Human Antibody-Dependent Cellular Cytotoxicity

ISOLATION AND IDENTIFICATION OF A SUBPOPULATION OF PERIPHERAL BLOOD LYMPHOCYTES WHICH KILL ANTIBODY-COATED AUTOLOGOUS TARGET CELLS

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ABSTRACT Antibody-dependent cellular cytotoxicity (ADCC), has been shown to be independent in vitro of thymus-derived lymphocytes, but the precise nature of the effector lymphocyte has not been fully clarified. To further study the identity of the ADCC effector cell type(s), peripheral blood leukocytes were purified by Ficoll-Hypaque density centrifugation and fractionated into surface immunoglobulin-positive [Ig(+)] and surface immunoglobulin-negative [Ig(−)] populations by chromatographic separation on Sephadex G-200 anti-human immunoglobulin columns. After column fractionation, the ADCC effector activity against antibody-coated autologous lymphocytes was predominantly and consistently found in the Ig(−) fraction.

This latter population was then further fractionated, by rosetting techniques, into two subpopulations. The first was depleted by lymphocytes with surface receptors for sheep red blood cells [E(+)] and the second was depleted of lymphocytes with receptors for sheep red blood cell-antibody-complement complexes [EAC-(+)]. Analysis of these populations showed that ADCC effector activity was predominantly a property of the Ig(−) lymphocytes which are E(−) but EAC(+). These lymphocytes have been referred to as "null lymphocytes" and probably represent a subset of bone marrow-derived (B) cells. In addition, variable and low levels of ADCC activity were observed in some Ig(+) populations (B cells). Further purification of the null cell population by filtration over nylon wool columns to reduce the number of contaminating latex ingesting monocytes did not reduce ADCC effector activity.

Isolated null cell ADCC effector activity was inhibited by either rabbit anti-human $F(ab)_2$ or normal pooled rabbit gamma globulin, but not by rabbit $F(ab)_2$ anti-human $F(ab)_2$ or media. This supports the contention previously suggested in studies using unfractionated lymphocyte populations that the ADCC effector cell recognizes the Fc portion of the antibody molecule. The variable and low level of activity noted in the Ig(+) populations is unexplained but possibly due to a variable population of null cell-derived Ig(+) lymphocytes within the whole Ig(+) population. In conclusion, these experiments demonstrate that, in vitro, the major ADCC effector activity of circulating human peripheral blood lymphocytes resides in the Ig(−), E(−), EAC-(+) subpopulation termed "null cells." Since it has been noted that in certain disease states, such as immunodeficiency syndromes, autoimmune disorders, and neoplasms, the percentage of this population of lymphocytes in the peripheral blood is elevated, it is speculated that these cells, perhaps through their ADCC function, may play an important pathophysiologic role in these diseases.

INTRODUCTION

Destruction of immunologically foreign cells is a major in vitro function of human peripheral blood lymphocytes. Two distinct mechanisms have been found by which lymphocytes directly manifest this cytotoxic function (1). The first, direct cell-mediated cytolysis, requires prior sensitization of the killer lymphocyte to the target cell and occurs in the absence of detectable antibody. Many studies have indicated that thymus-derived (T) [1] lymphocytes are the effector cells in this type of cytotoxicity (2–5). A second, more recently

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[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; B, bone marrow-derived; T, thymus-derived; TCC, transitional cell carcinoma.

studied mechanism, is termed "antibody-dependent cellular cytotoxicity" (ADCC) because the target cell must be coated with intact antibody or antigen-antibody complexes for effective cytolysis by the killer lymphocytes (6–9). Several studies have shown that the effector cell in ADCC is a non-T cell and does not require prior sensitization (10–13).

