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ADCC against human erythrocyte target cells: role of the anti-target cell antibodies in determining lymphocyte killer activity

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SUMMARY

Studies were undertaken to investigate the role of anti-target cell antibodies in determining whether lymphocytes can mediate antibody-dependent cellular cytotoxicity (ADCC) *in vitro*. Trinitrophenyl (TNP) modified Chang liver cells and human erythrocytes were employed as target cells and were coated with xenogeneic and allogeneic antibodies against TNP and natural cell surface antigens. Two cytotoxic effector cell populations were used: human peripheral blood mononuclear cells (PBMC) containing both lymphocytes and monocytes, and monocyte-depleted peripheral blood lymphocytes (PBL). With Chang targets, both PBMC and PBL mediated ADCC with xenogeneic anti-Chang and xenogeneic anti-TNP sera. With human erythrocyte targets, PBMC but not PBL mediated ADCC with human anti-blood group B serum, while both PBMC and PBL mediated ADCC with xenogeneic anti-TNP sera and also with a human anti-CD serum. These results demonstrate that the source of anti-target cell antibodies employed in ADCC reactions may determine whether or not lymphocytes are capable of mediating cytotoxicity.

INTRODUCTION

Cytoxicity occurring *in vitro* when non-immune effector cells are added to target cells in the presence of anti-target cell antibodies has been termed antibody-dependent cellular cytotoxicity (ADCC). When human peripheral blood mononuclear cells are used as effectors against antibody-coated chicken erythrocytes, it has been shown that both lymphocytes and monocytes are capable of mediating cytotoxicity (Perlmann & Perlmann, 1970; Nelson *et al.*, 1976; Macdonald *et al.*, 1975). In contrast, with human Chang targets and rabbit anti-Chang serum, ADCC was solely lymphocyte-mediated (Nelson *et al.*, 1976); and with human erythrocyte targets and human anti-blood group sera, ADCC was solely monocyte-mediated (Holm, 1972; Holm & Hammerström, 1973; Poplack *et al.*, 1976; Macdonald *et al.*, 1975). It is unclear whether the ability of human peripheral blood lymphocytes to kill Chang targets, but not erythrocyte targets, in ADCC results from the target cells or the anti-target cell serum employed for ADCC. To clarify this question, we used human peripheral blood mononuclear cells (PBMC) and monocyte-depleted peripheral blood lymphocytes (PBL) as effectors, human Chang cells and erythrocyte targets, and a variety of antisera reacting with both targets. Human PBL were found to mediate ADCC against human erythrocyte targets in the presence of several antisera suggesting that anti-target sera may play a critical role in determining which cell types are active as killer cells in ADCC.

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MATERIALS AND METHODS

Media and reagents. RPMI medium 1640 (1640) and 0·05 M phosphate buffered saline pH 7·2 (PBS) were obtained from the Media Production Unit, NIH. Foetal calf serum (FCS) was obtained from Microbiological Associates, Bethesda, Maryland and was heat-inactivated (56°C for 30 min) before use. Glutamine and penicillin–streptomycin were obtained from Grand Island Biological Co. Grand Island, New York, and all culture media were routinely supplemented with 4·0 mM glutamine, 100 u/ml penicillin, and 100 μg/ml streptomycin. 2,4,6-trinitrobenzene sulfonic acid (TNBS) was obtained from Sigma Chemical Company, St Louis, Missouri.

Anti-target cell sera. The rabbit anti-Chang cell serum used was the same as described previously (Nelson *et al.*, 1976). Human anti-Rh(CD) serum (Ripley) was the generous gift of Marion V. Waller, Medical College of Virginia, Richmond, Virginia. Human hyperimmune antiserum to group B human erythrocytes was obtained from DADE (Division of American Hospital Supply Corporation, Miami, Florida). Hyperimmune rabbit and monkey anti-TNP-KLH sera were from animals initially immunized intramuscularly with 1·0 mg of heavily conjugated TNP-keyhole limpet haemocyanin (KLH) in Freund's complete adjuvant and boosted several times at monthly intervals with 1·0 mg of antigen in incomplete adjuvant. Goat anti-DNP serum was purchased from Miles Laboratories, Elkhart, Indiana. When tested on TNP-modified human erythrocytes, the following haemagglutination titres were obtained: rabbit anti-TNP, 2^9; monkey anti-TNP, 2^{13}; goat anti-DNP, 2^{11}; Ripley anti-CD, 2^5; human anti-blood group B, 2^{10}. All antisera were heat-inactivated (56°C for 30 min) and were not lytic for target cells in the absence of added effector cells. Antisera with specificity for TNP were not lytic to non-TNBS modified target cells in the presence of added effector cells.

