Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 5137–5141, October 1978
Immunology

Spontaneous and antibody-dependent cell-mediated cytotoxicity by human T cell subpopulations

(killer cells/effector T cells/immunoglobulin receptors/T cell subsets)

SUDHIR GUPTA, GABRIEL FERNANDES, MADHAVAN NAIR, AND ROBERT A. GOOD

Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Contributed by Robert A. Good, July 24, 1978

ABSTRACT **Human peripheral blood non-T cells, T cells, and their subpopulations (Tμ, Tγ, Tϕ, Tγ-depleted cells, and Tμ-depleted cells) were assayed for their capacity to mediate spontaneous lymphocyte-mediated cytotoxicity (SLMC) or natural killer activity against K562 tumor cell line and antibody-dependent cellular cytotoxicity (ADCC) against chicken erythrocytes coated with antibody. Non-T cells, unseparated T cells, Tγ cells, and Tμ-depleted (Tγ-enriched) cells were found to have both SLMC (N_K activity) and ADCC. Tμ, Tϕ, and Tγ-depleted cells had minimal or no SLMC and ADCC activity. This study demonstrates that SLMC and ADCC activity in T cells is mediated by Tγ cell subpopulations. These two cytotoxic reactions were either mediated by two distinct subsets of Tγ cells or by a single effector cell using two different mechanisms.**

Human T lymphocytes are subdivided, according to the presence of receptors for immunoglobulin, into T lymphocytes with receptors for IgM (Tμ) or for IgG (Tγ) and T cells lacking receptors for either IgM or IgG (Tϕ) (1). Tμ and Tγ cells have been the subject of recent reviews (2–4) and are distinct with regard to their morphology, histochemical characteristics, response to phytomitogen, sensitivity to irradiation and corticosteroids, distribution in organs, RNA content, chemotactic properties, and lymphokine production as well as their immunoregulatory role in differentiation of B cells to plasma cells (1, 5–11). An imbalance of proportions of Tμ and Tγ cells has been reported in both primary and secondary immunodeficiency disorders (12–18). Recently, abnormalities of antibody-dependent cell-mediated cytotoxicity (ADCC) and spontaneous lymphocyte-mediated cytotoxicity (SLMC) have been reported in patients with certain primary immunodeficiencies or with certain malignancies (19–25). The cell-mediated immune responses to tumors are quite complex because of the findings that T cells, non-T cells, and macrophages all may be involved (26). ADCC and SLMC have been shown to be mediated by lymphoid cell populations that are lacking surface immunoglobulin but bear Fc receptors (27).

A number of investigators have found that SLMC and ADCC generally exist in parallel. However, two distinct cytotoxicity mechanisms seem to be involved, implicating two separate populations of cells that are immunoglobulin negative and Fc receptor positive or separate mechanisms utilizing a single population of effector cells. Many investigators have reported that both ADCC and SLMC are mediated by cells having Fc receptors of high affinity but lacking other markers for either T or B lymphocytes (27–31). These cells have also been termed "K" cells, "null" cells, or "third population" cells. Recently, West *et al.* (32, 33) have demonstrated the presence of SLMC and ADCC activity in thymic-dependent lymphocytes that have low-affinity receptors for sheep erythrocytes (SRBC), forming rosettes optimally only at 4° and not at 29°. In this investigation, we examined T lymphocytes and their subpopulations that possess receptors for Fc (Tμ and Tγ) and those without Fc receptors (Tϕ) in SLMC and ADCC assay systems.

MATERIAL AND METHODS

Isolation of Mononuclear Cells. Heparinized peripheral venous blood was obtained from three healthy donors free from both hepatitis antigen and antibody. Mononuclear cells were isolated on a Ficoll/Hypaque density gradient. Cells were washed three times in Hanks' balanced salt solution and resuspended in RPMI-1640 containing 15% heat-inactivated fetal calf serum at a concentration of 4×10^6 cells per ml. Lymphocyte separator reagent (Technicon Instrument Co., Tarrytown, NY) was added to the mononuclear cell suspension at a volume ratio of 1:2 and the mixture was incubated at 37° on a rotator for 30 min. Phagocytic cells were then depleted by subsequent centrifugation at $400 \times g$ for 20 min. Lymphoid cells depleted of phagocytic cells were then collected from the interface and washed three times with balanced salt solution and resuspended in RPMI-1640 at 4×10^6 cells per ml.

