Acta Medica Okayama

Volume 39, *Issue* 1 1985 *Article* 10

FEBRUARY 1985

Antibody-dependent cell-mediated cytotoxicity (ADCC) toward human O+ red cells coated with anti-D antibody: comparison between lymphocyte and monocyte ADCC activity.

Mitsutoshi Sunada* Shinya Suzuki†

Zensuke Ota‡

*Okayama University,

†Okayama University,

‡Okayama University,

Antibody-dependent cell-mediated cytotoxicity (ADCC) toward human O+ red cells coated with anti-D antibody: comparison between lymphocyte and monocyte ADCC activity.*

Mitsutoshi Sunada, Shinya Suzuki, and Zensuke Ota

Abstract

We investigated the antibody dependent cell-mediated cytotoxicity (ADCC) of lymphocytes and monocytes toward human O+ red cells coated with anti-D antibody using a 51Cr release assay. Lysis of sensitized red cells by lymphocytes occurred rapidly, but monocyte-mediated lysis occurred slowly. This difference might be due to postphagocytic 51Cr release by monocytes. ADCC of lymphocytes increased in proportion to the effector cell number, but large amounts of antibodies were required. In contrast, ADCC of monocytes was independent of the effector/target ratio and very small amounts of antibodies could produce red cell lysis. Large amounts of fluid phase IgG were required to inhibit the lymphocyte ADCC, whereas the monocyte ADCC was markedly inhibited by small amounts of IgG. Monocyte-mediated lysis was completely inhibited by the addition of 10% human AB serum, but lymphocyte-mediated lysis was only slightly inhibited. Purified IgG1 and IgG3 were much more inhibitory to the lysis by both effectors than IgG2 and IgG4 (IgG2 greater than IgG4). Erythrophagocytosis also was inhibited by IgG1 and IgG3. These studies demonstrate that lymphocytes as well as monocytes can cause the lysis of antibody sensitized red cells, and IgG1 and IgG3 subclasses are more important than IgG2 and IgG4 in causing lysis of anti-D coated red cells.

KEYWORDS: antibody dependent cell mediated cytotoxicity, lymphocyte, monocyte, IgG subclass, anti-D antibody

---

*PMID: 3923782 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 39, (2), 77-89 (1985)

ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC) TOWARD HUMAN O^+ RED CELLS COATED WITH ANTI-D ANTIBODY : COMPARISON BETWEEN LYMPHOCYTE AND MONOCYTE ADCC ACTIVITY

Mitsutoshi SUNADA, Shinya SUZUKI and Zensuke OTA

Third Depertment of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

Received August 29, 1984

Abstract. We investigated the antibody dependent cell-mediated cytotoxicity (ADCC) of lymphocytes and monocytes toward human O^+ red cells coated with anti-D antibody using a ^{51}Cr release assay. Lysis of sensitized red cells by lymphocytes occurred rapidly, but monocyte-mediated lysis occurred slowly. This difference might be due to postphagocytic ^{51}Cr release by monocytes. ADCC of lymphocytes increased in proportion to the effector cell number, but large amounts of antibodies were required. In contrast, ADCC of monocytes was independent of the effector/target ratio and very small amounts of antibodies could produce red cell lysis. Large amounts of fluid phase IgG were required to inhibit the lymphocyte ADCC, whereas the monocyte ADCC was markedly inhibited by small amounts of IgG. Monocyte-mediated lysis was completely inhibited by the addition of 10 % human AB serum, but lymphocyte-mediated lysis was only slightly inhibited. Purified IgG1 and IgG3 were much more inhibitory to the lysis by both effectors than IgG2 and IgG4 (IgG2 > IgG4). Erythrophagocytosis also was inhibited by IgG1 and IgG3. These studies demonstrate that lymphocytes as well as monocytes can cause the lysis of antibody sensitized red cells, and IgG1 and IgG3 subclasses are more important than IgG2 and IgG4 in causing lysis of anti-D coated red cells.

Key words : antibody dependent cell mediated cytotoxicity, lymphocyte, monocyte, IgG subclass, anti-D antibody.

It is well known that in warm reactive autoimmune hemolytic anemia (AIHA), antibody-coated red cell destruction is mediated by leukocytes bearing Fc-receptor. This destruction mechanism is now recognized as antibody-dependent, cell-mediated cytotoxicity (ADCC). The interactions between anti-D coated red cells and human leukocytes are of interest because these interactions *in vitro* are considered a prototype of ADCC.

Previous investigations concerning ADCC of human mononuclear cells toward human red cell targets indicated that monocytes, but not lymphocytes, were able to produce erythrocyte lysis (1, 2). But more recently, it was reported that lymphocytes can lyse human red cells by ADCC when the following combinations of target cells and antibodies are used (3-5): (I) -D- cells plus typical anti-D, (II) normal O^+ cells plus Riply anti-CD, (III) papain-pretreated normal O^+ cells plus small

amounts of anti-D and (IV) normal O^+ cells plus very large amounts of anti-D (5). However, 1-2 % monocytes contaminated these effector lymphocyte suspensions (3-6). We have completely separated lymphocytes from monocytes by removing silica phagocytosed cells and glass adherent cells using a new method. Lymphocyte suspensions obtained by this method were contaminated by less than 0.5 % monocytes. So, we reexamined the ADCC activity of lymphocytes and monocytes toward anti-D coated red cells using these purified cells, and evaluated the various conditions under which red cell lysis occurs.