Attempts to identify the effector population within the non-T cell populations have yielded seemingly conflicting results. van Boxel et al. (10), using anti-θ antisera, suggested the possibility that the effector cell is a bone marrow-derived (B) cell or a subpopulation of B cells. Consistent with this theory, both Perlmann et al. (9) and Schirrmacher et al. (11) reported that the effector cells from mouse spleens are absorbed onto anti-immunoglobulin columns. Subsequently, Perlmann et al. (14) reported that mature B cells with a high concentration of surface immunoglobulin were inactive as ADCC effector cells. In contrast, Greenberg et al. (12), using Sephadex anti-immunoglobulin columns, found that the effector cells remained in the effluent and were not absorbed onto the anti-immunoglobulin columns. They further proposed, on the basis of sedimentation studies, that the effector cell is a nonphagocytic monocyte. Wisloff and Froland (13), using nylon wool columns with human lymphocytes, also noted that effector cell activity increased after B cell depletion and concluded that ADCC in man is independent of B cells.

To further study this problem of the nature of the ADCC effector cell in circulating human peripheral lymphocytes, we have utilized a series of cell separation techniques recently reported from this laboratory (15–17). When applied to human peripheral lymphocytes, these techniques allow for the preparation of relatively pure subpopulations of Ig(−), E(+), EAC(−), T cells; Ig(+), E(−), EAC(+) B cells; and a third population of Ig(−), E(−), EAC(+) null cells. In the present report, utilizing autologous human lymphocytes as target cells, we demonstrate that in vitro the null cell population contains the major effector lymphocytes in ADCC.

METHODS

Fractionation of lymphocytes into non-immunoglobulin-bearing and immunoglobulin-bearing populations. Human peripheral blood mononuclear cells were isolated from normal volunteers by Ficoll-Hypaque density centrifugation (18). To separate the whole population of mononuclear cells into surface immunoglobulin positive [Ig(+)] and immunoglobulin negative [Ig(−)] subpopulations, unfractionated cells were filtered on a Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) column to which purified rabbit anti-human $F(ab)_2$ was covalently coupled by cyanogen bromide (15). The cells passing through the column routinely contained less than 2% Ig(+) lymphocytes by immunofluorescence with a polyvalent fluoresceinated anti-Fab reagent. Essentially complete recovery of the column-bound cells [Ig(+) B cells] was achieved by competitive inhibition with 1% pooled human gamma globulin and subsequent elution (15). The cells were washed three times in media 199 (Microbiological Associates, Bethesda, Md.) + 5% fetal calf serum and brought to a final concentration of 2×10^6 cells/ml.

Fractionation of non-immunoglobulin-bearing lymphocytes [Ig(−)] by rosette techniques into E or EAC rosette-positive populations. This technique has been described in detail (16). To prepare a population of Ig(−), E(−) cells, the Ig(−) lymphocytes obtained after column separation were depleted of sheep RBC rosette-forming [E(+)] lymphocytes. After Ficoll-Hypaque centrifugation, the isolated Ig(−), E(−) cell population was washed three times and brought to a concentration of 2×10^6 cells/ml. This population which was termed the "null cell population," contained less than 2% Ig(+) cells, less than 4% E(+) cells, and varied between 50 and 90% EAC(+).

To prepare a population of Ig(−), E(+) cells, the Ig(−) effluent cells obtained after column fractionation were depleted of EAC rosetting cells in a similar manner, and the remaining cells were washed three times and brought to 2×10^6 cells/ml. This cell population was less than 2% Ig(+), less than 2% EAC(+), and 80–95% E(+) and was designated the "T cell population." With both the E and EAC rosette depletion techniques, total cell recovery was between 50 and 70%. In some experiments latex-ingesting mononuclear cells were removed by passage over a nylon wool column as previously described (5).

Anti-human lymphocyte antiserum. Adult rabbits were injected intravenously with 3×10^6 Ficoll-purified human lymphocytes on three occasions, 1 wk apart. The serum was harvested at 4 wk, heat-inactivated at 56°C for 30 min, and stored at −30°C.

Target cells. Autologous Ficoll-purified mononuclear cells, isolated as above, were also used as target cells. 1.5×10^7 cells were labeled with 0.15 ml of $Na_2{}^{51}CrO_4$ (292 mCi/mg) (New England Nuclear, Boston), for 30 min at 37°C with slow continued inversion. The antibody-labeled target cells and control cells were further washed twice more to remove excess unbound antibody and brought to a final concentration of 10^5 cells/ml.