Preparation of effector cells. Human peripheral blood mononuclear cells (PBMC) were obtained from peripheral blood by density gradient centrifugation on Ficoll–Hypaque as described previously (Nelson *et al.*, 1976). Monocyte-depleted peripheral blood lymphocytes (PBL) were obtained following incubation of PBMC with carbonyl iron, treatment with a strong magnet, and repeat centrifugation on Ficoll–Hypaque (Nelson *et al.*, 1976).

Identification of monocytes. Monocytes were enumerated in the PBMC and PBL populations by staining for non-specific esterase as described previously (Koski, Poplack & Blaese, 1976).

Preparation of target cells. Chang liver cells grown in suspension cultures were labelled with ^{51}Cr as described earlier (Nelson *et al.*, 1976) and 10^7 cells were incubated in 1·0 ml of 1·0 mM TNBS in PBS for 15 min at 37°C. The TNP-modified ^{51}Cr-labelled cells were then washed three times and suspended in 1640 5% FCS for use in cytotoxicity assays. Fresh human erythrocytes from blood group B, Rh D positive donors were washed three times in PBS, and 10^9 erythrocytes were incubated with 100 μCi of $Na^{51}CrO_4$ for 30 min at 37°C in 1·0 ml 1640 5% FCS. After labelling, the cells were washed three times in PBS, incubated with TNBS as described above and suspended in RPMI 1640 5% FCS.

Cytotoxicity assays. Triplicate cultures were established by adding 100 μl of ^{51}Cr-labelled target cell suspension containing 10^4 Chang cells or 2×10^5 erythrocytes and 100 μl of various effector cell suspensions to the wells of U-bottom microculture plates (Linbro Scientific, Inc., Hamden, New Jersey). Then, 20 μl of 1640 or antisera diluted in RPMI 1640 was added to the appropriate cultures. Triplicate 100 μl aliquots of ^{51}Cr-labelled target cell suspensions were reserved for the determination of ct/min ^{51}Cr added per culture. The plates were then centrifuged at 55 *g* for 5 min and incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2 for 20 hr. The plates were then centrifuged at 550 *g* for 10 min, the supernatants havested and counted along with the target cell aliquots in a well-type gamma scintillation counter. Cytotoxicity manifest by ^{51}Cr release was determined by the following formula:

$$\text{Percentage } ^{51}\text{Cr release} = \frac{\text{supernatant ct/min}}{\text{total ct/min added per culture}} \times 100.$$

Values for the percentage ^{51}Cr release represent the mean ± 1 s.e.m. of triplicate cultures.

RESULTS

The role of lymphocytes in ADCC

Previous studies which demonstrated that human PBL mediated ADCC against Chang cells in the presence of rabbit anti-Chang serum (Nelson *et al.*, 1976), but not against human erythrocytes in the presence of human anti-blood group B sera (Poplack *et al.*, 1976), employed slightly different assay conditions. Therefore, experiments were performed employing PBMC and PBL from the same donor tested under identical assay conditions on both human Chang cells and erythrocytes using rabbit anti-Chang serum and human anti-blood group B serum, respectively. As can be seen in Fig. 1a, PBMC but not PBL mediated ADCC against erythrocyte targets. In contrast, when these same effector cell populations were tested against Chang targets (Fig. 1b) both PBMC and PBL mediated ADCC. Thus under the conditons of these assays, monocyte-depleted PBL possess a population of ADCC effector cells capable of lysing Chang cells in the presence of rabbit anti-Chang serum, but not erythrocytes in the presence of human anti-blood group B serum.