MATERIALS AND METHODS

Buffers and reagent. Hanks' balanced salt solution (HBSS) was purchased from Gibco Labo. The medium used was medium 199 (Gibco Labo) supplemented with 10 % fetal calf serum (FCS), 10,000 iu/dl penicillin and 10 mg/dl streptmycin. Macrophage separating plates (60 mm × 15 mm; MSP-P) and MSP-E (EDTA and FCS added to PBS) were obtained from Japan Immune Labo as were the silica suspensions (KAC-2). Anti-D serum was from Dade Diagnostic Co., Ltd. NH_4Cl was diluted to 0.87 %.

Target cells. Blood group O, Rh-positive red cells were obtained from a healthy donor. The heparinized blood was collected under sterile conditons, washed 4 times with isotonic saline and adjusted to a concentration of 5 %. One volume of the red cell suspension was incubated for 30 min at 37℃ with the same volume of an appropriately diluted anti-D serum. After incubation, the cells were washed 4 times and suspended in HBSS at a concentration of 5 %.

Chromium labelling of target red cells. Each 0.1-ml aliquot of the 5 % sensitized red cell suspensions (2×10^8 cells/ml) was incubated with 100 μci sodium chromate for 120 min at 37℃ in a water bath. Cells were washed 4 times, resuspended in medium, and adjusted to a concentration of 2×10^5 cells/ml. Radioactivity was measured with a gamma emmision spectrometer.

Preparation of the purified monocyte suspension. The heparinized blood was diluted with an equal volume of saline. Diluted blood (6 ml) was layered over 3 ml of Conray-Ficoll 400 in 13 mm × 105 mm glass tubes. The tubes were then centrifuged at 400 G for 20 min. After centrifugation, the mononuclear cells at the interface between the diluted plasma and the Conray-Ficoll 400 were harvested and washed 3 times in saline. After the final wash, the mononuclear cells were suspended in medium and adjusted to a concentration of 2-3 × 10^6 cells/ml. Four-ml aliquots of cell suspension were added to MSP-P plates and incubated at 37℃ for 60 min in a CO_2 incubator. Following incubation, the plates were washed gently 4 times with warm HBSS to remove the non-adherent cells. Then, 3 ml of MSP-E was added to each plate and incubated for 30 min at 4℃. The adherent cells were harvested by gentle pipeting and washed 3 times with medium. These adherent cell preparations were monocytes and consistenly contained less than 1 % lymphocytes, as determined by morphologic criteria and nonspecific esterase staining.

Preparation of the purified lymphocyte suspension. One volume of KAC-2 was added to ten volumes of heparinized blood in 13 mm × 105 mm glass tubes, which were then placed in a water bath at 37℃ for 60 min with occasional mixing of the contents. After incubation, the blood was layered over Conray-Ficoll 400 and centrifuged at 1200 G for 30 min to remove the phagocytic cells. Following centrifugation, the non-phagocytic cells at the interface were harvested, washed 3 times with HBSS and suspended in medium at a concentration of 2-3 × 10^6

cells/ml. Four-ml aliquots were added to MSP-P plates and incubated at 37℃ for 60 min in a CO_2 incubator to remove adherent cells. After incubation, the non-adherent cells were collected, washed 3 times with HBSS and suspended in medium at various concentrations prior to use. These non-phagocytic, non-adherent cell preparations were purely lymphocytes and contained less than 0.5 % monocytes, as determined by nonspecific esterase staining.

Antibody-dependent monocyte or lymphocyte-mediated ^{51}Cr release. ^{51}Cr release was measured after incubation of labelled antibody-coated cells with monocytes or lymphocytes. Antibody-coated red cells in suspension (0.5 ml, 2 × 10^5 cells/ml) and purified monocytes or lymphocytes in suspensions of varying concentrations (0.5 ml, 1.2-64 × 10^5 cells/ml) were mixed together in 13 mm × 105 mm glass tubes in triplicate and incubated in a moisturized 5 % CO_2 incubator at 37℃ for 4 h. Some experiments were done with different incubation times to examine the time course of ADCC. The cell suspensions were centrifuged at 200G for 3 min prior to incubation. After incubation, each tube was centrifuged at 400G for 10 min to sediment unlysed cells. Supernatant fluid (0.5 ml) was then carefully removed from each tube, and the radioactivity was determined using a gamma emission spectrometer. Chromium release from antibody-coated red cells without effector cells and chromium release from antibody-coated red cells with effector cells were measured in each experiment. Monocyte or lymphocyte-mediated red cell lysis was calculated using the formula: Net % ^{51}Cr release $= \frac{A - B}{C} \times 100$, where A = the mean cpm in the supernatant of tubes containing antibody-coated tragct plus effectors, B = the mean cpm in the supernatant of tubes containing only antibody-coated targets (without effectors) and C = the mean total cpm of targets added to each tube. The spontaneous release of ^{51}Cr was always less than 2 %, and release from non-antibody coated targets incubated with effectors did not exceed spontaneous release. Student's t test was used to determine the significance of the differences among the tubes.

Postphagocytic ^{51}Cr release assay. Purified monocyte suspensions (0.5 ml, 1 × 10^5 cells/tube) and antibody coated labelled red cell suspensions (0.5 ml, 1 × 10^5 cells/tube) were mixed together and centrifuged at 200G for 3 min prior to incubation. After 1 h of incubation, the tubes were centrifuged at 400G for 10 min. The supernatant was removed and large amounts of 0.87 % NH_4Cl were added to lyse the non-ingested red cells. After washing 3 times with 0.87 % NH_4Cl, 1.0 ml of medium was added, and the cells were further incubated under the same conditions. Postphagocytic ^{51}Cr release was determined at various incubation times.

