclear) and rabbit complement (1:10; Cedarlane Laboratories Limited, Hicksville, N.Y.) as described elsewhere (26). An IgM-purified mouse myeloma protein, MOPC 104 E (Litton Bionetics, Inc., Kensington, Mass.) was used as a control reagent for this procedure. Cells were treated with either anti-Thy 1.2 plus complement or MOPC 104E plus complement, washed in HBSS, resuspended in complete medium, and used as effector cells in standard NK assays.

Treatment with anti-asialo GM 1 and complement. Rabbit anti-asialo GM 1 antiserum was graciously provided by Vinary Kumar (University of Texas Health Science Center at Dallas) and was used to deplete NK cells from spleen cell suspensions. Briefly, 2×10^6 spleen cells were incubated for 45 min at 37°C in 0.2 ml of anti-asialo GM 1 in microtiter plates. Plates were centrifuged ($330 \times g$ for 10 min), the supernatant was aspirated, and the spleen cells were suspended in 0.2 ml of rabbit complement (1:10). After an additional 45-min incubation (37°C), the plates were centrifuged, and the supernatant was replaced with 0.2 ml of complete medium containing 2×10^4 ^{51}Cr-labeled YAC-1 tumor cells. Plates were incubated for 4 h (37°C) and processed for standard NK assays as described above.

RESULTS

Increased NK cell activity in experimental encephalitozoonosis. Panels of C57BL/6 and BALB/c mice were infected i.p. with 10^7 parasites, and the splenic NK cell activity was determined at various intervals thereafter. BALB/c and C57BL/6 mice were used for com-

parative purposes because they differ markedly in their susceptibility to experimental encephalitozoonosis (24). BALB/c mice have been shown to be relatively resistant, whereas C57BL/6 mice experience more severe manifestations of encephalitozoonosis (24). I.p. infection with *E. cuniculi* resulted in markedly enhanced activation of NK cells (Fig. 1). The NK cellular cytotoxicity peaked 3 to 4 days after infection, dwindled gradually, and returned to normal control levels by day 10 postinfection. Although BALB/c mice experienced milder infections with *E. cuniculi*, they demonstrated greater augmentation of splenic NK activity during the first 7 days after infection compared with infected C57BL/6 mice. On day 14 postinfection, both mouse strains demonstrated slightly depressed NK cell function; however, NK cytolysis returned to normal levels in both mouse strains by day 21 postinfection.

Further experiments showed that the augmented NK cell responses were correlated with

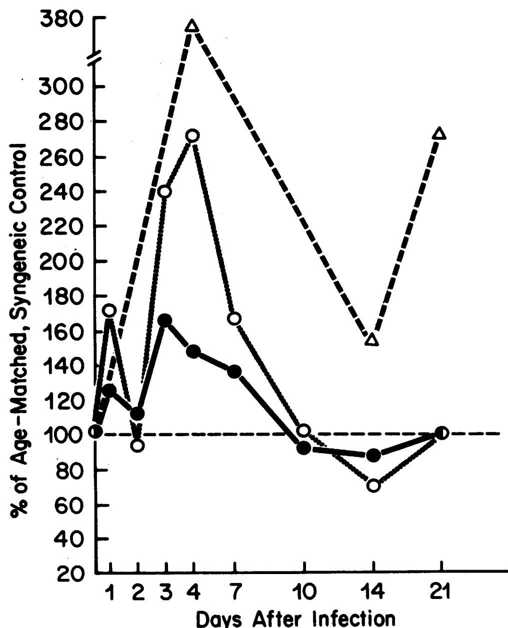

FIG. 1. Cytotoxic activity of spleen cells from mice infected with *E. cuniculi*. Groups of BALB/c (○), C57BL/6 (●), and athymic, nude BALB/c (△) mice were infected i.p. with 10^7 viable parasites, and the spleen cells were collected at various times thereafter. At least three separate experiments were performed at each time point. Standard NK cytotoxicity assays employed YAC-1 tumor target cells as described in the text. Data are plotted as percentage of cytotoxicity of uninfected, age-matched, syngeneic control mice; data for infected nude mice are compared with those for uninfected, nude mouse controls. The effector-to-target cell ratio was 200:1.

the dose of parasites injected. Panels of BALB/c mice were infected i.p. with 10^7, 10^6, 10^5, or 10^4 parasites, and the splenic NK cell activity was determined 5 days later. The data show that the enhanced splenic anti-YAC-1 cytotoxicity was directly correlated with the number of parasites inoculated 5 days earlier. That is, inoculation of 10^7 parasites gave maximum enhancement (i.e., 276% of normal, age-matched control mice; $P < 0.0005$), whereas 10^5 and 10^4 parasites had an insignificant ($P > 0.05$) effect compared with that in uninfected controls. An intermediate effect was found afer inoculation of 10^6 parasites. In these mice, splenic cytolysis of YAC-1 tumor cells was 180% of that found in uninfected control mice.

