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Antibody-Dependent Cellular Cytotoxicity of *Trypanosoma cruzi*: Characterization of the Effector Cell from Normal Human Blood

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The antibody-dependent cellular cytotoxicity activity of normal human blood cells against epimastigotes of *Trypanosoma cruzi* was measured by the release of incorporated [^{3}H]uridine. Sera from patients with chronic Chagas' disease were used to sensitize the parasites to the lytic activity of the effector cells. Different steps of peripheral blood cell purification were employed, and different cell subpopulations were tested as effectors in the system. The main cytotoxic activity was detected in the granulocyte-rich fraction.

Antibody-dependent cellular cytotoxicity (ADCC) to protozoa and metazoa has been described in models using *Schistosoma mansoni* and *Trypanosoma cruzi* as targets (4, 5). In both situations the effector cells seem to be different from the ones that kill tumor cells (12, 14) and nucleated erythrocytes (8). Butterworth et al. (3, 4), by using Ficoll/Hypaque-purified cells and with the aid of an anti-eosinophil serum, have shown that the effector cell in human ADCC against schistosomula is the eosinophil. These authors have pointed out that eosinophils from patients with schistosomal infections are unable to act as effectors (3, 4). Sanderson et al. (15), by means of density gradient, nylon wool adherence, and carbonyl iron techniques of cell purification, have shown that rat eosinophils are active effectors against antibody-coated epimastigote forms of *T. cruzi*. Abrahamsohn and da Silva (1), by using loss of motility as a criterion of cytotoxicity, found that mouse splenic lymphocytes are the effector cells against *T. cruzi* epimastigotes, and Mkwananzi et al. (10) showed that human peripheral blood lymphocytes are cytotoxic to cultures of *Trypanosoma dionisii*.

No information is available concerning the ability of normal human cells or cells from Chagas' disease patients to kill *T. cruzi* in an antibody-dependent system.

In this paper we study the ADCC activity of normal human peripheral blood cell subpopulations against epimastigotes of *T. cruzi*. The cytotoxic activity was found in the granulocyte-rich blood fraction.

MATERIALS AND METHODS

Medium. RPMI-1640 (Flow Laboratories, Inc., Rockville, Md.), containing 10 mM HEPES (*N*-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid; Sigma Chemical Co., St. Louis, Mo.), 1% bovine serum albumin, fraction V (Sigma), 100 U of penicillin per ml, and 100 μg of streptomycin per ml, was used throughout the study.

Target cells. Epimastigotes of the Y-OC strain of *T. cruzi* labeled with [^{3}H]uridine (43 Ci/mmol; The Radiochemical Centre, Amersham, England) were used as target cells. The labeling technique has already been described (15). Briefly, epimastigotes were grown at 28°C in LIT modified medium (7) to which 10 μCi of a high-specific-activity (58 Ci/mmol) [^{3}H]uridine was added per ml. After 5 days of incubation the parasites were throughly washed in medium and resuspended to a final concentration of 10^6 forms per ml.

Antisera. Sera from three chronic Chagas' disease patients were used to sensitize the target cells. One of the patients had the cardiac form of the disease, and the other two were asymptomatic. They had positive serology for Chagas' disease (direct agglutination without and with 2-mercaptoethanol treatment of the serum, indirect hemagglutination, and indirect immunofluorescence). Anti-meningococcal and anti-brucella sera were used as controls of specificity. Normal human serum was used as a control when indicated. All of the above-mentioned sera were heat inactivated and membrane filtered (Millipore Corp., Bedford, Mass.) before use.

Effector cells. The steps involved in purification of human blood cell subpopulations to be used as effector cells are depicted in Fig. 1. Five parts of heparinized (preservative free; Roche Lab) peripheral blood from normal human donors (laboratory personnel with negative serology for Chagas' disease) was incubated with 1 part of a 4% solution of dextran (molecular weight, 200,000; Sigma) for 30 min at 37°C. The leukocyte-rich supernatant was collected, and the cells were washed and centrifuged, first at 400 × *g* for 10 min and then at 200 × *g* for 5 min. After resuspension in RPMI-1640 medium, a sample of the cell sus-