ADCC assay. Into 10×75-mm tubes, 0.1 ml of ^{51}Cr-labeled target cells at 10^5 cells/ml was pipetted in triplicate. 0.2 ml of the appropriate suspension of killer cells was added, and the tubes were centrifuged at 1,000 *g* for 10 min and incubated at 37°C in 5% CO_2 overnight. After 16 h incubation, 1.7 ml of fresh media was added to each tube, the tubes centrifuged at 1,000 *g* for 10 min, and 1 ml of supernate was removed and assayed for γ radiation. Maximal ^{51}Cr release was determined by alternate freezing and thawing of target cells. Percent cytotoxicity was determined by the formula: percent cytotoxicity = [^{51}Cr released by experimental − ^{51}Cr released from control (spontaneous)]/[^{51}Cr released by freeze-thaw − ^{51}Cr released from control], and results were expressed as percent cytotoxicity±SEM.

Anti-human Fab immunoglobulin. Rabbit antibody against human immunoglobulin $F(ab)_2$ and rabbit $F(ab)_2$ anti-human $F(ab)_2$ were prepared as described (15).

RESULTS

Dependence of cytotoxicity upon effector-to-target ratio. To determine the quantitative requirement for effector cells, initial experiments were performed with variable numbers of unfractionated lymphocytes as ef-

fector cells against an aliquot of the same cells prepared as targets by ^{51}Cr labeling and sensitization with rabbit antibody. As seen in Table I, there is a direct relationship between the number of unfractionated effector cells and the amount of lysis. In the absence of effector cells, spontaneous ^{51}Cr release after 16 h average 15–30% of the total releasable counts. Control target cells, labeled in a similar manner with ^{51}Cr, but not sensitized with antibody, failed to be lysed by autologous effector cells.

Activity of immunoglobulin-bearing [Ig(+)] and non-immunoglobulin-bearing [Ig(−)] subpopulations. To determine whether ADCC effector activity is a property of Ig(+) or Ig(−) lymphocytes, Ficoll-purified unfractionated lymphocytes were divided into two aliquots. The first was assayed directly without further fractionation. The second aliquot was fractionated over a Sephadex G-200 anti-F(ab)$_2$ column. The unretained fraction (approximately 80% of the starting population) was less than 2% Ig(+) by direct fluorescent antibody technique. In contrast, the retained cells, which were then eluted with 1% human gamma globulin, were virtually all Ig(+) by the same technique. Each of the populations was washed three times and assayed for ADCC activity. As seen in Table II, the Ig(−) population, depleted of the Ig(+) cells, contained most of the ADCC activity present in the unfractionated population. In contrast, the activity of the Ig(+) population was variable, and when present, was always less than that of the corresponding whole population. Because the Ig(+) cells are eluted with 1% human gamma globulin, additional controls were performed in which both whole cells and Ig(−) lymphocytes were similarly incubated with 1% human gamma globulin. The results, after the cells were washed, indicated that the gamma globulin elution had no effect on the ADCC activity of the whole cells or the Ig(−) cells. Thus, the low and variable activity of the Ig(+) cells does not appear to be an artifact of the isolation procedure.

Effect of rosette depletion upon ADCC activity of Ig(−) cells. As seen above, it appears that the major part of the ADCC activity observed in the unfractionated population resides in the Ig(−) populations. It has been shown in previous studies that the Ig(−) effluent is heterogeneous: it contains both E(+) and (−) subsets. To determine which of these cell types contained the major ADCC effector cells, Ig(−) cells, after filtration through an anti-F(ab)$_2$ column, were divided into three aliquots. The first was depleted of E rosette-forming lymphocytes; the second was depleted of EAC rosette-forming lymphocytes; and the third received no further treatment. All three aliquots were then assayed for ADCC activity. As seen in Fig. 1, depletion of E(+) lymphocytes (T cells) from the Ig(−) populations markedly enriched the effector activity of the remaining null cells and monocytes. In distinct contrast, depletion of the EAC(+) cells (null cells and monocytes) essentially abolished ADCC effector activity. Thus, it appears that ADCC effector activity resides in the EAC(+) fraction of the Ig(−) population. This fraction is composed of 90% null cells and 10% latex-ingesting monocytes.