Quantitation of phagocytosis of monocytes. Mononuclear cells suspended in medium (0.2 ml, 5 × 10^6 cells/ml) were gently layered onto coverslips (12 mm × 32 mm) in Leighton tubes and incubated in a moisturized 5 % CO_2 incubator for 60 min at 37℃. After incubation, the coverslips were rinsed 3 times with warm HBSS to remove non-adherent cells, and 1.0 ml of a 0.5 % red cell suspension was added to Leighton tubes. The suspension was incubated for another 60 min under the same conditions. After incubation, the coverslips were washed with warm HBSS, dried, fixed in methanol for 3 min, stained with Giemsa and examined for erythrophagocytosis under a light microscope. Percent phagocytosis was defined as the number of phagocytized monocytes counted. The mean and standard error of triplicate samples were calculated. Student's t test was used to assess the significance of differences between sets of samples.

Inhibition of lysis or phagocytosis by IgG and its subclasses. Purified IgG, prepared from polyethylene glycol treated human normal immunoglobulins, was kindly suplied by The Green Cross Corporation. IgG1, IgG2, IgG3, and IgG4 were purified by DEAE Sephacel column chromatography in 0.0175 M phosphate buffer (pH 6.8) from sera containing monoclonal IgG

subclasses obtained from patients with multiple myeloma. The sera were kindly donated by Dr T. Sezaki of Okayama National Hospital. The IgG solution was concentrated and stored in small aliquots. In inhibition tests, target cells and effector cells were suspended in medium with and without IgG or IgG subclasses and were assayed for red cell destruction as described above.

RESULTS

We ran the ADCC assay at various incubation times to examine the time course of ADCC. Fig. 1 shows that lymphocyte-mediated, antibody-coated red cell (coated with twofold diluted anti-D serum) lysis occurred rapidly and was nearly completed within 4 h. Monocyte-mediated lysis occurred slowly, and increasing ^{51}Cr release was observed upto 15 h. This difference may be due to the phagocytic activity of monocytes, so we examine the postphagocytic ^{51}Cr release from monocytes (Fig. 2). It was demonstrated that about 40 % of all the labelled erythrocytes added were phagocytized by monocytes within 1 h and about 50 % of the ingested cells were lysed and released into the supernatant within 4-6 h. We chosed rapid ^{51}Cr release assay of 4 h in the following experiments for comparing the ADCC activity of lymphocytes and monocytes.

Fig. 3 shows the comparison of ADCC activity of lymphocytes and monocytes at various effector/target (E/T) ratios. The ADCC of lymphocytes showed a direct relationship between effector cell number and number of red cells lysed. On

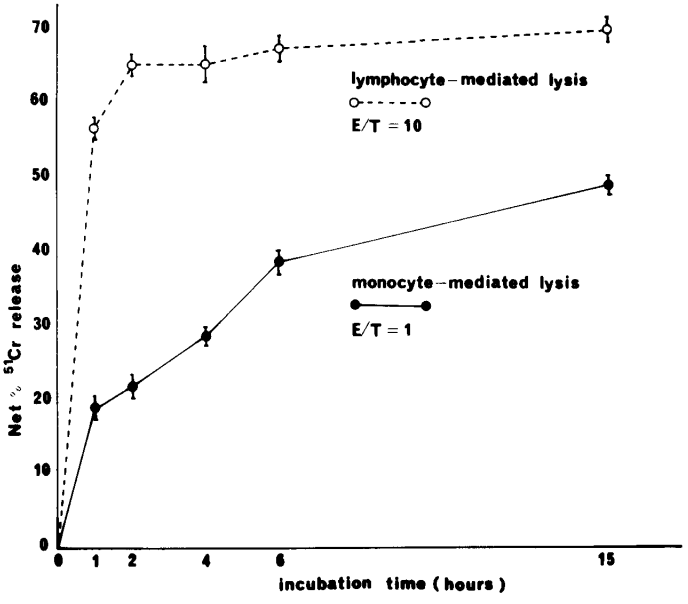

Fig. 1. Time course of ADCC of lymphocytes (○-----○) and monocytes (●——●). Target red cells were sensitized with 2 × diluted anti-D serum (1 × 10^5 cells/tube).

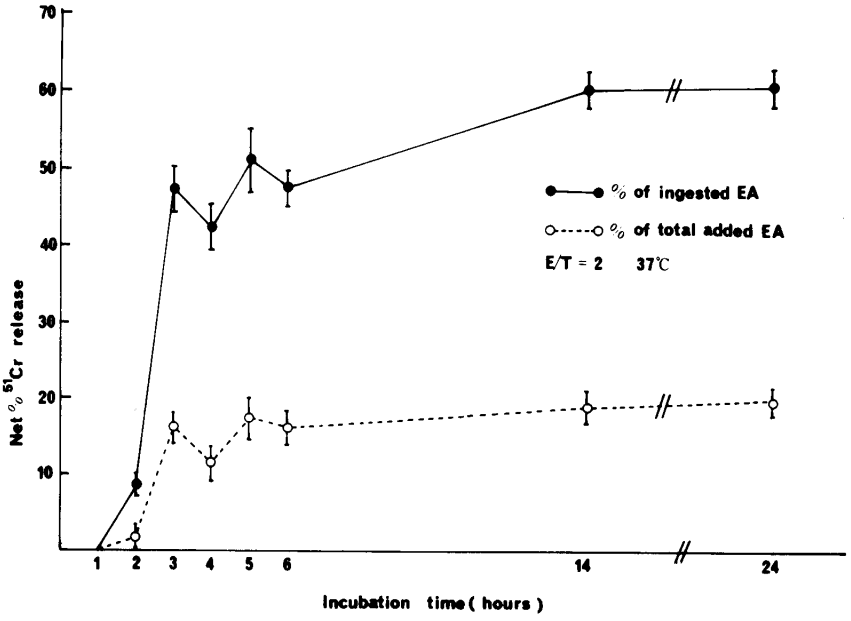

Fig. 2. Postphagocytic ^{51}Cr release by monocytes. % of ingested EA (●——●) and % of total EA added (○------○) were calculated as described under MATERIALS AND METHODS. Target red cells were sensitized with 2 × diluted anti-D serum.