pension was collected and considered as total cells (T_t) when used as effectors in the assay. After the dextran treatment, T_t cells were separated in a Ficoll/Hypaque (Sigma; Winthrop Products Inc., New York, N.Y.) gradient (2) by centrifugation for 40 min at 400 × *g*. The interface fraction (I) and the pellet (P) were washed as described for the total cells. The I and P cells were counted in a model ZBI Coulter Counter and resuspended in medium to the desired final concentration. The I fraction, when indicated, was incubated with 100 μg of carbonyl iron (Fe) per ml (GAF Corp., New York, N.Y.) for 15 min at 37°C. The cells were resuspended every 3 min during incubation with iron. The cells were then immersed in an ice bath for 5 min, and the iron-containing cells were separated by means of a magnet as described by Sher and Glover (16). The interface population after treatment with carbonyl iron was called I_{Fe}. Cell viability was assessed by trypan blue (J. T. Baker Chemical Co., Phillipsburg, N.J.) exclusion, and only subpopulations with 100% viability were used as effector.

Assay system. The cytotoxicity reaction mixture consisted of 100 μl of diluted serum, 100 μl of a suspension of [^{3}H]uridine-labeled *T. cruzi*, and 100 μl of a suspension of effector cells in the appropriate concentration. The mixture was incubated for 4 h at 37°C, and, after centrifugation at 400 × *g* for 10 min, 200 μl of the supernatant was counted in a Liquid Scintillation System (Beckman LS-250). The total isotope release was obtained by lysis of 10^5 parasites with Triton X-100 (Sigma), and the spontaneous release was given by a mixture of *T. cruzi* with medium. In all the experiments, results of immune serum without effector cells and effector cells without immune serum are shown. The spontaneous release is subtracted from all the data presented. All isotope release data are the means of triplicate determinations. The percent of isotope release was calculated as described (15). The statistical difference between different treatments was assessed by means of a one-way analysis of variance, using the Newman-Keuls test of multiple comparisons after an angular transformation of the data.

RESULTS

Figure 2 shows the titration of immune sera from different Chagas' disease patients at an effector/target ratio of 30:1. Total blood leukocytes were used as effector cells. A good cytotoxicity effect was obtained up to a dilution of 1:64 with almost all the tested sera. This serum dilution was then used throught the studies. Figure 3 shows the titration of the effector/target cell ratio with a serum dilution of 1:64. An effector/target cell ratio of 15:1 was chosen as the one to be used in all the experiments. A lytic activity was observed with effector/target ratios as low as 1.5:1. Figure 4 shows a typical experiment where the activity of the different cell subpopulations is presented as the percent of isotope release. Both total cells and cells from the pellet were able to induce lysis of *T. cruzi* in the presence of immune serum. Cells from the interface of a Ficoll/Hypaque gradient displayed

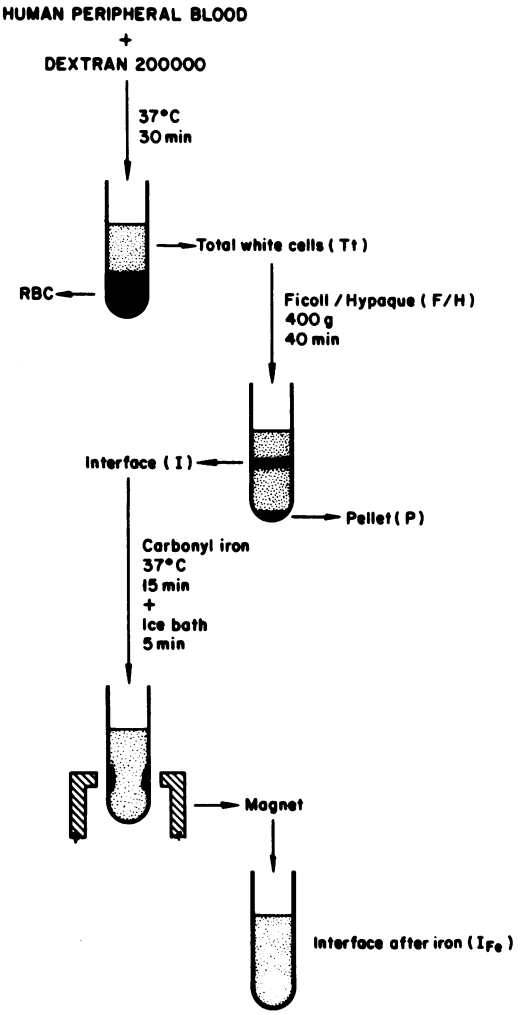

FIG. 1. *Schematic representation of the cell fractionation procedures employed in the study. The cell subpopulations used as effector were: total leukocytes (T_t), cells from the interface (I) and from the pellet (P) of a Ficoll/Hypaque gradient, and cells from the interface after carbonyl iron treatment (I_{Fe}).*