TABLE I

Dependence of Cytotoxicty on Effector Cell Concentration

Effector-to-target cell ratio	Percent cytotoxicity
40/1	42.3±4.7
20/1	33.6±2.9
10/1	23.8±2.6
5/1	17.3±1.9
1/1	3.4±2.3
0	0.6±0.5
40/1	−7.0±2.7*

10^4 ^{51}Cr-labeled, antibody-sensitized target lymphocytes were incubated with varying concentrations of autologous effector lymphocytes. After 16 h, the ^{51}Cr released into the supernate from triplicate cultures was assayed and expressed as the mean percent±SEM of the maximum release from controls. The freeze-thaw value was 273.6±6.2 and the spontaneous release value was 74.3±5.2.

* Target cells without antibody.

TABLE II

Fractionation of Effector Cells on Sephadex Anti-Immunoglobulin Columns

Exp.	Effector-to-target cell ratio	Percent cytoxicity		
		Unfractionated	Ig(−)	Ig(+)
1	40/1	42.3±3.0	61.8±3.8	31.0±3.3
2	20/1	26.6±3.4	52.7±2.7	10.9±2.4
3	40/1	25.5±3.7	31.4±1.7	1.4±1.6
4	40/1	30.8±3.0	ND	29.8±1.4
5	40/1	41.5±4.7	ND	21.0±2.3
6	40/1	20.6±4.0	ND	−12.0±1.9
7	40/1	69.0±1.9	51.2±3.4	ND
8	40/1	41.9±1.3	44.7±4.8	−5.7±2.6

Aliquots of Ficoll-purified lymphocytes were fractionated into Ig(−) and Ig(+) populations. Unfractionated, Ig(−) and Ig(+) effector cells were each incubated with 10^4 ^{51}Cr-labeled, antibody-sensitized autologous unfractionated target lymphocytes. In each experiment, incubation of effector cells with autologous target cells lacking sensitizing antibody failed to produce ^{51}Cr release above spontaneous background release. The mean freeze-thaw value in these experiments was 283±46 and the mean percent spontaneous release was 23.9. ND, not done.

Effect of nylon wool filtration. To determine whether ADCC activity was due to the monocyte population, the isolated null-monocyte preparation was depleted of nylon wool-adherent monocytes by passage over a nylon wool column. The activity of the nonadherent null cells, after passage through the nylon wool, was compared to the activity of the same population of null cells and monocytes before nylon wool filtration. After filtration, the percent of monocytes was reduced to less than 1% latex-ingesting cells. Despite this marked depletion of phagocytic cells, there was no effect, or possibly even enhancement, on the ADCC activity (Fig. 2). Thus, as demonstrated by this sequence of experiments ADCC activity parallels the content of Ig(−), E(−), nonphagocytic cells or null cells.

Inhibition of effector cells. Prior studies on mouse spleen ADCC effector cells have noted that these cells have a surface receptor for the Fc portion of IgG antibody, and if this receptor is blocked, ADCC activity is inhibited (1, 9, 12). To see if a similar mechanism was present in the autologous human system, the following experiment was performed: column effluent cells, from which the Ig(+) cells had been removed, were divided into four aliquots and incubated with equivalent concentrations of rabbit anti-human $F(ab)_2$, rabbit $F(ab)_2$ anti-human $F(ab)_2$, normal pooled rabbit gamma globulin, and media. After a 30-min incubation, the effector cells were washed three times and assayed for ADCC activity. As seen in Table III, incubation with either whole rabbit anti-human $F(ab)_2$ or pooled rabbit gamma globulin markedly inhibited the effector cells, while a similar concentration of rabbit $F(ab)_2$ anti-human $F(ab)_2$ had minimal effect. Thus, as in the mouse system, inhibition was dependent upon the presence of the Fc portion of the immunoglobulin molecule. This supports the concept that this population of Ig(−), E(−), effector cells is also characterized by an Fc surface receptor and furthermore, that this receptor plays a critical role in ADCC function.