Purification of T Lymphocytes. Aliquots (1 ml) of phagocytic cell-depleted lymphoid cells were mixed with 0.25 ml of fetal calf serum (heat inactivated and absorbed with SRBC) and 1 ml of 1% neuraminidase-treated SRBC (25 units/ml of 5% SRBC). The mixture was incubated at 37° for 5 min, centrifuged at $200 \times g$ for 5 min, and then incubated at 4° for 1 hr. The pellets were resuspended and left on ice for another 15 min. Rosetting T lymphocytes were separated from non-T lymphocytes on Ficoll/Hypaque density gradient by centrifugation at $480 \times g$ for 20 min at 20°. SRBC attached to T lymphocytes (pellet) were lysed with distilled water and double-strength minimal essential medium (Microbiological Associates, Bethesda, MD). T cells were washed three times in RPMI-1640 and were shown to contain 96–98% T cells as determined by rosette formation with SRBC and lack of surface immunoglobulin. Almost no peroxidase-positive cells were found in such preparations and viability was more than 98% as determined by trypan blue dye exclusion. Non-T cells contained 10% T cells, 60% B cells, and 30% third population or "K" cells.

Tγ cells were isolated either on the same day or after overnight incubation of T cells in medium containing 20% fetal calf serum; Tμ cells were isolated after overnight incubation of purified T cells in medium containing fetal calf serum. These populations were separated by using ox erythrocytes (ORBC) coated with anti-ORBC IgM or IgG antibody.

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; SLMC, spontaneous lymphocyte-mediated cytotoxicity; SRBC, sheep erythrocytes; CRBC, chicken erythrocytes; Tμ, T cells with receptors for IgM; Tγ, T cells with receptors for IgG; Tϕ, T cells without receptors for IgM or IgG; ORBC, ox erythrocytes; EA_m, erythrocytes coated with antierythrocyte IgM; EA_g, erythrocytes coated with antierythrocyte IgG; E-RFC, T cells forming rosettes with SRBC.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be herby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

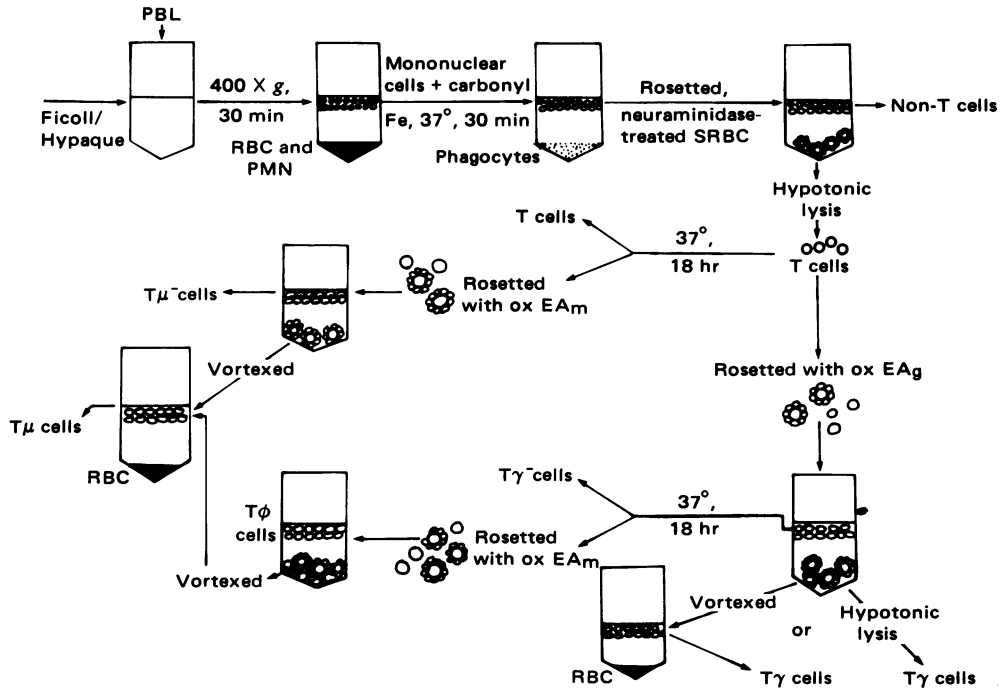

FIG. 1. Methods for purification of lymphocyte subpopulations. PBL, peripheral blood lymphocytes; PMN, polymorphonuclear leukocytes.