a lower but still significant lytic activity. As can be seen in the inset of Fig. 4, a differential cell count shows an enrichment of eosinophils and neutrophils in the pellet fraction and an enrichment of lymphocytes in the interface. A contamination of neutrophils in the interface was also seen. This pattern of cell distribution was observed in all experiments performed. Experiments using human anti-meningococcal and anti-brucella sera gave results identical to the ones performed with normal human serum (results not shown). To compare the T_t and P cell fraction activities and to learn about the kinetics of the reaction in this system, a temporal study

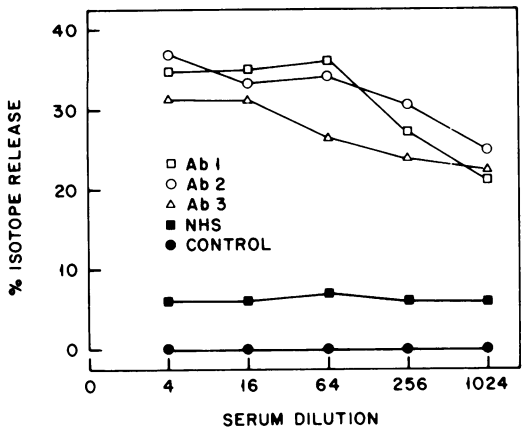

FIG. 2. *Sera titration curves at an effector/target ratio of 30:1. Ab 1, Ab 2, and Ab 3 refer to sera from three different Chagas' disease patients. The control level refers to the reaction with normal human serum (NHS). The spontaneous release has been subtracted from all the experimental points.*

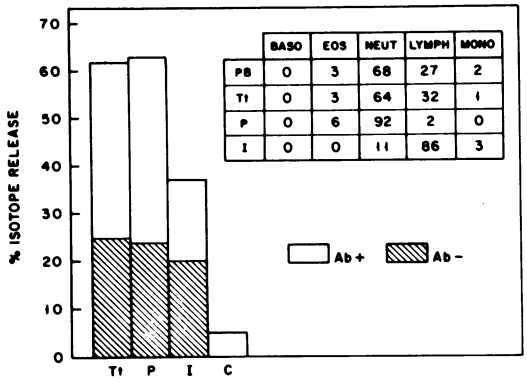

FIG. 4. *ADCC with different cell subpopulations from normal human blood, measured as the percentage of [^{3}H]uridine release from T. cruzi epimastigotes. The inset shows leukocyte differential count. The effects of T_t and P are not statistically significant ($P > 0.05$). $P < 0.01$ between P and I and between I and C.*

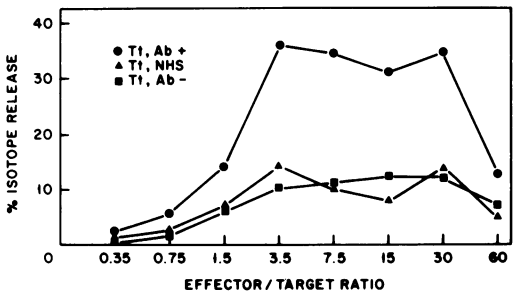

FIG. 3. *Effector/target ratio curve with total leukocytes (T_t) as the effector population. Normal (NHS) and immune (Ab+) sera were used at a dilution of 1:64. Ab− refers to the reaction in the total absence of serum.*