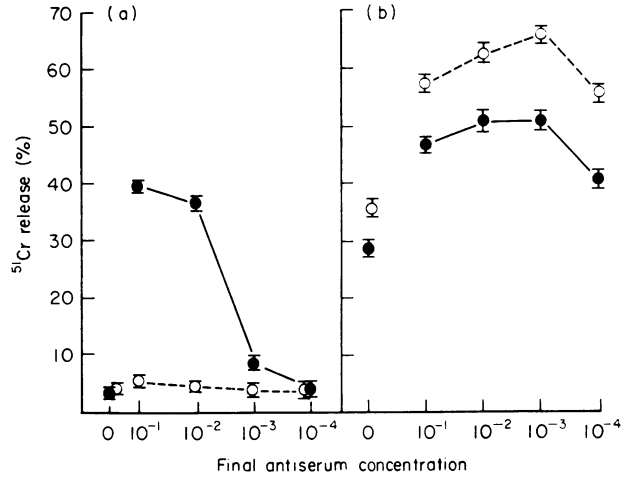

FIG. 1. ADCC effector cell activity of human peripheral blood mononuclear cells (PBMC) and peripheral blood lymphocytes (PBL). Cytotoxicity cultures were established employing PBMC (●———●) and PBL (○ - - - ○) from a single donor simultaneously tested on (a) human erythrocyte targets in the presence of human anti-blood group B serum and (b) Chang cell targets in the presence of rabbit anti-Chang serum. Attacker to target ratios: (a) 1:1, (b) 25:1. PBMC and PBL contained 15·5 and < 0·5% esterase positive cells, respectively.

The role of various heterologous anti-target cell sera in ADCC against human erythrocyte targets

Studies were next performed to determine whether the lack of ADCC by PBL for human erythrocyte targets in the presence of human anti-blood group B serum was due to the nature of the target cell or to the anti-target cell antibodies. For these experiments, PBMC and PBL effector cells from the same donor were tested against TNP-modified human blood group B erythrocytes with heterologous anti-TNP sera, anti-DNP serum and human anti-blood group B serum as sources of anti-target cell antibody. As can be seen in Fig. 2a, PBMC mediated cytotoxicity in the presence of human anti-blood group B serum, as well as all three antisera reacting with TNP. With PBMC effectors and heterologous anti-TNP or anti-DNP sera, target cell lysis equivalent to that observed with the anti-blood group B serum could be

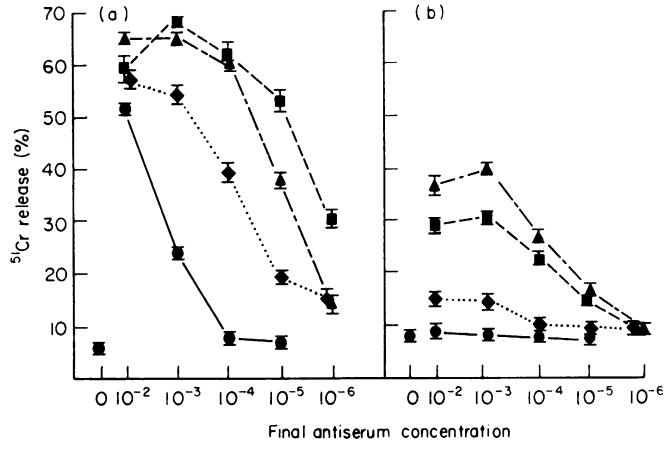

FIG. 2. ADCC effector cell activity for human erythrocyte targets. Cytotoxicity cultures were established employing (a) PBMC and (b) PBL effectors, TNP-modified human erythrocyte targets, and human anti-blood group B serum (●———●), rabbit anti-TNP serum (▲ - - - - - ▲), monkey anti-TNP serum (■ - - - ■), and goat anti-DNP serum (◆ · · · · · ◆). Attacker to target ratio: 1:1. PBMC and PBL contained 13·0 and < 0·8% esterase positive cells, respectively.

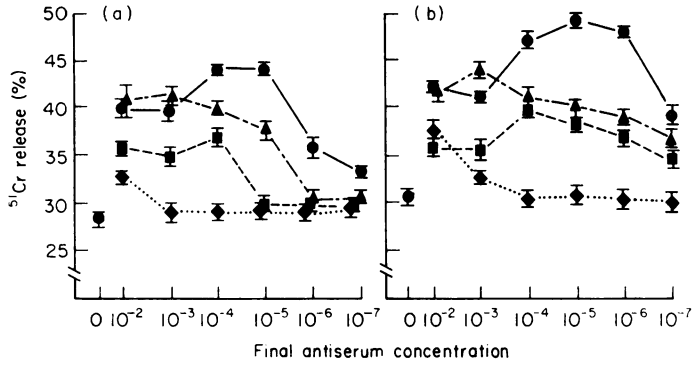

FIG. 3. ADCC effector cell activity for human Chang targets. Cytotoxicity cultures were established employing (a) PBMC and (b) PBL effectors, TNP-modified Chang targets and rabbit anti-Chang serum (●———●), rabbit anti-TNP serum (▲ - · - · - ▲), monkey anti-TNP serum (■ - - - ■), and goat anti-DNP serum (◆ · · · · · ◆). Attacker to target ratio: 25:1. PBMC and PBL contained 13·0 and < 0·8% esterase positive cells, respectively.

