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Destruction of *Trypanosoma cruzi* by Natural Killer Cells

Abstract. *Mice infected with* Trypanosoma cruzi *or stimulated with poly(inosinic · cytodylic acid) were found to possess splenic and peritoneal exudate cells with enhanced cytotoxic activity against epimastigote and trypomastigote forms of* Trypanosoma cruzi. *By use of specific alloantiserums it was determined that the effector cells responsible for this cytotoxic activity were typical natural killer cells.*

Trypanosoma cruzi is the obligate intracellular protozoan parasite that causes American trypanosomiasis (Chagas' disease). The parasite infects diverse species of mammals in addition to man, and elicits both humoral and cell-mediated immune responses. These immune responses are generally effective in protecting the host against lethal acute infections. Humans who survive the acute infection may develop chronic disease and frequently display various cardiopathies or enlargement of the hollow viscera (megasyndrome). The nature of the specific or nonspecific immune mechanisms that protect hosts from *T. cruzi* are not understood, although it appears that several interacting mechanisms collectively contribute to protective, but not complete, immunity (*1*). Assays in vitro show that direct destruction of the extracellular stage of the parasite (trypomastigote) is effected by antibody-dependent, complement-mediated lysis and antibody-dependent, cellular cytotoxicity involving granulocytes, lymphocytes, and monocytes (*1*). Cytotoxic lymphocytes from infected experimental animals or humans can detect and destroy *T. cruzi*–infected host cells (*2*, *3*). We have shown previously (*4*) that mice infected with *T. cruzi* exhibit augmented levels of apparently nonspecific killer cell activity against allogeneic tumor cells and that two distinct populations of killer cells are induced at separate times during the course of acute infection. Within 48 hours of the initiation of infection with *T. cruzi* (10^2 to 5×10^4 trypomastigotes, injected intraperitoneally) in mice, a significant increase occurs in natural killer (NK) cell activity in cells from the spleen or peritoneal cavity (*5*). This early phase of cytotoxic activity is followed at 16 to 19 days after infection by the expression of another phase of increased killer cell activity mediated by Thy 1^+, NK 1^- effector cells against several tumor cell lines (*6*). The significance of these observations on immunity to *T. cruzi* was not determined in our earlier work. In the studies reported here, we examined the ability of NK cells from two strains of mice, which differ in their susceptibility to *T. cruzi*, to destroy epimastigotes and trypomastigotes (from blood or from infected fibroblast cultures).

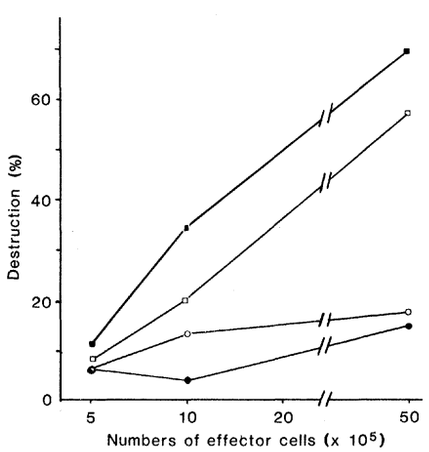

Fig. 1. Cytotoxicity assays with epimastigotes of *T. cruzi*. Spleen cells were obtained from normal (○, ●) or poly(I · C)–stimulated (□, ■) mice. Cells that were unfractioned (○, □) or nonadherent (●, ■) to plastic (primarily lymphocytes) were incubated with the parasites for 6 hours and then evaluated for their cytotoxic activity. Various concentrations of effector cells were tested with 2×10^4 parasites (see Table 1). Similar results were obtained when peritoneal exudate cells were used as effectors (data not shown).