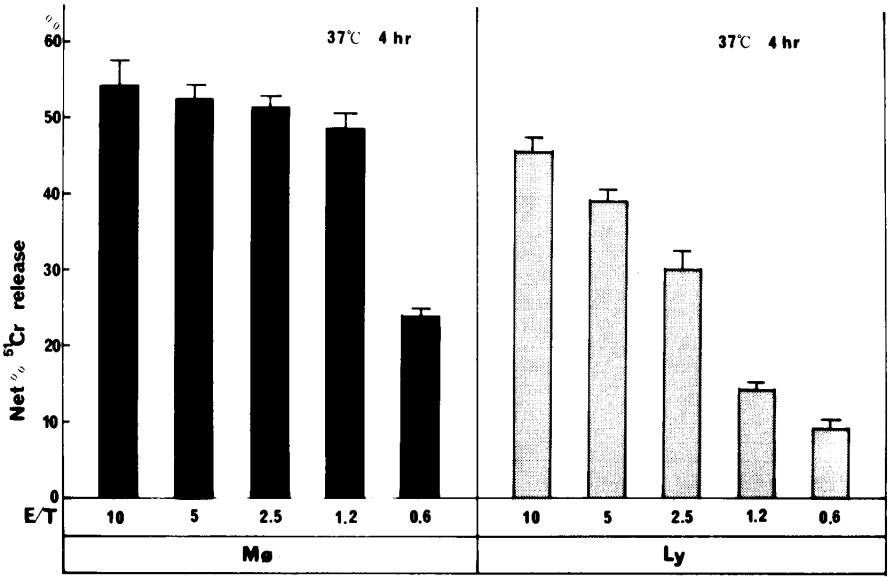

Fig. 3. Effect of the E/T ratio on monocyte and lymphocyte-mediated lysis of anti-D coated red cells. Target red cells were sensitized with 2 × diluted anti-D serum.

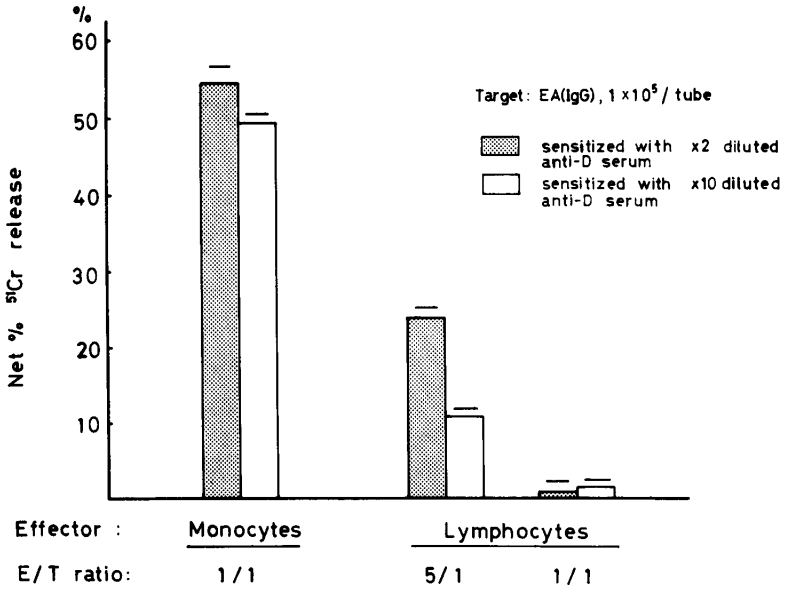

Fig. 4. Comparison of antibody-dependent lysis of human red cells by monocytes and lymphocytes.

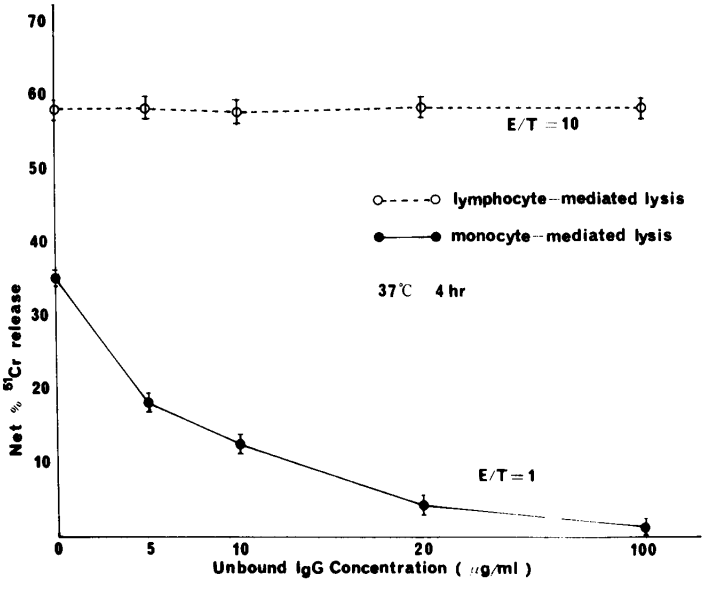

Fig. 5. Inhibition of monocyte and lymphocyte-mediated lysis of anti-D coated red cells by fluid phase whole molecule IgG. Target red cells were sensitized with 4 × diluted anti-D serum.