obtained with approximately 1·5–3 $\log_{10}$ lower antisera concentrations. As shown in Fig. 2b, PBL did not lyse these target cells in the presence of anti-blood group B serum; however, PBL did mediate cytotoxicity in the presence of heterologous anti-TNP and anti-DNP sera. Furthermore, PBL effected lysis equivalent to that by PBMC in the presence of heterologous anti-TNP sera, but PBL required 2–4 $\log_{10}$ higher antisera concentrations to achieve the same amount of target cell lysis. The lysis of TNP-modified erythrocytes by PBL was not due to the non-specific effects of chemical modification on the erythrocyte membrane, since PBL did not mediate ADCC against TNP-erythrocytes in the presence of anti-blood group B antisera. Thus, PBL were capable of mediating ADCC against TNP-modified human erythrocytes in the presence of xenogeneic anti-hapten sera but not human anti-blood group B sera.

The role of various heterologous anti-target cell sera in ADCC against Chang cell targets

Studies were also performed to determine whether sera other than rabbit anti-Chang serum would sensitize Chang cells to be lysed by lymphocyte effectors. As can be seen in Fig. 3a, when TNP-modified Chang cells were employed as targets, PBMC mediated cytotoxicity in the presence of the heterologous anti-TNP and anti-DNP sera, as well as the rabbit anti-Chang serum. When PBL were employed as effectors (Fig. 3b) cytotoxicity was observed in the presence of all three heterologous antisera recognizing TNP, as well as the rabbit anti-Chang serum. PBL produced lysis equivalent to that by PBMC at 1–2 $\log_{10}$ lower heterologous anti-TNP or anti-DNP serum concentrations. Thus, PBL mediated ADCC against TNP-modified Chang cell in the presence of all the anti-target cell sera tested.

The role of different human anti-erythrocyte sera in ADCC by PBL

Since lymphocytes did not mediate ADCC in the presence of the human anti-blood group B serum but did so in the presence of xenoantisera, it appeared that human antibodies, or antibodies reacting with native membrane antigens, might not be active in effecting lysis. However, in view of the high activity of the monkey anti-TNP serum and the homology between monkey and human immunoglobulins, other human anti-erythrocyte sera were tested. For these experiments, an immune human anti-Rh (CD) serum (Ripley) was chosen since the human PBL mediating ADCC are known to bind Ripley-coated erythrocytes forming EA rosettes (Wisloff, Frøland & Michaelson, 1974). As can be seen in Fig. 4, PBMC mediated ADCC against group B, Rh D positive erythrocytes in the presence of human anti-blood group B serum and anti-CD serum. In addition, PBL mediated ADCC with human anti-CD serum, but not anti-blood group B serum. Thus, the ability of PBL to mediate ADCC against human erythrocyte

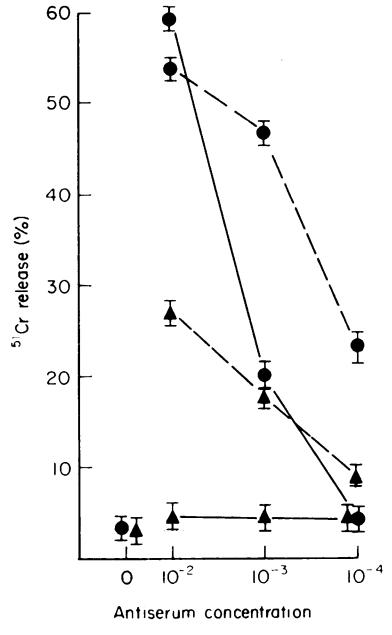

FIG. 4. ADCC effector activity of human PBMC and PBL. Cytotoxicity cultures were established employing PBMC (●) and PBL (▲) in the presence of human anti-blood group B serum (———), or human anti-CD serum (– – – –). Attacker to target ratio: 1:1. PBMC and PBL contained 15·5 and < 0·5% esterase positive cells, respectively.

targets was not dependent on the use of xenogeneic antisera or the reactivity of such antisera with non-native, chemically produced antigenic specificities.