ORBC-Antibody Complexes. Purified rabbit IgM and IgG antiORBC antibodies were prepared as described (7). ORBC-antibody complexes were prepared by incubating an equal volume of 2% ORBC and anti-ORBC IgM (1:20 dilution) or IgG antibody (1:200 dilution) at room temperature for 90 min. The complexes, EA_m and EA_g, respectively, were washed three times with balanced salt solution and resuspended to a concentration of 1%.

Isolation of T Cell Subpopulations. The procedures are summarized in Fig. 1. Purified T cells (4×10^6 per ml) were incubated with an equal volume of 1% ox EA_g, or ox EA_m, centrifuged at $200 \times g$ for 5 min, and incubated at 4° for 60 min. The Tγ and Tμ cells formed rosettes with ox Ea_g and ox Ea_m, respectively. The pellets were resuspended, layered on Ficoll/Hypaque gradients and centrifuged at $400 \times g$ for 20 min, thus separating the rosetted ($T\gamma^+$ and $T\mu^+$) from nonrosetted ($T\gamma^-$ and $T\mu^-$) cells. Some of the $T\gamma^-$ and $T\mu^-$ cells were set aside. The remaining $T\gamma^-$ and $T\mu^-$ cells from the interface were then incubated with an equal volume of ox EA_m and ox EA_g, respectively, centrifuged at $200 \times g$ for 5 min, and incubated at 4° for 30 min. The pellets were resuspended, layered on Ficoll/Hypaque gradients, and centrifuged at $400 \times g$ for 20 min, separating rosetted (Tγ and Tμ) cells at the bottom and null T cells (Tφ) at the interface. The Tγ and Tμ rosetted cells were freed from ORBC by either vortexing and then separating on a Ficoll/Hypaque gradient or lysing the ORBC with distilled water and double strength medium. Isolated Tμ and Tγ cells had only 1–2% contamination with Tγ and Tμ cells, respectively. Non-T, T, Tμ, Tγ, Tφ, $T\gamma^-$ and $T\mu^-$ cells were then assayed for ADCC and SLMC activity.

Assay for ADCC. The ADCC activity was determined by the original method of Perlmann (34) as modified by Handwerger and Koren (35). Briefly, varying concentrations of cells under study were added to 50 μl of RPMI containing 5×10^4 ^{51}Cr-labeled chicken erythrocytes (CRBC) and 100 μl of a 1:10,000 final dilution of rabbit anti-CRBC antibody (Cappel Laboratories, Cochranville, PA). As a control, 100 μl of medium was added for every dilution of T cells in place of antiCRBC antibody. After centrifugation ($40 \times g$ for 2 min), the plates were incubated for 3 hr at 37° in 95% air/5% CO_2. Then the plates were centrifuged at $500 \times g$ for 5 min, and the ^{51}Cr released was collected in 100 μl of supernatant and counted in a well type Auto-Gamma scintillation spectrophotometer. The percentage of cytotoxicity was calculated by: $(E - C)/(T - S)$, in which E = counts released in experimental group, C = counts released in control group containing T lymphocyte cells under study, labeled CRBC, and medium instead of anti-CRBC antibody, T = total counts in 10^5 ^{51}Cr-labeled CRBC, and S = counts released by 10^5 ^{51}Cr-labeled CRBC in the presence of anti-CRBC antibody without cells.

Assay for SLMC. K562 myeloid tissue culture cell line (36), obtained from Y. B. Kim and maintained in RPMI-1640 with 10% fetal calf serum, was used as target cell after labeling with sodium [^{51}Cr]chromate (NEZ-0305, New England Nuclear) as described (37). A fixed number of viable, nucleated cells of the appropriate subpopulation in RPMI-1640 supplemented with 10% fetal calf serum, penicillin, streptomycin, and glutamine (Grand Island Biological Co., Grand Island, NY) were prepared in varying dilutions to give a 1:50, 1:25, 1:12.5, and 1:6.0 target ($1–2 \times 10^4$)/effector cell ratios. The leukocytes were added in triplicate to ^{51}Cr-labeled target cells in a total volume of 0.2 ml in V-bottom microfilter plates (1-220-25A, Microbiological Associates). After centrifugation ($40 \times g$ for 2 min), the plates were incubated at 37° in 5% CO_2 in air for 4 hr. Then the plates were centrifuged at $500 \times g$ for 5 min and 100 μl of supernatant was collected and assayed for ^{51}Cr released in a well-type Auto-Gamma scintillation spectrophotometer.