Thus, experimental encephalitozoonosis evokes enhanced, albeit transient, splenic NK cellular cytotoxicity that is dose dependent and influenced by the strain of the mouse host.

Phenotype of spleen cells mediating cytolysis of YAC-1 tumor cells. The augmented cytolysis of YAC-1 tumor cells by spleen cells from infected mice was presumably due to stimulation of splenic NK cells. This hypothesis was tested by a series of selective cell depletion experiments. Panels of BALB/c mice were infected i.p. with 10^7 parasites, and the spleens were harvested 4 days later. Pooled spleen cell suspensions were treated by each of the following: (i) passage over nylon wool columns; (ii) anti-Thy 1.2 plus complement; and (iii) anti-asialo GM 1 plus complement.

Depletion of nylon-wool adherent cells (i.e., B cells and macrophages) did not appreciably affect the augmented anti-YAC-1 lysis by spleen cells from infected mice. The fact that nylon wool-passed spleen cells were more efficient in lysing YAC-1 target cells (Table 1) argues strongly against a role for macrophage-mediated cytolysis in this phenomenon. Similarly, removal of T lymphocytes by treatment with anti-Thy 1.2 plus complement did not significantly influence the cytolysis of YAC-1 target cells. Therefore, the parasite-induced augmentation of tumor cytolysis was not mediated by either cytotoxic T lymphocytes or tumoricidal macrophages. By contrast, treatment of spleen cell suspensions with anti-asialo GM 1 plus complement abrogated splenic cytolysis of YAC-1 tumor cells (Table 1). Thus, the augmented cytolysis of YAC-1 tumor cells is mediated by nonadherent, non-T cells which express asialo GM 1 surface determinants; that is, NK cells.

Additional experiments reinforced this conclusion. The beige mutant of the C57BL/6 mouse strain lacks normal NK cell functions (25) but demonstrates normal T-cell-mediated and macrophage-mediated cytolytic activities (26). In the present study, beige mice infected 4 days previ-

TABLE 1. Anti-YAC cytotoxic activity exhibited by spleen cells from 4-day-infected mice after selective cell depletion procedures

Expt	Treatment of effector cells	Donor strain	% ^{51}Cr release[a]	
			Infected mice	Uninfected mice
I	None	BALB/c	60.9 ± 5.5	24.3 ± 0.5
	Anti-asialo GM 1 + complement	BALB/c	1.8 ± 0.4	0.0
	Complement alone	BALB/c	58.9 ± 0.5	20.5 ± 1.1
	Nylon wool	BALB/c	69.9 ± 2.3	13.1 ± 1.6
II	None	BALB/c	45.7 ± 3.1	19.2 ± 2.8
	Anti-Thy 1.2 + complement	BALB/c	44.5 ± 2.8	24.2 ± 2.5
	MOPC 104E + complement	BALB/c	38.1 ± 1.7	19.4 ± 3.3
	Complement alone	BALB/c	45.7 ± 1.8	19.2 ± 1.0
III	Anti-Thy 1.2 + complement	C57BL/6	34.4 ± 2.1	24.0 ± 1.0
	MOPC 104 E + complement	C57BL/6	30.5 ± 1.7	22.4 ± 1.4
	Complement alone	C57BL/6	32.3 ± 2.4	18.8 ± 4.6

[a] Standard 4-h ^{51}Cr release assay, using YAC-1 tumor target cells (see the text). The effector-to-target cell ratio was 200:1. There were three to five mice per experimental group. Values indicate mean ± standard deviation.

ously failed to develop significant anti-YAC cytolytic activity (% ^{51}Cr release = 5.5 ± 0.24) compared with uninfected beige mouse controls (% ^{51}Cr release = 4.3 ± 0.46; $P > 0.05$). By contrast, similarly infected normal C57BL/6 mice developed significantly elevated splenic anti-YAC cytotoxicity (% ^{51}Cr release = 45.0 ± 1.49; $P < 0.005$) compared with the cytolysis by spleen cells from uninfected C57BL/6 controls (% ^{51}Cr release = 17.2 ± 0.98). Thus, the parasite-induced augmentation of tumor cytolysis requires a functional NK cell repertoire and is neither directy nor indirectly mediated by cytotoxic T lymphocytes or activated macrophages.