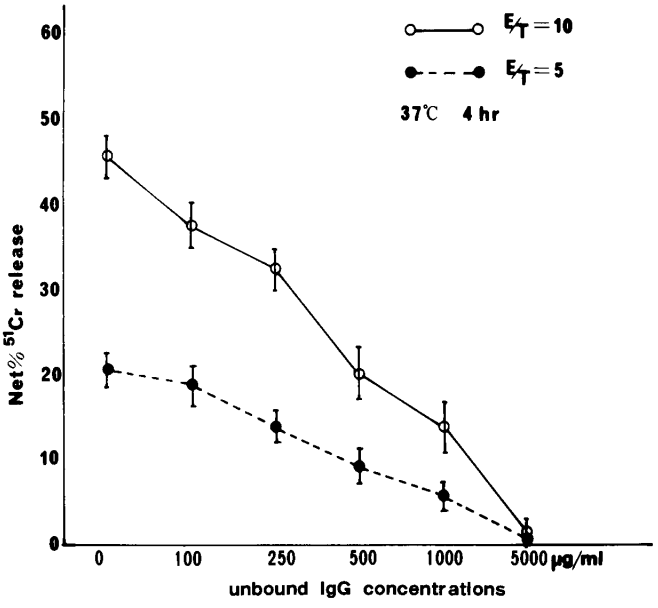

Fig. 6. Inhibition of lymphocyte-mediated lysis of anti-D coated red cells by fluid phase whole molecule IgG. Target red cells were sensitized with 4 × diluted anti-D serum.

the other hand, the ADCC of monocytes showed no notable relationship with E/T ratios from 1.2 to 10. Fig. 4 illustrates the ADCC of lymphocytes and monocytes toward normal O^+ red cells sensitized with twofold diluted anti-D serum. At a low E/T ratio (1 : 1), ADCC of lymphocytes did not occur even though target red cells were strongly sensitized with large amounts of antibodies. However, when the E/T ratio was increased (5 : 1), ADCC occurred moderately with lymphocytes toward red cells sensitized with either low or high concentrations of antisera. In contrast, ADCC of monocytes occurred equally against both targets at a low E/T ratio.

It was indicated that both effector cells can cause lysis of target red cells, but that the ADCC of monocytes was stronger than that of lymphocytes because the ADCC of monocytes required only small amounts of antibody and a small number of effector cells.

We examined the inhibitory effect of fluid phase IgG on ADCC of monocytes and lymphocytes. Fig. 5 shows the inhibition of lymphocyte or monocyte-mediated lysis of anti-D coated red cells by fluid phase whole molecule IgG. ADCC of lymphocytes was not inhibited even at a concentration of 100 μg/ml, but ADCC of monocytes was markedly inhibited by small amounts of IgG (10-20 μg/ml). Very large amounts of whole molecule IgG were required to inhibit lysis by lymphocytes (Fig. 6). The inhibition by IgG was dose-dependent, and lymphocyte-mediated red cell lysis was

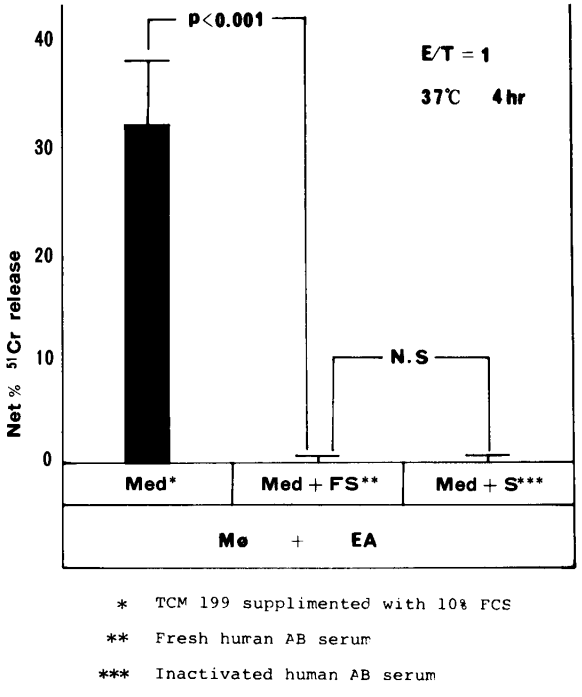

Fig. 7. Inhibition of monocyte-mediated lysis of anti-D coated red cells by human AB serum. Fresh and inactivated human AB sera were added to the medium to a final concentration of 10 %. Target red cells were sensitized with 4 × diluted anti-D serum.