The percentage of cell lysis was calculated as: mean cpm released in the presence of effector cells minus mean cpm spontaneously released by target cells incubated with medium alone, divided by cpm released after treating target cells with Triton X-100 (1:100 dilution) minus cpm spontaneously released and the quotient multiplied by 100.

RESULTS

The results of analyses of SLMC by subpopulations of lymphocytes are shown in Fig. 2. Non-T lymphocytes (containing ~60% Ig-bearing B cells, ~10% T cells, and ~30% third population cells) yielded strong lysis of the target cells (K562).

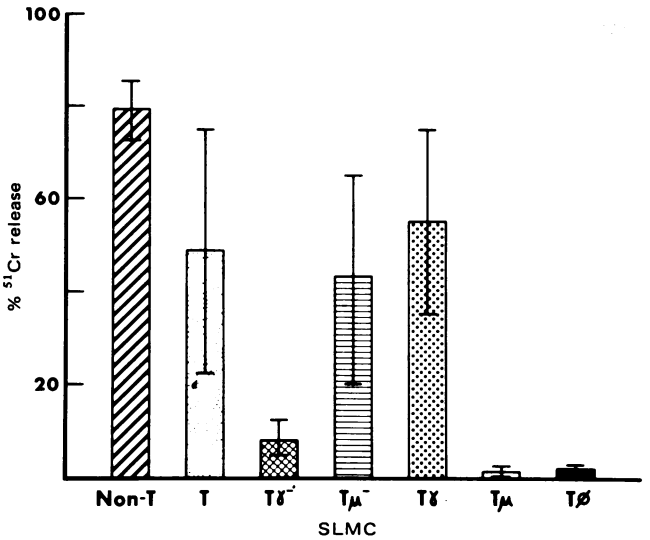

FIG. 2. SLMC in different peripheral blood lymphocyte subpopulations against K562. Mean ± SD for three experiments.

SLMC by purified T cells was 47%. When Tγ cells were removed from the purified T cells, the SLMC in the remaining T cells (Tγ⁻) was markedly decreased (~8%). However, depletion of Tμ cells (Tμ⁻) did not decrease SLMC significantly. Purified Tγ cells resulted in a greater SLMC (55%) than that exhibited by purified T cells. Tμ and Tφ produced almost no SLMC.

Results of ADCC by lymphocyte subpopulations against CRBC coated with antibody are shown in Fig. 3. Non-T cells caused approximately 70% lysis of target cells. Purified T cells resulted in 48% lysis; when Tγ cells were depleted from purified T cells, the remaining T cells (Tγ⁻) had almost no ADCC activity (~3% lysis). Tμ⁻ cells had enhanced ADCC activity (~65% lysis); Tγ cells, Tμ cells, and Tφ cells produced 8, 6, and 1% lysis, respectively.

Fig. 4 compares SLMC and ADCC by Tγ cells purified by two different methods: (*i*) by vortexing Tγ cells and then separating freed Tγ cells from the ORBC to which they had been attached, by Ficoll/Hypaque density gradient centrifugation; or (*ii*) by lysing ORBC with distilled water and double-strength medium (hypotonic lysis). The SLMC activity was similar in

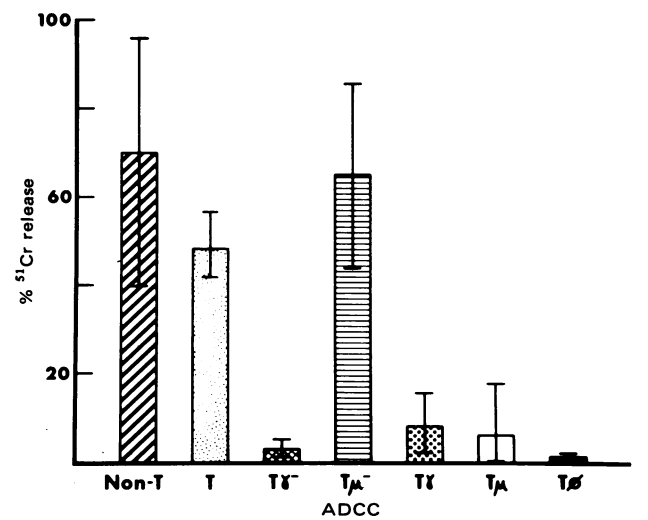

FIG. 3. ADCC in different peripheral blood lymphocyte subpopulations against CRBC coated with anti-CRBC IgG. Mean ± SD for three experiments.