Effect of viable and killed parasites on splenic NK responses. It was important to determine whether the parasite-induced augmentation of NK cell cytotoxicity was due to direct activation of NK cells by constitutive products of the parasites or, conversely, an active infectious process indirectly activated NK cells. Accordingly, panels of C57BL/6 and BALB/c mice were infected with either viable parasites or killed parasites. Parasites were killed by incubation in 95% ethanol for 30 min at room temperature (29). Injection of ethanol-killed parasites failed to evoke a significant ($P > 0.05$) increase in splenic NK cell cytotoxicity in either mouse strain tested (Table 2). By contrast, injection of viable parasites evoked significant increases ($P < 0.005$) in NK lysis of YAC-1 tumor cells by both C57BL/6 and BALB/c mice. Thus, parasite-induced NK cell enhancement requires viable parasites and presumably active infection of host.

Elevated NK activity in infected nude mice. The previous experiments indicated that the parasite-induced NK cell augmentation was a consequence of an infectious process. Active infection with *E. cuniculi* might enhance NK activity by stimulating T lymphocytes to elaborate either gamma interferon or interleukin-2, which in turn stimulates NK cell cytotoxicity (11). Conversely, infected macrophages or other non-T cells might elaborate significant amounts of interferon or an interferon-like molecule which then directly stimulates NK cells. To distinguish between these two possible mechanisms, nude BALB/c mice were infected i.p. with 10^7 viable parasites, and the splenic NK activity was determined 4, 14, and 21 days later. Infected nude BALB/c mice had significantly higher levels of NK cytotoxicity than did uninfected controls (Fig. 1).

TABLE 2. Effect of viable and ethanol-killed parasites on splenic NK activity in mice challenged 4 days previously

Mouse strain	% ^{51}Cr release in mice receiving[a]:		
	Viable parasites[b]	Ethanol-killed parasites[c]	HBSS
BALB/c	18.0 ± 0.62	7.2 ± 0.33	9.5 ± 0.78
C57BL/6	36.0 ± 0.53	15.5 ± 1.07	17.0 ± 0.54

[a] Mice received either 10^7 viable parasites or 10^7 ethanol-killed parasites injected i.p. on day zero. Spleen cells were harvested on day 4, and standard NK assays were performed by using YAC-1 target cells in a 4-h ^{51}Cr release assay.

[b] Significantly different from ethanol-killed parasite group ($P < 0.005$) ± standard error of the mean.

[c] Not significantly different from uninfected (HBSS) group ($P > 0.05$) ± standard error of the mean.

Unlike infected euthymic BALB/c mice, nude mice maintained elevated NK cell responses 14 and 21 days postinfection. Thus, the parasite-induced augmentation of NK function is a T-cell-independent phenomenon. Moreover, the persistent NK cell stimulation in nude mice is correlated with an active infection; these mice are unable to restrain *E. cuniculi* infections, whereas euthymic BALB/c mice recover quickly and eliminate similar experimental infections (24).

DISCUSSION

In the present study, we have demonstrated enhanced spontaneous splenic cytolysis of YAC-1 tumor cells in mice infected with *E. cuniculi*. The enhanced cytolysis peaked within 7 days of infection, gradually diminished, and finally returned to normal control levels by day 21 postinfection.

We have shown previously that several mouse strains differ in their relative susceptibility to encephalitozoonosis (24). In particular, C57BL/6 mice were found to be highly susceptible to infection, whereas BALB/c mice experience only mild infections. In the present study, we compared these two mouse strains and found that infection with *E. cuniculi* evoked a greater augmentation of NK cell cytolysis in BALB/c hosts than in C57BL/6 mice.

Selective cell depletion experiments showed that the dominant cell population mediating cytolysis of YAC-1 tumor cells expressed the characteristic phenotype of murine NK cells. Treatment of the spleen cell suspensions with antibodies directed against asialo GM 1, in the presence of complement, abrogated spleen cell-mediated cytolysis of YAC-1 tumor target cells. Although the asialo GM 1 determinant is found on cells other than NK cells (15), it is highly unlikely that other cytotoxic cells participated in the lysis of YAC-1 tumor cells. For example, the role of cytotoxic T lymphocytes was ruled out because removal of T cells from the spleen cell suspensions, by treatment with anti-Thy 1.2 plus complement, did not alter the level of cytolysis of YAC-1 tumor cells. Moreover, parasite-induced enhancement of cytolysis was markedly higher in T-cell-deficient nude mice compared with euthymic mice, thus ruling out either direct or indirect participation of T cells in this phenomenon. It might be argued that activated macrophages were lysing YAC-1 tumor cells in our assays, since other protozoan parasite infections have been shown to induce tumoricidal and tumoristatic macrophages (16, 22). This explanation is untenable because removal of adherent spleen cells (i.e., B cells and macrophages) by nylon wool column fractionation did not reduce tumor cell cytolysis. Experiments employing NK-defective beige mice provided further evidence that parasite-induced enhancement of cytolysis was referrable to NK cells. Beige mice manifest normal T-cell and macrophage functions (26), yet they were not stimulated to lyse YAC-1 tumor targets after infection with *E. cuniculi*. Thus, the enhanced splenic cytolytic activity associated with *E. cuniculi* infections required a functional NK cell repertoire and did not necessitate the participation of T-cell elements.