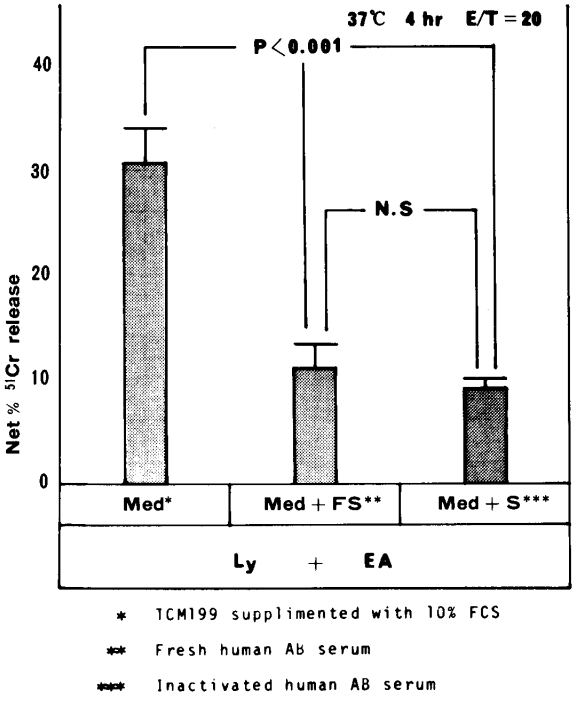

Fig. 8. Inhibition of lymphocyte-mediated lysis of anti-D coated red cells by human AB serum. Fresh and inactivated human AB sera were added to a final concentration of 10 %. Target red cells were sensitized with 4 × diluted anti-D serum.

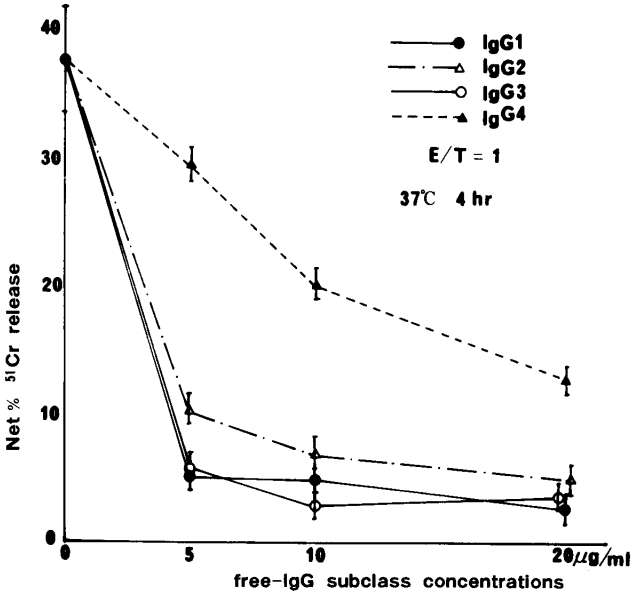

Fig. 9. Inhibition of monocyte-mediated lysis of anti-D coated red cells by free IgG subclasses. Target red cells were sensitized with 4 × diluted anti-D serum.

completely inhibited by high concentrations of IgG (5,000 μg/ml) (Fig. 6).

To determine the inhibitory effect of fresh human AB serum and heat-inactivated human AB serum, we studied the ADCC of both effector cells when either of these two sera were added at a final concentration of 10 % in the assay system. Monocyte-mediated lysis was completely inhibited by these two sera (Fig. 7). In contrast, lymphocyte-mediated lysis was only moderately inhibited by the same two sera (Fig. 8).

Finally, we investigated the inhibition of ADCC by IgG subclasses. Monocyte-mediated lysis of anti-D coated red cells was markedly inhibited by 20 μg/ml of IgG1 and IgG3. Equal concentrations of IgG2 moderately inhibited the lysis, but IgG4 at the same concentration produced minimal inhibition (Fig. 9).

In the same experiment, purified IgA (32 mg/dl) and IgM (4.0 mg/dl) did not inhibit lysis by monocytes (data not shown). Inhibition tests of monocyte erythrophagocytosis by fluid phase IgG subclasses yieldid results similar to those of the

TABLE 1. INHIBITION OF MONOCYTE-MEDIATED ERYTHROPHAGOCYTOSIS OF ANTI-D COATED RED CELLS BY FREE IgG SUBCLASSES.

				IgG subclasses**	% phagocytosis
Monocyte	+	EA*	+	(-)	75.0 ± 0.5 %
Monocyte	+	EA	+	IgG1	1.8 ± 0.7 %
Monocyte	+	EA	+	IgG2	8.3 ± 0.4 %
Monocyte	+	EA	+	IgG3	2.1 ± 0.3 %
Monocyte	+	EA	+	IgG4	32.0 ± 1.0 %

*: Normal human O, Rh-positive red cells sensitized with 4 × diluted anti-D serum.

**: adjusted to 20 μg/ml with medium.

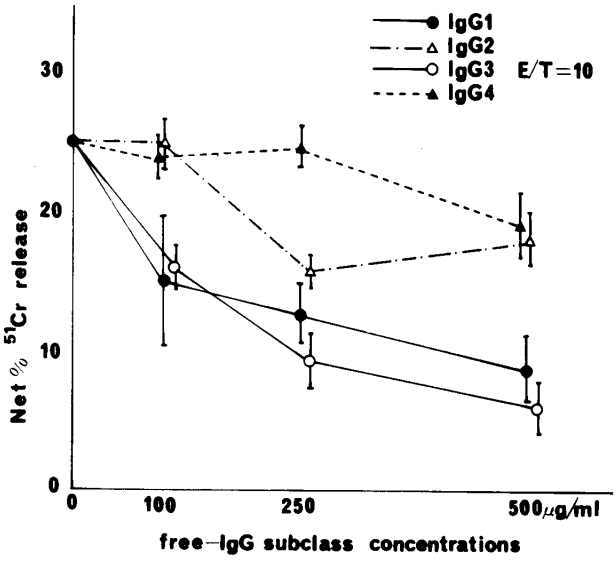

Fig. 10. Inhibition of lymphocyte-mediated lysis of anti-D coated red cells by free IgG subclasses. Target red cells were sensitized with 4 × diluted anti-D serum.