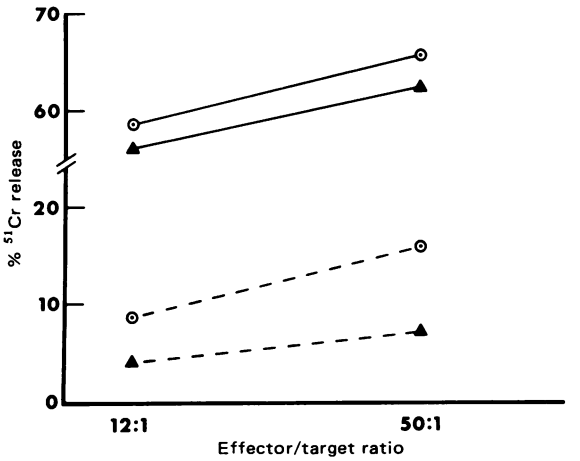

FIG. 4. Influence of method of detaching/lysing OxRBC from Tγ cells on SLMC (—) and ADCC (- - -). ⊙, Separation by vortexing; ▲, separation by lysis of erythrocytes.

both preparations of Tγ cells. However, ADCC activity was low in both preparations of Tγ cells, and no difference in ADCC activity among Tγ cells was obtained by the two different methods.

DISCUSSION

In this study we have demonstrated that both non-T cells and purified T lymphocytes may mediate both ADCC (against antibody-coated CRBC) and SLMC (against K562). When T cells are depleted of Tγ cells, both activities are lost almost completely. Depletion of Tμ cells did not either influence or enhance SLMC and ADCC activities, indicating that neither of the cytotoxic activities is mediated by Tμ and that these cytotoxicities are dependent on Tγ cells. Isolated Tμ cells and Tφ cells did not demonstrate significant ADCC or SLMC activity. Tγ cells demonstrated strong SLMC; however, this subpopulation of isolated T lymphocytes failed to demonstrate significant ADCC activity.

The failure of Tγ cells to demonstrate ADCC activity when assayed directly as an isolated population could be due to a number of factors. First, the treatment of Tγ cells with distilled water and double-strength medium or with Tris/ammonium chloride for lysing of attached ORBC may have inhibited ADCC (38). Second, the Tγ cells were used in ADCC assay soon after lysis and not enough time may have been allowed for regeneration of Fc receptors required for mediating the ADCC activity. Third, it is also possible that the IgG antibodies from the EA complex were still attached to the Fc receptors even after the ORBC were lysed, thus blocking the effector function of Fc receptors in ADCC. Furthermore, it is likely that, when Tγ cells are separated from EA_g by spontaneous dissociation at 37° over an 18-hr period, they lose their Fc receptors and therefore their ADCC activity. Of these possible explanations the first and last seem most likely. Cordier *et al.* (39) have also found that, when separated from EA by spontaneous dissociation at 37° for 18 hr and then washed three times in cold balanced salt solution, K cells do not form EA rosettes and lose their effector capacity in ADCC. Recently, Moretta *et al.* (40) have found that, after reacting with EA complexes and cultured overnight at 37°, Tγ cells lose their Fc receptors. In our experiments also, only a few Tγ cells were found to rosette a second time with ox EA_g, although all the cells readily bound SRBC. However, depletion data showing almost complete abrogation of ADCC in remaining T cells after removal of Tγ cells clearly link the ADCC to Tγ cells.

A number of studies have attempted to demonstrate

tumor-specific lymphocyte-mediated cytotoxicity in man as well as in experimental animals (41–43). Definition of the effector cells in ADCC and SLMC has been the subject of intensive investigations and much literature. However, much of the data are conflicting. Akira and Takasugi have shown that SLMC may be mediated by cytophilic antibodies (44) bound to effector cells possibly bearing B cell alloantigens (45). Eremin *et al.* (46) reported that immunoglobulins are involved in SLMC. They demonstrated abrogation of SLMC activity of depletion of cells that rosette with rabbit antihuman immunoglobulin-coated SRBC. Troye *et al.* (47) demonstrated that Fab fragments of rabbit anti-human immunoglobulin inhibit SLMC when added to the system. However, it has been shown, by experiments involving passing cells over nylon wool columns (48, 49) or through the columns coated with rabbit $F(ab')_2$ IgG reagents directed against $F(ab')_2$ of human IgG (50), that B cells are not necessary for SLMC. Nelson *et al.* (51), Pross and Jondal (27), and Jondal and Pross (48) have demonstrated that most of the SLMC is mediated by surface immunoglobulin-negative Fc receptor-positive lymphocytes. Pross and Jondal (27) and Pross *et al.* (49) also demonstrated that most of the SLMC activity is mediated by non-T, non-B lymphocytes (K cells or null cells). Chess and Schlossman (52) also reported SLMC and ADCC activity to be present in a null cell fraction but not in a B lymphocyte population. Study of patients with primary immunodeficiency disorders in which B lymphocytes are lacking and serum immunoglobulin levels are very low showed SLMC to be intact (19). This finding clearly demonstrates that (*i*) B cells are not essential for SLMC and (*ii*) immunoglobulins are not required for this form of cytotoxicity.