Parasite-induced enhancement of NK activity was dependent upon viable parasites because injection of killed parasites failed to stimulate a significant increase in NK activity. An active infectious process appears to be an important component of this process. The enhancement of NK activity in euthymic BALB/c mice is closely correlated with the active stages of infection; likewise, the reduction of NK activity in these hosts corresponds to our previous observations regarding recovery from infection in euthymic BALB/c mice (24). By day 21 postinfection, euthymic mice show no evidence of active infection (24), and, as shown here, they have normal levels of splenic NK activity. By contrast, *E. cuniculi* infections are fatal in nude mice and are characterized by unrestrained parasite proliferation (24). Interestingly, these hosts, unlike euthymic mice, maintain persistently elevated NK responses, suggesting that ongoing infectious processes promote persistent activation of NK cells. The exact mechanisms of parasite-induced NK cell activation await identification; however, the present data indicate that the NK activation occurs in the absence of T cells. A previous report demonstrated that rabbit fibroblasts elaborate an interferon-like molecule after in vitro infection with *E. cuniculi* (2). Therefore, it seems plausible that in vivo infection leads to the release of beta (fibroblast) interferon which might play a major role in the parasite-induced activation of NK function reported in the present study.

The present data add to a growing body of evidence demonstrating NK cell activation by protozoan parasites (10, 17, 19). We assume, although we have no direct evidence, that NK cells do not play a pivotal role in resistance to encephalitozoonosis in mice. Several observations support this assumption. For example, NK-defective beige mice do not die from *E. cuniculi* infection (data not shown). By contrast, encephalitozoonosis is fatal in athymic nude mice despite persistently elevated levels of splenic NK activity. Compared with BALB/c hosts, C57BL/6 mice demonstrate significantly higher base-line levels of NK cell responses, yet these same hosts (C57BL/6 mice) experience

more severe manifestations of encephalitozoonosis.

In summary, infection with *E. cuniculi* results in a transient augmentation of splenic NK activity in mice. This phenomenon is (i) T cell independent, (ii) dose dependent, (iii) time dependent, (iv) dependent upon the presence of viable parasites, and (v) influenced by the strain of the host.

The importance of *E. cuniculi*-induced augmentation of NK cell function remains to be elucidated, but it may be an important consideration in animal colonies which harbor encephalitozoonosis and which also serve as sources of subjects for experimental immunological and tumor research.

ACKNOWLEDGMENTS

This work was supported in part by grant no. RR00890 from the National Institutes of Health and by funds from the Nobel Foundation.

We thank Smita Ghanekar for excellent technical assistance on anti-asialo GM 1 experiments. We thank Vinay Kumar for graciously providing various antisera and for his helpful discussions. We also thank Michael Bennett for kindly providing YAC-1 tumor cultures.