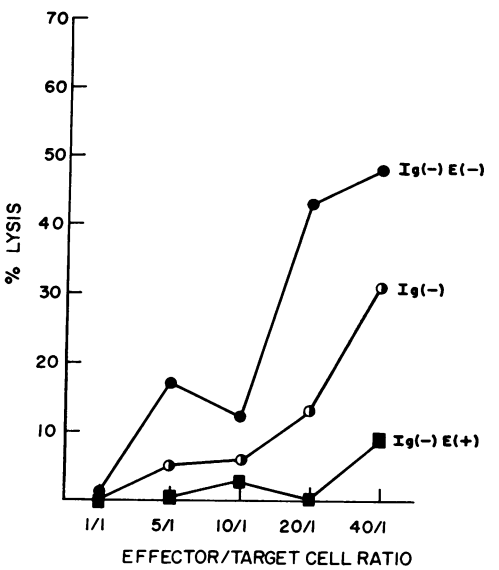

FIGURE 1 Effect of rosette depletion upon ADCC activity of Ig(−) cells. Aliquots of Ig(−) cells were separated into E(−) and E(+) populations by rosette depletion. Cells from each population were incubated with autologous unfractionated ^{51}Cr-labeled, antibody-sensitized target cells for 16 h. The results were calculated on the basis of a freeze-thaw value of 253±25 and a spontaneous release of 72±8.

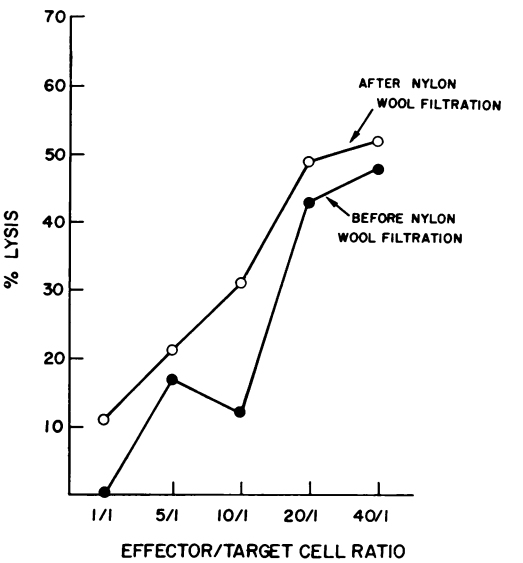

FIGURE 2 Effect of nylon wool filtration upon ADCC effector activity of the Ig(−), E(−) lymphocyte population. After nylon wool filtration, the percent of latex-ingesting monocytes was reduced from 10% to less than 1%. Cells from each population were incubated with autologous unfractionated ^{51}Cr-labeled, antibody-sensitized target cells for 16 h. The freeze-thaw value in this experiment was 373±51 and the spontaneous release 106±15.

DISCUSSION

Recent advances in the ability to isolate subpopulations of human peripheral blood lymphocytes has facilitated our understanding of their unique functions. The ability to make definitive interpretations, of course, depends in part upon the homogeneity of the isolated cell populations and the amount of cell loss during the isolation procedure. In the present studies we have isolated three subpopulations of human lymphocytes which are readily distinguished by surface characteristics: an Ig(−), E(−), EAC(+) (null cell) population; an Ig(+), E(−), EAC(+) (B cell) population; and an Ig(−), E(+), EAC(−) (T cell) population. Using these subpopulations, we have analyzed the problem of which