Recently, West *et al.* (33) demonstrated SLMC in a subpopulation of human T lymphocytes that had low-affinity receptors for SRBC. Our study clearly demonstrates that SLMC activity is mediated by T lymphocytes and that, among the T cell subpopulations, the activity is almost exclusively mediated by Tγ cells. Tμ and Tϕ cells lacked SLMC activity almost completely.

ADCC is considered synonymous with K or null (non-T, non-B cell) cell cytotoxicity. Most investigators have demonstrated the effector cells of ADCC to be negative for surface Ig and lacking receptors for SRBC but to have high-affinity Fc receptors and perhaps a weak receptor for C3. Pross and Jondal (27) showed marked reduction of ADCC activity when EAC rosette-forming cells were removed. Lack of B cell requirement in this assay is supported by the presence of normal ADCC in patients with X-linked agammaglobulinemia (19). West *et al.* (32) recently reported ADCC activity in a T-cell subpopulation with low-affinity receptors for SRBC. These low-affinity SRBC receptor-bearing T cells were found to have receptors for IgG. Perlmann *et al.* (30) have also presented evidence that K cells may have a relationship to SRBC rosette-forming cells. Our study clearly demonstrates that T cells can mediate ADCC against CRBC coated with IgG antibody. Among T cell subpopulations, almost all ADCC activity seems to be mediated by Tγ cells and no ADCC is found in Tμ or Tϕ cells. Depletion of Tγ cells from T cells profoundly reduced the ADCC activity but depletion of Tμ did not.

From our study and the studies reported by West *et al.* (32, 33) it is evident that significant ADCC and SLMC are mediated by a subpopulation of T cells with low-affinity receptors for SRBC and with receptors for IgG (Tγ). Studies by other investigators have demonstrated both ADCC and SLMC in K or null cell populations. It seems to us that ADCC and SLMC in so-called null cells may be mediated by a subpopulation that is a precursor of mature T cells. Chess and Schlossman (52) have demonstrated the presence of certain cells with T cell influence among the null cells.

Both ADCC and SLMC are mediated by Tγ cells and also by a population of non-T cells, most likely by the third population or K cells. It has not yet been shown whether these cytotoxic activities are mediated by two different subpopulations of Tγ cells or by the same subpopulations of T cells but by distinct mechanisms. Nelson *et al.* (51) have demonstrated that treatment of effector cells with trypsin significantly inhibited SLMC but did not inhibit ADCC. The inhibition of SLMC by trypsin is corrected by incubation of treated cells in RPMI-1640 containing 5% fetal calf serum. Treatment of fractions containing T cells forming rosettes with SRBC (E-RFC) with ammonium chloride solution to lyse SRBC profoundly inhibits ADCC, an influence that is corrected at least in part by incubation overnight in the absence of ammonium chloride. Three experiments in our study showed that purified Tγ cells obtained by lysing the attached ORBC with distilled water and double-strength medium (hypotonic lysis) had no effect on SLMC but profoundly inhibited ADCC. It has also been shown that protein A will block ADCC but not SLMC (53). Our study and that reported by Nelson *et al.* (51) demonstrate quite clearly that SLMC and ADCC are mediated by separate cytotoxic mechanisms but the findings are compatible with the view that either Tγ cells contain two distinct subsets of cytotoxic cells with separate functional capabilities or that a single effector cell exists that is capable of mediating two different cytotoxicity reactions. This perspective is further supported by the finding that suppressor factor(s) generated by concanavalin A-treated T lymphocyte subpopulations inhibited ADCC but not SLMC (unpublished observations).

West *et al.* (32) and Revillard *et al.* (54) have demonstrated that ADCC activity and low-affinity T cells forming rosettes with SRBC(E-RFC) are restricted to spleen and peripheral blood and little ADCC can be found in thymus and lymph nodes. This is in agreement with our present observation that Tγ cells mediate ADCC and Tγ cells are almost completely lacking in normal human thymus and lymph nodes (7).