LITERATURE CITED

1. **Arison, R. N., J. A. Cassaro, and M. P. Pruss.** 1966. Studies on a murine ascites-producing agents and its effect on tumor development. Cancer Res. **26**:1915–1920.
2. **Armstrong, J. A., Y-H., Ke, M. C. Breinig, and L. Ople.** 1973. Virus resistance in rabbit kidney cell cultures contaminated by a protozoan resembling *Encephalitozoon cuniculi*. Proc. Soc. Exp. Biol. Med. **142**:1205–1208.
3. **Bismanis, J. E.** 1970. Detection of latent murine nosematosis and growth of *Nosema cuniculi* in cell cultures. Can. J. Microbiol. **16**:237–242.
4. **Cox, J. C.** 1977. Altered immune responsiveness associated with *Encephalitozoon cuniculi* infection in rabbits. Infect. Immun. **15**:392–395.
5. **Flatt, R. E., and S. J. Jackson.** 1970. Renal nosematosis in young rabbits. Pathol. Vet. **7**:492–497.
6. **Gilund, M., A. Orn, H. Wigzell, A. Senik, and I. Gresser.** 1978. Enhanced NK cell activity in mice injected with interferon and interferon inducers. Nature (London) **273**: 759–761.
7. **Glimcher, L., F. W. Shen, and H. Cantor.** 1977. Identification of cell surface antigen selectively expressed in the natural killer cell. J. Exp. Med. **145**:1–9.
8. **Haller, O., M. Gidlund, J. Kurnick, and H. Wigzell.** 1978. In vivo generation of mouse natural killer cells; role of the spleen and thymus. Scand. J. Immunol. **8**:207–213.
9. **Haller, O., R. Kiessling, A. Orn and H. Wigzell.** 1977. Generation of natural killer cells; an autonomous function of the bone marrow. J. Exp. Med. **145**:1411–1416.
10. **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.
11. **Henney, C. S., K. Kuribayashi, D. E. Kern, and S. Gillis.** 1981. Interleukin-2 augments natural killer cell activity. Nature (London) **291**:335–338.
12. **Herberman, R. B.** 1982. Natural killer cells and their possible relevance to transplantation biology. Transplantation **34**:1–7.
13. **Herberman, R. B., S. Bartram, and J. S. Haskill.** 1977. Fc receptors on mouse effector cells mediating natural cytotoxicity against tumor cells. J. Immunol. **119**:322–326.
14. **Herberman, R. B., E. E. Nunn, and H. T. Holden.** 1978. Low density of Thy 1 antigen on mouse effector cells mediating natural cytotoxicity against tumor cells. J. Immunol. **121**:304–309.
15. **Herberman, R. B., and J. R. Ortaldo.** 1981. Natural killer cells: their role in defense against disease. Science **214**: 24–30.
16. **Hibbs, J. B., Jr., L. H. Lanbert, Jr., and J. S. Remington.** 1972. Possible role of macrophage mediated nonspecific cytotoxicity in tumor resistance. Nature (London) New Biol. **235**:48–50.
17. **Hunter, K. W., Jr., T. M. Folks, P. C. Sayles, and G. C. Strickland.** 1981. Early enhancement followed by supression of natural killer cell activity during murine malarial infections. Immunol. Lett. **2**:209–212.
18. **Julius, M. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for isolation of functional thymus derived murine lymphocytes. Eur. J. Immunol. **3**:645–649.
19. **Kamiyama, T., and T. Hagiwara.** 1982. Augmented followed by suppressed levels of natural cell-mediated cytotoxicity in mice infected with *Toxoplasma gondii*. Infect. Immun. **36**:628–636.
20. **Kiessling, R., and H. W. Wigzell.** 1979. An analysis of the murine NK cell as to structure, function, and biological relevance. Immunol. Rev. **44**:165–208.
21. **Koller, L. D.** 1969. Spontaneous *Nosema cuniculi* infection in laboratory rabbits. J. Am. Vet. Med. Assoc. **155**:1108–1114.
22. **Krahenbuhl, J. L., and J. S. Remington.** 1974. The role of activated macrophages in specific and nonspecific cytostasis of tumor cells. J. Immunol. **113**:507–516.
23. **Minato, M., L. Reid, and B. Bloom.** 1981. On the heterogeneity of murine natural killer (NK) cells. J. Exp. Med. **154**:750–762.
24. **Niederkorn, J. Y., J. A. Shadduck, and E. C. Schmidt.** 1981. Susceptibility of selected inbred strains of mice to *Encephalitozoon cuniculi*. J. Infect. Dis. **144**:249–253.
25. **Roder, J.** 1979. The beige mutation in the mouse. I. A stem cell predetermined impairment in natural killer cell function. J. Immunol. **123**:2168–2173.
26. **Roder, J. C., M. L. Lohmann-Matthes, W. Domizig, and H. Wigzell.** 1979. The beige mutation in the mouse. II. Selectivity of the natural killer (NK) cell defect. J. Immunol. **123**:2174–2181.
27. **Shadduck, J. A.** 1969. *Nosema cuniculi*: in vitro isolation. Science **166**:516–517.
28. **Shadduck, J. A., and S. P. Pakes.** 1971. Encephalitozoonosis (nosematosis) and toxoplasmosis. Am. J. Pathol. **64**:657–674.
29. **Shadduck, J. A., and M. B. Polley.** 1978. Some factors influencing the in vitro infectivity and replication of *Encephalitozoon cuniculi*. J. Protozool. **25**:491–496.
30. **Young, W. W., Jr., S. I. Hakomori, J. M. Durdik, and C. S. Henney.** 1980. Identification of ganglio-N-tetraosylceramide as a new cell surface marker for murine natural killer (NK) cells. J. Immunol. **124**:199–201.