ADCC inhibition tests (Table 1). IgG1 and IgG3 inhibited more strongly the phagocytosis of anti-D coated red cells by monocytes than IgG2 and IgG4 (IgG2 > IgG4). The inhibitory effect of fluid phase IgG subclasses on antibody dependent lymphocyte-mediated lysis was similar to that on monocyte-mediated lysis, but larger amounts of IgG subclasses (above 500 μg/ml) were needed for inhibition (16).

DISCUSSION

Early studies indicated that human lymphocytes did not show ADCC toward human anti-D coated red cells (1-2). However, recent investigations have shown that ADCC of lymphocytes occurs if special combinations of target cells and antibodies are selected (3-6). We compared the ADCC activity between human lymphocytes and monocytes and showed that lymphocytes can produce lysis of anti-D coated red cells without particular target cells or antibodies. Lymphocyte preparations used in our experiments contained less than 0.5 % monocytes, so we could ignore the effect of contamination by monocytes. In our study, lymphocytes caused red cell lysis under the usual conditions, but larger amount of antibodies and a larger number of effector cells were needed for lysis than with monocytes (Fig. 3 and 4). These facts indicate that monocytes play a more important role in ADCC than lymphocytes *in vitro*. In the human body, however, there are so many circulating lymphocytes that a high enough E/T ratio might be reached for lymphocytes to play a role in destroying red cells in the acute phase of AIHA.

The results of the time course study of ADCC of both effector cells were in approximate agreement with the results of Shaw *et al.* (4). ADCC of lymphocytes occurred within 4 h, and monocyte-mediated lysis occurred slowly and was observed

upto 15 h (Fig. 1). These different time courses may be ascribable to the phagocytosis and postphagocytic ^{51}Cr release by monocytes.

It has been proposed that the ADCC of monocytes is due to either phagocytosis or extracellular cytolysis (7). Fleer *et al.* demonstrated in their studies using Cytochalasin B (CB), which is known to inhibit phagocytosis but enhance the release of lysosomal enzymes from monocytes, that the lysis of anti-D coated red cells by monocytes did not depend on phagocytosis, because CB enhanced the lysis of monocyte-mediated antibody-coated red cells (8). On the other hand, we have found that CB inhibited phagocytosis and extracellular lysis by monocytes and lymphocytes (data unpublished). The reason why such different results were obtained is not clear, but the differences might be due to the different amounts of CB used and the different experimental systems.

In our experiment, about 50 % postphagocytic ^{51}Cr release from monocytes was observed within 4-6 h. We have found in two patients with AIHA that ADCC of monocytes toward autologous red cells did not occur, even though significant phagocytosis of red cells was observed. The dissociation of phagocytosis and ADCC may depend on the quality or quantity of red cell bound antibodies, but it is not known exactly how much phagocytosis participates in the ADCC of monocytes.

Recent investigators have shown that ADCC of monocytes and lymphocytes is inhibited by fluid phase whole molecule IgG (3, 6). These inhibitory effects were presumed to be the results of competition between the fluid phase IgG and membrane-bound IgG antibody for binding to a limited number of Fc-receptors of effector cells. In these studies, large amounts of whole molecule IgG were required to inhibit ADCC of lymphocytes, but only small amounts of IgG completely inhibited ADCC of monocytes (3, 6). The results of our experiments agree with these observations.

To determine the effect of human serum containing large amounts of whole molecule IgG, we performed an inhibition study of ADCC using human serum. Figs 7 and 8 show that ADCC of monocytes was completely inhibited by AB serum, but ADCC of lymphocytes was only moderately inhibited. There was no difference between fresh and heat-inactivated serum. These results indicate that complement, other immunoglobulins, *i.e.*, IgM and IgA, and other unknown components of serum did not affect ADCC of either of the effector cells. Since monocyte ADCC was completely inhibited by 10 % human serum *in vitro*, it is doubtful that monocyte ADCC occurs *in vivo*, although it is reported that monocytes can produce significant red cell destruction in undiluted human serum if effector and target cells are mixed and centrifuged prior to incubation (9). As to *in vivo* interactions between monocytes and antibody coated red cells, kurlander *et al.* have postulated that antibody coated red cells are concentrated in macrophage-rich splenic cords by the process of plasma skimming, followed by the developement of intimate macrophage-red cell contact and subsequent red cell destruction in the reticuloendothelial system (10).

In our experiments, lysis by lymphocytes was moderately inhibited by the

addition of 10 % human AB serum. On the other hand, Kurlander *et al.* demonstrated that lysis by lymphocytes of sensitized red cells in the presence of undiluted human serum was equal to or greater than lysis in medium alone (3). They attributed this augmentation of lysis to unknown factors present in a fraction of the serum, separated on a Sephadex G-200 column. The precise nature of this component is unknown (3). They used autologous undiluted serum together with effector lymphocytes, but we used homologous human AB serum. This difference may be the cause of the disagreement between their results and ours. The finding that monocyte-mediated hemolysis is completely inhibited by serum, whereas lymphocyte-mediated hemolysis is only moderately inhibited or augmented, suggests that lymphocytes play a more important role in the destruction of red cells *in vivo* than was previously thought.