The possible relevance of SLMC against tumor *in vivo* has remained speculative. Further studies of mechanisms of SLMC and their alterations in pathological states may be helpful in understanding the relationship of this form of cytotoxic activity to immune surveillance in man.

We thank Ms. Judith Miller, Mr. Joseph Cavallo, and Mr. Jerry Vogel for their skillful technical assistance. This work was supported in part by Grants CA-08748, CA-17404, CA-19267, AI-11843, NS-11457, and AG-00541 from the National Institutes of Health and by the Zelda R. Weintraub Cancer Fund, the Fund for Advanced Study of Cancer, the Earl M. Coleman Laboratories, and the Richard Molin Memorial Foundation.

1. Moretta, L., Webb, S. R., Grossi, C. E., Lydyard, P. M. & Cooper, M. D. (1977) *J. Exp. Med.* **146**, 184–200.
2. Gupta, S. (1978) *Clin. Bull.*, in press.
3. Gupta, S. & Good, R. A. (1978) in *Human Lymphocyte Differentiation: Its Application to Human Cancer*, eds. Serrou, B. & Rosenfeld, C. (North Holland, Amsterdam, Netherlands), pp. 367–374.
4. Gupta, S. (1978) *Pol. Arch. Med. Wewn.*, in press.
5. Grossi, C. E., Webb, S. R., Zicca, A., Lydyard, P. M., Moretta, L., Mingari, M. C. & Cooper, M. D. (1978) *J. Exp. Med.* **147**, 1405–1417.
6. Moretta, L., Ferrarini, M., Mingari, M. C., Moretta, A. & Webb, S. R. (1976) *J. Immunol.* **17**, 2171–2174.
7. Gupta, S. & Good, R. A. (1977) *Cell. Immunol.* **34**, 10–18.
8. Gupta, S. & Good, R. A. (1978) *Cell. Immunol.* **36**, 263–270.
9. Andreeff, M., Beck, J. D., Darzynkiewicz, Z., Traganos, F., Gupta, S., Melamed, M. R. & Good, R. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1923–1927.