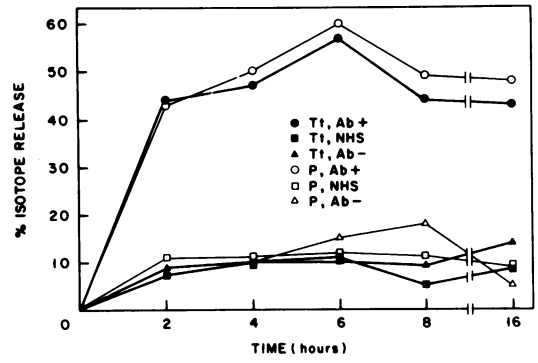

FIG. 5. *ADCC kinetics of T_t and P cell populations at an effector/target ratio of 15:1. The kinetics in the presence (Ab+) or absence (Ab−) of immune serum were exactly the same for both cell populations.*

of their ADCC activity was undertaken (Fig. 5). The activities of both cell populations were identical, increasing up to 6 h of reaction and then plateauing up to 16 h. The apparent decrease in their cytotoxic activity after 6 h of incubation was due to the increase in the spontaneous release of isotope. Since the I cells present a significant ADCC activity, and since, with the classical Ficoll/Hypaque separation technique, a contamination of granulocytes in this cell population is always observed, they were submitted to carbonyl iron treatment as a further purification step, to determine to which cell population (granulocyte or lymphocyte) their activity was due. The activity of the I cells, after carbonyl iron treatment, fell to control level (Fig. 6). A drastic reduction of the percentage of neutro-

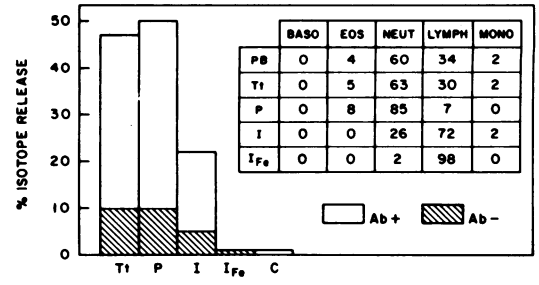

FIG. 6. *As in Fig. 4, showing also the absence of ADCC activity of the I_{Fe} population. $P > 0.05$ between T_t and P. $P < 0.01$ between P and I, and I and I_{Fe}. No difference between I_{Fe} and C.*

phils and a high efficiency of enrichment of lymphocytes was obtained in the I population after the iron treatment (inset, Fig. 6).

DISCUSSION

The results presented in this paper show that human normal peripheral blood cells are able to lyse epimastigote forms of *T. cruzi* coated with antibody present in the serum of chronic Chagas' disease patients. The consistent presence of a high lytic activity in the P fraction of a Ficoll/Hypaque gradient, compared to a much lower activity in the I fraction, strongly suggests that the effector cell in this system is not a mononuclear cell but rather a granulocyte, as has been shown to be the case with schistosomula (3–5). On the other hand, the consistently significant lytic activity displayed by the I population implies that mononuclear cells might also be effective in this system or that a very small fraction of contaminating granulocytes are the cells responsible for this activity. If the latter alternative holds true, one must admit that granulocytes are extremely active killers, operating efficiently at very low effector/target ratios. The carbonyl iron treatment of the I population, while depleting it of contaminating granulocytes, simultaneously reduced its lytic activity to control levels (Fig. 6). These results were always obtained with a neutrophil-rich population sometimes completely depleted of eosinophils. This, of course, implies that normal neutrophils are able to lyse epimastigotes of *T. cruzi* in the presence of specific antibodies. Since we have no tested cell population enriched for eosinophils, we cannot exclude these cells as effectors in this system.

The remaining possibility, that the carbonyl iron treatment damages the lymphocytes making them unable to act as efficient killers (6), seems very unlikely in view of the results of the cell viability assays and with the known examples of lymphocyte activity after this treatment (1, 8–11, 16). The kinetic studies showing an absolute similarity between the curves generated by total cells and cells from the pellet are compatible with the interpretation that the same lytic mechanism is operating in both cell populations. The small but still significant lytic activity observed with cells in the absence of specific immune serum has been described in several ADCC systems (1, 3). No definitive explanation exists for this phenomenon. In our experiments this activity has been observed in the I fraction while contaminated with neutrophils but not after carbonyl iron treatment of this cell fraction.

Our results reinforce the idea of a granulocyte as the cell responsible for the lytic activity against *T. cruzi* and emphasizes its efficiency, since a rather small contaminating population (of the order of 11%) is still sufficient to induce a measurable lysis of the parasites.

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