TABLE III

Inhibition Effector Cells by Immunoglobulins

Immunoglobulin	40:1*		20:1*		10:1*	
	Percent cytotoxicity	Percent inhibition	Percent cytotoxicity	Percent inhibition	Percent cytotoxicity	Percent inhibition
Media	44.7±3.7	0	28.5±2.6	0	18.4±1.9	0
Rabbit anti-human $F(ab)_2$	18.9±1.9	57.7	7.2±3.3	74.7	8.4±2.7	54.5
Rabbit $F(ab)_2$ anti-human $F(ab)_2$	38.1±3.4	14.8	21.5±3.5	24.6	20.7±20.7	0
Normal pooled rabbit gamma globulin	21.7±1.8	51.5	14.4±3.2	49.5	12.9±2.0	30

Aliquots if Ig(−) effector cells (20×10^6) were incubated with either 0.5 ml media, rabbit $F(ab)_2$ anti-human $F(ab)_2$, rabbit anti-human $F(ab)_2$, or normal pooled rabbit gamma globulin. All antibodies were diluted $\frac{1}{5}$ before incubation with effector cells. After 30 min incubation, the effector cells were washed three times and added to autologous ^{51}Cr-labeled, antibody-coated target cells.

* Effector-to-target cell ratio.

cell types function as effector cells in ADCC with homologous target cells.

Several points emerge from these experiments: (*a*) ADCC effector activity was found in the whole lymphocyte preparation from each normal individual studied. (*b*) After fractionation of lymphocytes, ADCC activity was not found to any significant degree in isolated T cell fractions. (*c*) In each case studied, the null cell fractions showed the highest activity and any procedure which enriched the null cell population increased the ADCC activity of the population. This was even true when macrophages were depleted from the null cells. (*d*) The activity of the B cell population was variable. In many experiments B cells were active but never to a degree comparable to null cells. In a number of experiments, no B cell killing was observed even when null cell killing was present.

This variability of B cell killing could have several explanations. There may be an ADCC effector cell subpopulation of B cells, independent of null cells, which is variably present. We have no further evidence to support this hypothesis. It is possible that variable contamination of the B cells by monocytes resulted in the observed ADCC activity. In these experiments, however, when monocytes were removed from the null cells, the remaining activity was enhanced. Thus, there is indirect evidence that monocytes are relatively inactive in this system. Unfortunately, attempts to remove monocytes from B cell populations depleted most (80%) of the B cells as well, so that the resulting drop in ADCC activity could be due to a loss of either cell type.

A more likely explanation may stem from recent work exploring the relation between null cells and B cells (19, 20). Studies of multiple in vitro functions of null cells and B cells have shown identical proliferation patterns in response to specific antigens and mitogens and in ADCC function with xenogeneic target cells. In addition, although null cells are Ig(−) by fluoresceinated antibody techniques, recent data obtained with a sensitive ^{125}I-Fab-anti-Fab binding radioimmunoassay has shown that null cells do have detectable immunoglobulin on their surface when compared to T cells, although 1/50–1/100 the quantity on B cells. Furthermore, when placed in culture, a subset of the null cell population develops surface immunoglobulin as assayed by the fluoresceinated antibody technique and secretes immunoglobulin into the medium (19, 20). In addition, preliminary data show that by day 3 in culture, when more than 50% of the null cells are Ig(+), the null cell population still retains its ADCC effector activity.

Taken together, this data suggests two alternative relationships of null cells and B cells: (*a*) The null cell is an immature form of the B cell. It initially has low surface immunoglobulin and very efficient ADCC activity. As it matures into a B cell, it passes through a stage in which it has increasingly large amounts of surface immunoglobulin and ADCC activity, and finally reaches an end stage of maximal surface immunoglobulin but little or no ADCC activity. (*b*) A second alternative is that the null cell develops into a distinct subpopulation of B cells which is characterized by both ADCC activity and large amounts of surface immunoglobulin. Thus, this alternative necessarily implies the existence of at least two subpopulations of mature B cells, one with and one without ADCC activity. Both postulates are consistent with the observation that null cells develop into Ig(+) cells when in culture. Both also postulate the existence of a null cell derivative that is both (Ig(+) and ADCC(+).