DISCUSSION

Several human peripheral blood leucocyte populations have been shown to mediate ADCC. Depending on the exact assay conditions employed, cytotoxicity may be mediated by lymphocytes, monocytes and neutrophils (Perlmann & Perlmann, 1970; Macdonald *et al.*, 1975; Nelson *et al.*, 1976; Holm, 1972; Holm & Hammerström, 1973; Poplack *et al.*, 1976). The present studies were undertaken to define better the role played by the anti-target cell antibody in determining which effector cells are capable of mediating ADCC. Two assays of human peripheral blood mononuclear ADCC effector cell activity were examined simultaneously using PBMC and PBL as effectors. In the first assay employing human erythrocyte targets and human anti-blood group B serum, ADCC was mediated by monocyte-containing PBMC but not by PBL. In the second assay using human Chang targets and rabbit anti-Chang serum, ADCC was mediated by both PBMC and PBL. Thus, lymphocytes mediated ADCC against Chang but not erythrocyte targets. Such results have been interpreted as showing that the target cells have a critical role in determining which effector cells mediate ADCC. To assess the role of the anti-target cell sera in determining the ability of lymphocytes to mediate ADCC in each assay, antisera capable of reacting with determinants chemically produced on both target cells were employed in the two cytotoxicity assays. When TNP-modified Chang cells were employed as targets, both PBMC and PBL mediated ADCC in the presence of rabbit, monkey and goat antisera reacting with TNP. Thus, using other xenogeneic antisera reacting with determinants on the Chang cell did not prohibit PBL from mediating cytotoxicity. When TNP-modified erythrocytes were used as targets in the presence of xenogeneic antisera reacting with TNP instead of human anti-blood group sera, PBL were able to mediate ADCC. The ability of PBL to mediate ADCC against erythrocyte targets was further shown not to be dependent on the use of xenogeneic antisera since, when a human anti-CD serum (Ripley) was used, cytotoxicity by PBL also

occurred. Moreover, with the anti-CD serum, PBL-mediated ADCC was demonstrated with anti-target cell serum reacting with native membrane constituents.

The observation that human Chang cell targets are susceptible to lysis by both PBL and PBMC in the presence of xenogeneic anti-target cell sera is in agreement with several studies in this same assay system (MacLennan, 1972; Nelson *et al.*, 1976). With regard to human erythrocyte targets, our observation that monocyte-containing PBMC but not monocyte-depleted PBL are capable of mediating ADCC in the presence of human anti-blood group serum is in agreement with the results of others (Holm, 1972; Holm & Hammerström, 1973; Macdonald *et al.*, 1975). Considerable controversy exists, however, as to whether lymphocytes can mediate ADCC against human erythrocyte target cells. The present studies demonstrating ADCC by PBL for human erythrocyte targets in the presence of xenogeneic and allogeneic anti-target cell sera show that the antiserum is critical in determining the mononuclear effector cells capable of mediating ADCC. In previous studies, Holm (1972) and Holm & Hammerström (1973) found that human PBL did not mediate ADCC for human erythrocyte targets in the presence of rabbit anti-erythrocyte serum or several anti-Rh (D) sera. Similarly, Milgrom & Shore (1977) were unable to demonstrate PBL-mediated ADCC for human erythrocyte targets in the presence of anti-Rh (D) serum. In contrast with these studies, Hinz & Chickosky (1972) were able to demonstrate PBL-mediated ADCC for human erythrocyte targets with high concentrations (1:4) of anti-blood group B serum or anti-Rh (D) serum, at high attacker to target ratios (20:1) and with prolonged incubation times (24–48 hr). Sanal & Buckley (1978) were also able to demonstrate PBL-mediated ADCC against human erythrocyte targets with anti-Rh (D) serum. The most likely explanation for the discrepancies among these results is that the anti-target cell serum is critical in determining whether PBL mediate cytotoxicity. These differences among antisera may relate to the titre of anti-target cell antibody within the antisera, to the class(es) of anti-target cell antibody in the sera, to the target cell specificities recognized by the antisera, or to a combination of these possible causes. In considering the results with human erythrocyte targets, it is possible that the chemical nature of the antigens to which anti-target cell antibodies are bound may influence the display of antibody molecules on the cell surface and hence its recognition by effector cell Fc receptors. For example, the reagent TNBS reacts covalently with the exposed erythrocyte protein band 3 and glycophorin, as well as with some membrane lipid components which are probably not externally exposed (Steck, 1972). The ABO blood group antigens are 'macroglycolipids' (Dejter-Juszynski *et al.*, 1977) which may be more mobile in the target cell membrane in that they are not transmembrane components whose mobility is restricted by the spectrin–actin complex. The chemical nature of the Rh antigens is as yet unknown (Hakomori & Kobata, 1974). In part, these differences in antigenic determinants or the erythrocyte membrane may influence the lysis of these cells by monocytes or PBL.

Although the precise reasons for the observed differences among anti-target cell sera in ADCC are not known, the present studies demonstrate that the anti-target cell serum has a critical role to play in determining which effector cells mediate ADCC.

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