It has been reported by Engelfreit *et al.* (11) and Dacie (12) that IgG1 and IgG3 play a more important role in destroying red cells than other IgG subclasses. They concluded that if these antibody classes were bound to the red cell membrane, severe hemolytic anemia would develope *in vivo.* Many investigators have demonstrated the specificity of human monocyte Fc-receptors to the different IgG subclasses by inhibition tests using myeloma proteins (13, 14). In these studies, IgG1 and IgG3 preferentially bound to monocyte Fc-receptors, whereas the binding of IgG2 and IgG4 was much weaker.

In our experiments, the ADCC of both monocytes and lymphocytes was more strongly inhibited by IgG1 and IgG3 than IgG2 and IgG4 (Figs. 9-10). However, it has been reported that the latter two subclasses can bind to monocytes after aggregation (15). Furthermore, the red cells coated with IgG2 antibody were phagocytized by monocytes, but these interactions were completely inhibited by IgG1 and IgG3 (16). These facts suggest the presence of a single common Fc-receptor for all human IgG subclasses that triggers ADCC and phagocytosis.

It may be that IgG1 and IgG3 bind completely to Fc-receptors without modification of their Fc-portion, whereas IgG2 and IgG4 require modification of their Fc-portion to bind to Fc-receptors. More recently, it has been reported that human lymphocytes possess Fc-receptors for IgG3 but not IgG1 (17). In our study, however, ADCC of lymphocytes was inhibited by both IgG1 and IgG3 (Fig. 10). This result indicates that IgG1 and IgG3 have a high affinity for Fc-receptors on lymphocytes. Phagocytosis by monocytes also was inhibited by IgG1 and IgG3 (Table 1). These results are interesting, considering that IgG1 and IgG3 anti-erythrocyte antibody can produce overt hemolytic anemia *in vivo.*

Acknowlegement. I am grateful to Dr. Y. Namba for his help in the purification of IgG subclasses from patients with multiple myeloma.

REFERENCES

1. Holm, G.: Lysis of antibody-treated human erythrocytes by human leukocytes and macrophages

in tissue culture. *Int. Arch. Allergy Appl. Immunol.* **43**, 671-682, 1972.

2. Poplack, D.G., Bonnard, G.D., Holiman, B.J. and Blaese, R.M.: Monocyte-mediated antibody-dependent cellular cytotoxicity: A clinical test of monocyte function. *Blood* **48**, 809-816, 1976.
3. Kurlander, R.J. and Rosse, W.F.: Lymphocyte-mediated lysis of antibody coated human red cells in the presence of human serum. *Blood* **53**, 1179-1202, 1979.
4. Shaw, G.M., Levy, P.C. and LoBuglio, A.F.: Human lymphocyte, monocyte, and neutrophil antibody-dependent cell-mediated cytotoxicity toward human erythrocytes. *Cell. Immunol.* **41**, 122-133, 1978.
5. Shaw, G.M., Levy, P.C. and LoBuglio, A.F.: Human lymphocyte antibody-dependent cell-mediated cytotoxicity (ADCC) toward human red blood cells. *Blood* **52**, 696-704, 1979.
6. Kurlander, R.J., Rosse, W.F., and Ferreira, E.: Quantitative evaluation of antibody-dependent lymphocyte-mediated lysis of human red cells. *Am. J. Hematol.* **6**, 295-311, 1979.
7. Hersey, P.: Macrophage effector function. An *in vitro* system of assesment. *Transplantation* **15**, 282-290, 1973.
8. Urbaniak, S.J.: ADCC (K-cell) lysis of human erythrocytes sensitized with rheusus alloantibodies. 1. Investigation of *in vitro* culture *variables*. *Br. J. Haematol.* **46**, 447-453, 1980.
9. Kurlander, R.J. and Rosse, W.F.: Monocyte-mediated destruction in the presence of serum of red cells coated with antibody. *Blood* **54**, 1131-1139, 1979.
10. Kurlander, R.J., Rosse, W.F. and Logue, G.L.: Quantitative influence of antibody and complement coating of red cells on monocyte-mediated cell lysis. *J. Clin. Invest.* **61**, 1309-1319, 1978.
11. Engelfreit, C.P., Borne, A.E.G.Kr.V.D., Do Beckers and Van Loghem, J.J.: Autoimmune haemolytic anaemia: Serological and immunochemical characteristics of the antibodies; Mechanism of cell destruction. *Ser. Haematol.* **7**, 328-347, 1974.
12. Dacie, J.V.: Autoimmune hemolytic anemia. *Arch. Intern. Med.* **135**, 1293-1300, 1975.
13. Abramson, N., Gelfand, E.W., Jandle, J.H. and Rosen, F.S.: The interaction between human monocyte and red cell. Specificity for IgG subclasses and IgG fragments. *J. Exp. Med.* **132**, 1207-1215, 1970.
14. Huber, H. and Fudenderg, H.H.: Receptor sites of human monocytes for IgG. *Int. Arch. Allergy* **34**, 18-31, 1968.
15. Spiegel, H.L.: Biological activities of immunoglobulins of different classes and subclasses. *Adv. Immunol.* **19**, 259-294, 1974.
16. Ohlander, C., Larsson, A. and Perlman, P.: Monocyte mediation of immunoglobulin Fc receptor interactions; In *Macrophage-Mediated Antibody-dependent Cellular Cytotoxicity*, Immunology series 21, ed. H.S. Koren, Marcel Dekker, Inc. New York and Basel, pp. 97-112, 1983.
17. Zupanska, B., Maslanka, K. and Loghem, V.: Importance of IgG subclasses of antibodies for the detection of Fc-receptor bearing human lymphocytes. *Vox. Sang.* **43**, 243-247, 1982.