If such cells were present in vivo in peripheral blood, they would probably segregate with the whole B cell population during our fractionation precedures. Hence, the variability of B cell killing previously described may really be a reflection of the percentage of these

cells which are circulating in any donor at the time of phlebotomy. Further studies of null cell-B cell relationships are in progress. For the present, to avoid semantic arguments, it seems fair to conclude that ADCC effector activity in human peripheral blood lymphocytes is a property of null cells and their progeny. The importance of the circulating monocyte is unclear, but in view of this and prior work (13, 21) in which depletion of monocytes enhanced the ADCC activity of the residual population, it appears that circulating monocytes play a minor role in vitro with regard to this immunologic function.

ADCC killing of target cells has now been demonstrated against xenogeneic red cells (9), cultured tumor cell lines (22), allogeneic myeloblasts (23), virus-infected cells (24, 25), allogenic (26), and now autologous lymphocytes. In addition, recent studies have also shown that patients with transitional cell carcinoma (TCC) can develop antibody which specifically renders cultured TCC cell lines susceptible to ADCC killing by patient lymphocytes (27, 28). Thus, evidence is mounting that ADCC is an ubiquitous immune cytotoxic mechanism, at least in vitro. Additionally, it has been noted that in certain disease states, such as immunodeficiency syndromes, autoimmune disorders, and neoplasms, the percentage of null cells in the peripheral blood is elevated (29). The emerging concept of these null cells as major effectors in ADCC suggests the possibility that null cells, perhaps through their ADCC function, play an important pathophysiologic role in these diseases. These speculations, as well as the biological significance of null cells and ADCC, require further study.

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REFERENCES

1. Cerottini, J., and K. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. *Adv. Immunol.* **18**: 67–132.
2. Cerottini, J. C., A. A. Nordin, and K. T. Brunner. 1970. Specific *in vitro* cytotoxicity of thymus-derived lymphocytes sensitized to alloantigens. *Nature (Lond.).* **228**: 1308–1309.
3. Wagner, H., A. W. Harris, and M. Feldmann. 1972. Cell mediated immune response *in vitro*. II. The role of thymus and thymus-derived lymphocytes. *Cell. Immunol.* **4**: 39–50.
4. Häyry, P., L. C. Anderson, S. Nordling, and M. Virolainen. 1972. Allograft response *in vitro*. *Transplant. Rev.* **12**: 91–140.
5. Sondel, P., L. Chess, R. MacDermott, and S. Schlossman. 1975. Immunologic functions of isolated human lymphocyte subpopulations. III. Specific allogeneic lympholysis mediated by human T cells alone. *J. Immunol.* **114**: 982–987.
6. Moller, E. 1965. Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens. *Science (Wash. D. C.).* **147**: 873–879.
7. Granger, G. A., and W. P. Kolb. 1968. Lymphocyte *in vitro* cytotoxicity mechanisms of immune and non-immune small lymphocyte mediated target L cell destruction. *J. Immunol.* **101**: 111–120.
8. MacLennan, I. C. M., and G. Loewi. 1968. Effect of specific antibody to target cells on their specific and non-specific interactions with lymphocytes. *Nature (Lond.).* **219**: 1069–1070.
9. Perlmann, P., H. Perlmann, and H. Wigzell. 1972. Lymphocyte mediated cytotoxicity *in vitro*. Induction and inhibition by humoral antibody and nature of effector cells. *Transplant. Rev.* **13**: 91–114.
10. van Boxel, J. A., J. D. Stobo, W. E. Paul, and I. Green. 1972. Antibody-dependent lymphoid cell-mediated cytotoxicity: no requirement for thymus-derived lymphocytes. *Science (Wash. D. C.).* **175**: 194–195.
11. Schirrmacher, V., B. Rubin, P. Golstein, H. Wigzell, and B. Andersson. 1973. Cytotoxic immune cells with specificity for defined soluble antigens. III. Separation from helper cells and from antibody-forming cell precursors. *Transplant. Proc.* **5**: 1447–1450.
12. Greenberg, A. H., L. Hudson, L. Shen, and I. M. Roitt. 1973. Antibody-dependent cell-mediated cytotoxicity due to a "null" lymphoid cell. *Nat. New Biol.* **242**: 111–113.
13. Wisløff, F., and S. S. Frøland. 1973. Antibody-dependent lymphocyte-mediated cytotoxicity in man: no requirement for lymphocytes with membrane-bound immunoglobulin. *Scand. J. Immunol.* **2**: 151–157.
14. Perlmann, P., H. Wigzell, P. Golstein, E. W. Lanun, A. Larsson, C. O'Toole, H. Perlmann, and E. A. J. Svedmyr. 1974. Cell mediated cytolysis in vitro. Analysis of active lymphocyte subpopulations in different experimental systems. *Adv. Biosci.* **12**: 71.
15. Chess, L., R. P. MacDermott, and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. *J. Immunol.* **113**: 1113–1121.
16. Chess, L., R. MacDermott, P. Sondell, and S. Schlossman. 1974. Isolation of cells involved in human cellular hypersensitivity. *Prog. Immunol.* **3**: 125–132.
17. MacDermott, R., L. Chess, and S. Schlossman. 1975. Immunologic functions of isolated human lymphocyte subpopulations. V. Isolation and functional analysis of surface Ig negative E rosette negative subset. *Clin. Immunol. Immunopathol.* In press.
18. Boyum, A. 1968. Separation of leucocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* **21** *Suppl. 97*: 1–109.
19. Ryser, J-E., and P. Vassalli. 1974. Mouse bone marrow lymphocytes and their differentiation. *J. Immunol.* **113**: 719–728.
20. Chess, L., H. Levine, R. P. MacDermott, and S. F. Schlossman. 1975. The maturation of human Null cells into B cells. *Fed. Proc.* **34**: 1031. (Abstr.)
21. Forman, J., and G. Möller. 1973. The effector cell in antibody-induced cell mediated immunity. *Transplant. Rev.* **17**: 108–149.