Spleen cells and peritoneal exudate cells were removed from normal C57B1/6 or C3H(He)Dub female mice (8 to 12 weeks of age), from mice stimulated 18 to 20 hours previously with poly(inosinic · cytodylic acid) [poly(I · C), 100 μg in 0.1 ml of phosphate-buffered saline, injected intraperitoneally], or from mice infected with *T. cruzi* for 48 hours as previously described (*4*). Treatment of mice with either poly(I · C) or *T. cruzi* augments NK cell activity in various lymphoid organs as well as the peritoneal cavity of mice (*5*, *7*). We placed these cells in RPMI-1640 medium (supplemented with fetal bovine serum, 10 percent) containing epimastigotes (from axenic cultures in liver-infusion tryptose broth) or blood-form trypomastigotes [derived from infected mice as described in (*4*)] and determined their ability to destroy the parasites. As shown in Table 1, the ability to destroy both the trypomastigotes and epimastigotes was increased in cells from mice stimulated with poly(I · C) or infected with *T. cruzi*. The stimulation of cytotoxic activity by infection with *T. cruzi* was similar to that stimulated by poly(I · C), and both the highly susceptible C3H and the resistant C57B1/6 mice were stimulated to approximately the same degree (Table 1).

The assay system used to determine the destruction of *T. cruzi* is dependent upon the difference in numbers of parasites at the beginning and the end of the incubation period. It was therefore possible that the observed reduction in numbers of parasites (percentage destruction) was due to the phagocytic activity of macrophages in the cell populations. To test this possibility we conducted experiments with spleen and peritoneal exudate cells that had been depleted of adherent macrophages prior to their use in the cytotoxicity assays. On a cell to cell basis, cells that were nonadherent to plastic (predominantly lymphocytes) from poly(I · C)–stimulated mice were as active, or more so, as the unfractionated cells (Fig. 1). These results also demonstrated that increased numbers of effector cells mediated increased destruction of the parasite, as would be expected for active cytotoxic cells.

We studied the kinetics of the destruction of *T. cruzi* by incubating effector cells with epimastigotes for 3, 6, and 18 hours before assessing cytotoxic activity. The numbers of viable parasites cultured with cells from poly(I · C)–stimulated mice at the end of these periods

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were reduced to 41.3 ± 4.3, 58.4 ± 8.7, and 47.5 ± 5.4 percent, respectively. When cells from poly(I · C)–stimulated mice were incubated for 5 hours before the epimastigotes were added, the number of parasites killed at 6 hours was 36.3 ± 5.3 percent. If the initial 5-hour incubation period was omitted, 66.3 ± 1.8 percent of the parasites were killed. These results suggest that the effector cells stimulated by poly(I · C) were somewhat sensitive to the conditions of culture in vitro. Parasites incubated for 5 hours before exposure to spleen or peritoneal exudate cells from poly(I · C)–stimulated mice did not become more resistant to destruction than parasites exposed to the effector cells without the initial 5-hour incubation period. Furthermore, the supernatant fluids from cultured cells of poly(I · C)–stimulated or normal animals had no effect on parasites with which they were incubated for 6 hours. These results indicate that the destruction of *T. cruzi* in these assays is due to a direct interaction between the effector cells and the parasite.

The cytotoxic effector cells obtained from mice infected with *T. cruzi* or stimulated with poly(I · C) are nonadherent to plastic and exert their cytotoxic effects early during the 6-hour incubation period with extracellular forms of *T. cruzi*. These characteristics are similar to those of murine NK cells active against YAC-1 tumor cells (*5*) induced during the first 48 hours of infection with *T. cruzi*.

Table 1. Effect of stimulation of mice with poly(I · C) or infection with *T. cruzi* on the killing of epimastigotes and trypomastigotes by spleen and peritoneal cells in vitro. Spleen cells (SC) and peritoneal exudate cells (PEC) were obtained from normal mice, mice stimulated with poly (I · C) (100 μg), or mice infected with *T. cruzi* (10^3 trypomastigotes). Epimastigotes or blood-form trypomastigotes were washed in RPMI-1640 medium containing 10 percent heat-inactivated fetal bovine serum (RPMI-1640f). We then added 2×10^4 parasites in 0.1 ml of medium to microtiter wells containing 10^6 SC or 5×10^5 PEC in 0.1 ml of RPMI-1640f. After mixing the parasites and centrifuging them at 300*g* for 5 minutes, we incubated the plates for 6 hours at 37°C at which time we determined the number of surviving parasites by counting them in a hemacytometer chamber. Each culture was done in duplicate and the numbers in parentheses indicate the number of separate experiments performed. The index of destruction was calculated by subtracting the number of parasites in cultures containing mouse cells from those containing parasites in medium alone (controls), dividing this by the number in controls and multiplying by 100. The mortality of parasites in medium alone for 6 hours was less than 5 percent. S.D., standard deviation.