10. Parrott, D. M. V., Good, R. A., O'Neill, G. J. & Gupta, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2392–2395.
11. Kapadia, A., O'Reilly, R. J., Good, R. A. & Gupta, S. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1365.
12. Gupta, S. & Good, R. A. (1977) *Clin. Exp. Immunol.* **30**, 222–228.
13. Gupta, S. & Good, R. A. (1978) *Cell. Immunol.* **39**, 18–26.
14. Gupta, S. & Good, R. A. (1978) *Clin. Immunol. Immunopathol.*, in press.
15. Gupta, S., Kapadi, A., Kapoor, N. & Good, R. A. (1978) *Ind. J. Med. Res.*, in press.
16. Moretta, L., Mingari, M. C., Webb, S. R., Pearl, E. R., Lydyard, P. M., Grossi, C. E., Lawton, A. R. & Cooper, M. D. (1977) *Eur. J. Immunol.* **7**, 696–700.
17. Moretta, L., Mingari, M. C., Moretta, A. & Lydyard, P. M. (1977) *Clin. Immunol. Immunopathol.* **7**, 405–419.
18. Beck, J. D., Mertelsmann, R., Haghbin, M., Good, R. A. & Gupta, S. (1978) *Proc. Am. Assn. Clin. Res.* **19**, 172.
19. Pross, H. F., Gupta, S., Good, R. A. & Baines, M. G. (1978) *Cell. Immunol.*, in press.
20. Rosenberg, E. B., McCoy, J. L., Green, S. S., Donnelly, F. C., Siwarski, D. F., Levine, P. H. & Herberman, R. B. (1974) *J. Natl. Cancer. Inst.* **52**, 345–352.
21. Behelak, Y., Banerjee, D. & Richter, M. (1976) *Cancer* **38**, 2274–2277.
22. Ting, A. & Tarasaki, P. I. (1974) *Cancer Res.* **34**, 2694–2698.
23. Pross, H. F. & Baines, M. G. (1976) *Int. J. Cancer* **18**, 593–604.
24. Koren, H. S., Amos, D. M. & Buckley, R. H. (1978) *J. Immunol.* **120**, 796–799.
25. Sanal, S. O. & Buckley, R. H. (1978) *J. Clin. Invest.* **61**, 1–10.
26. Herberman, R. B. (1974) in *Advances in Cancer Research*, eds. Klein, G. & Weinhouse, S. (Academic, New York), pp. 207–263.
27. Pross, H. F. & Jondal, M. (1975) *Clin. Exp. Immunol.* **21**, 226–235.
28. Wisloff, F., Froland, S. S. & Michaelsen, T. E. (1974) *Int. Arch. Allergy Appl. Immunol.* **47**, 139–154.
29. Cordier, G., Samarut, C., Brochier, J. & Revillard, J. P. (1976) *Scand. J. Immunol.* **5**, 233–242.
30. Perlmann, P., Biberfield, P., Larsson, A., Perlmann, H. & Wahlin, B. (1975) in *Membrane Receptors of Lymphocytes*, eds. Seligmann, M., Preudhomme, J. L. & Kourilsky, F. M. (North-Holland, Amsterdam, Netherlands), pp. 161–170.
31. Nelson, D. L., Bundy, B. M., Pitchon, H. E., Blaese, R. M. & Strober, W. (1976) *J. Immunol.* **117**, 1472–1481.
32. West, W., Boozer, R. B. & Herberman, R. B. (1978) *J. Immunol.* **120**, 90–95.
33. West, W., Cannon, G. B., Kay, H. D., Bonnard, G. D. & Herberman, R. B. (1976) *J. Immunol.* **118**, 355–364.
34. Perlmann, P. & Perlmann, H. (1970) *Cell. Immunol.* **1**, 300–315.
35. Handwerger, B. S. & Koren, H. S. (1976) *Clin. Immunol. Immunopathol.* **5**, 272–281.
36. Klein, E., Ben-Bassat, H. & Newmann, H. (1976) *Int. J. Cancer* **18**, 421–431.
37. Fernandes, G., Yunis, E. J. & Good, R. A. (1975) *Clin. Immunol. Immunopathol.* **4**, 304–313.
38. Yust, I., Smith, R. W., Wunderlich, J. R. & Mann, D. L. (1976) *J. Immunol.* **116**, 1170–1172.
39. Cordier, G., Samarut, C. & Revillard, J. P. (1977) *J. Immunol.* **119**, 1943–1948.
40. Moretta, L., Mingari, M. C. & Romanzi, C. A. (1976) *Nature (London)* **272**, 618–620.
41. Perlmann, P. & Holm, G. (1969) *Adv. Immunol.* **11**, 117–193.
42. Hellstrom, I., Hellstrom, K. I., Sjogren, H. O. & Warner, G. A. (1971) *Int. J. Cancer* **7**, 1–16.
43. Takasugi, M., Ward, P. H., Mickey, M. R. & Terasaki, P. I. (1972) *Natl. Cancer Inst. Monograph* **35**, 251–257.
44. Akira, D. & Takasugi, M. (1977) *Int. J. Cancer* **19**, 747–755.
45. Koide, Y., Takasugi, M. & Billing, R. (1977) *Eur. J. Immunol.* **7**, 731–736.
46. Eremin, O., Coombs, R. R. A., Plumb, D. & Ashby, J. (1978) *Int. J. Cancer* **21**, 42–50.
47. Troye, M., Perlmann, P., Pape, G. R., Spiegelberg, H. L., Naslund, I. & Gidlof, A. (1977) *J. Immunol.* **119**, 1061–1967.
48. Jondal, M. & Pross, H. F. (1975) *Int. J. Cancer* **15**, 596–605.
49. Pross, H. F., Baines, M. G. & Jondal, M. (1977) *Int. J. Cancer* **20**, 353–358.
50. Pape, G. R., Troye, M. & Perlmann, P. (1977) *J. Immunol.* **118**, 1919–1924.
51. Nelson, D. L., Bundy, B. M. & Strober, W. (1977) *J. Immunol.* **119**, 1401–1405.
52. Chess, L. & Schlossman, S. F. (1977) in *Contemporary Topics in Immunobiology*, ed. Werner, N. (Plenum, New York), pp. 363–379.
53. Kay, M. D., Bonnard, G. D., West, W. H. & Herberman, R. B. (1977) *J. Immunol.* **118**, 2058–2066.
54. Revillard, J. P., Samarut, C., Cordier, G. & Brochier, J. (1975) in *Membrane Receptors of Lymphocytes*, eds. Seligmann, M., Preudhomme, J. L. & Kourilsky, F. M. (North-Holland, Amsterdam, Netherlands), pp. 171–184.