22. MacLennan, I. C. M., G. Loewi, and B. Harding. 1970. The role of immunoglobulins in lymphocyte-mediated cell damage, in vitro. I and II. *Immunology.* **18:** 397–412.
23. Hersey, P., I. C. M. MacLennan, A. C. Campbell, R. Harris, and C. B. Freeman. 1973. Cytotoxicity against human leukaemic cells. I. Demonstration of antibody-dependent lymphocyte killing of human allogeneic myeloblasts. *Clin. Exp. Immunol.* **14:** 159–166.
24. Shore, S. L., A. J. Nahmias, S. E. Starr, P. A. Wood, and D. E. McFarlin. 1974. Detection of cell-dependent cytotoxic antibody to cells infected with herpes simplex virus. *Nature (Lond.).* **251:** 350–352.
25. Rager-Zisman, B., and B. R. Bloom. 1974. Immunological destruction of herpes simplex virus I infected cells. *Nature (Lond.).* **251:** 542–543.
26. Hersey, P., P. Cullen, and I. C. M. MacLennan. 1973. Lymphocyte-dependent cytotoxic antibody activity against human transplantation antigens. *Transplantation (Baltimore).* **16:** 9–16.
27. O'Toole, C., V. Stejskal, P. Perlmann, and M. Karlsson. 1974. Lymphoid cells mediating tumor-specific cytotoxicity to carcinoma of the urinary bladder. *J. Exp. Med.* **139:** 457–466.
28. Hakala, T. R., P. H. Lange, A. E. Castro, A. Y. Elliott, and E. E. Fraley. 1974. Antibody induction of lymphocyte-mediated cytotoxicity against human transitional-cell carcinomas of the urinary tract. *N. Engl. J. Med.* **291:** 637–641.
29. Frøland, S., and J. B. Natvig. 1973. Identification of three different human lymphocyte populations by surface markers. *Transplant. Rev.* **16:** 114–162.