Group	Ratio of effector cells to parasites	Percentage destruction (± S.D.)	
		Epimastigotes	Blood-form trypomastigotes
Normal mice			
C57B1/6 SC	50:1	10.3 ± 5.5 (4)	15 ± 2.6 (3)
C57B1/6 PEC	25:1	5.4 ± 4.0 (3)	6.6 ± 1.1 (3)
C3H SC	50:1	15.2 ± 2.1 (2)	10.1 ± 2.5 (2)
C3H PEC	25:1	6.3 ± 3.4 (2)	9.3 ± 1.7 (2)
Mice stimulated with poly(I · C)			
C57B1/6 SC	50:1	57.5 ± 4.4 (5)	43.1 ± 4.6 (3)
C57B1/6 PEC	25:1	50.8 ± 6.3 (4)	40.0 ± 4.4 (3)
C3H SC	50:1	63.6 ± 4.3 (2)	47.1 ± 4.1 (2)
C3H PEC	25:1	47.6 ± 4.8 (2)	50.6 ± 3.1 (2)
Mice infected with *T. cruzi*			
C57B1/6 SC	50:1	57.9 ± 5.4 (3)	39.9 ± 3.6 (3)
C57B1/6 PEC	25:1	65.2 ± 1.9 (2)	Not done

Table 2. The effect of treating effector cells with antiserum to NK 1.2 antigen or to Thy 1.2 antigen plus complement on the destruction of *T. cruzi* in vitro. The effector cells from C3H mice to be used in subsequent cytotoxic assays were treated with complement alone or with the appropriate antiserum and complement in a two-step cytotoxic depletion procedure as described previously (*5*). Effector cells assayed against ^{51}Cr-labeled YAC-1 tumor cells were from normal mice (8 weeks of age) and were assayed for NK cytotoxic activity as described (*5*) at an effector-target ratio of 100:1. Epimastigote target parasites were used as described in Table 1 with cells from poly(I · C)–stimulated mice in a 6-hour assay (effector-parasite ratio of 50:1). Results from our laboratory and others (*9*) have confirmed the specificity of the antiserum to NK 1.2 for murine NK cells.

Treatment of effector cells	Percentage destruction (± S.D.) of	
	YAC-1	*T. cruzi*
Complement alone	20.5 ± 2.1	50.9 ± 3.0
Antiserum to NK 1.2 plus complement	6.4 ± 1.8	5.2 ± 4.7
Antiserum to Thy 1.2 plus complement	15.3 ± 3.5	33.5 ± 6.3

To better define the cell population responsible for the destruction of *T. cruzi*, we treated effector cells with complement alone, antiserum to NK 1.2 antigen plus complement, or antiserum to Thy 1.2 antigen plus complement before adding the parasites in cytotoxicity assays (*5*) (Table 2). Treatment of splenic or peritoneal exudate cells from poly(I · C)–stimulated mice with antiserum to NK 1.2 antigen plus complement eliminated all the activity against *T. cruzi*. Treatment of the cells with antiserum to Thy 1.2 plus complement also reduced the cytotoxic activity against *T. cruzi* but to a lesser extent. The cytotoxic activity of normal splenic NK cells against YAC-1 tumor cells was similarly reduced by treatment with antiserum to NK 1.2 or Thy 1.2 in the presence of complement. Taken together, these results indicate that the cells with increased cytotoxic activity against *T. cruzi* have characteristics that are typical of murine NK cells. To our knowledge, this is the first report of the destruction of protozoan parasites by NK cells.

The actual contribution of NK cells to resistance against cellular antigens (parasites, tumor cells, or allogeneic cells) is not known, although NK cells may be important in immunosurveillance (*8*). The early enhancement of NK activity in mice infected with *T. cruzi* is indicative of a mobilization of this part of the immune system, but the importance of this mobilization is yet to be investigated.

FRANK M. HATCHER
Department of Microbiology,
Meharry Medical College,
Nashville, Tennessee 37208

RAYMOND E. KUHN
Department of Biology,
Wake Forest University,
Winston-Salem, North Carolina 27109

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