

INHIBITION OF ANTIBODY-DEPENDENT CELLULAR
CYTOTOXICITY AND IMMUNOGLOBULIN
SYNTHESIS BY AN ANTISERUM PREPARED AGAINST A
HUMAN B-CELL Ia-LIKE MOLECULE*

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A subset of human peripheral blood lymphocytes is cytolytic for a variety of antibody-coated target cells. This cytotoxic phenomenon occurs without complement (C) and is called antibody-dependent cellular cytotoxicity (ADCC).¹ Several points emerge from extensive studies of ADCC: (a) The predominant effector lymphocyte is not a T cell and does not bear readily detectable amounts of surface-bound immunoglobulin (SIg) (1-5). (b) Although macrophages are effector cells in some systems (6, 7), the lymphocyte effector is nonphagocytic, does not adhere to nylon wool, and does not differentiate into macrophage-like cells in culture (5, 8, 9). (c) The effector lymphocyte bears Fc and C receptors. Although the interaction between the Fc receptor and Fc portion of the antibody bound to target cells is required to initiate the cytolytic reaction (9-11), the role, if any, of the C receptor in the triggering of ADCC is unknown.

In a model system using chromium-labeled human lymphocytes and rabbit antihuman lymphocyte sera to coat these target cells, we have previously shown that the ADCC effector cell population in humans can be enriched and isolated by a combination of separation procedures including: anti-Fab immunoabsorbent column chromatography to remove immunoglobulin-bearing B cells, E rosette depletion to remove T cells, and nylon adherence to remove phagocytic cells (5, 12). The resulting population which is SIg negative and E rosette negative, has been termed "Null" cells. This subpopulation is responsible for most of the ADCC activity present in peripheral human blood. Furthermore, a subset of this Null cell population, on continued culture, matures to develop surface immunoglobulins and the capacity to secrete immunoglobulins (13). Whether the same subset of the Null cell population possesses both ADCC activity and the potential for immunoglobulin synthesis, was not determined in these prior studies.

To resolve this question, we used in the current studies, a rabbit antiserum raised to a purified glycoprotein complex of mol wt 23,000 and 30,000 (p23,30), which was isolated from a human lymphoblastoid B-cell line (14). This antise-

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¹ *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; ALS, antihuman lymphocyte sera; G/R Fab, goat antirabbit Fab; NRS, normal rabbit serum; SIg, surface immunoglobulin.

rum (anti-p23,30) reacts with all SIg-positive B cells and with a subset of human Null cells. The serum does not react with human T cells, thymocytes, or platelets and has functional properties analogous to murine anti-Ia sera (14). Our data show that depletion of p23,30-bearing cells by C-mediated lysis or on cellular immunoabsorbent columns, markedly reduces ADCC reactivity and abrogates the production of immunoglobulin in cell culture. Moreover, specific binding of anti-p23,30 serum to the cell surface inhibits ADCC. These results thus provide evidence that the same Null subset of cells destined to differentiate into B cells is responsible for ADCC and, perhaps more importantly, suggest a role of Ia antigens in this form of cell-mediated cytotoxicity.

Materials and Methods

Preparation of Antisera. The isolation and purification of p23,30 from the human lymphoblastoid B-cell line IM-1 and the preparation of anti-p23,30 serum is described in the accompanying paper (14). This antiserum reacts with all SIg-positive B lymphocytes, with virtually no human T cells and with approximately 20% of cells within the Ig-negative, E rosette-negative subpopulation (Null cells) (14). Antihuman lymphocyte sera (ALS), used to coat target cells for the ADCC reaction, was prepared by injecting adult male rabbits intravenously with purified human lymphocytes (3×10^6) on three occasions at 1-wk intervals and bleeding at 6 wk. The preparation and iodination of rabbit antihuman Fab has been previously described (5, 15). Antisera against sheep erythrocytes were prepared by immunizing adult rabbits with sheep erythrocytes (10^8) (Microbiological Associates, Bethesda, Md.) intravenously three times at 0, 1, and 4 wk, with bleeding at 6 wk. Fetal calf serum was obtained from Microbiological Associates. All serum were heat inactivated at 56°C for 45 min.

Analysis of Surface Properties of Human Lymphocyte Subsets. SIg was detected with fluoresceinated rabbit antihuman Fab in the direct fluoresceinated antibody technique (15). The percentage of lymphocytes forming spontaneous rosettes with sheep erythrocytes (E rosettes) (Microbiological Associates) and sheep erythrocytes coated with antisheep erythrocyte antibody and C components 1, 4, 2, and 3 (EAC cells) (Cordis Laboratories, Miami, Fla.) was determined as previously described (12). For determination of EA-rosetting cells a 0.5% suspension of sheep erythrocytes was incubated with a 1:10 dilution of antisheep erythrocyte antibody at room temperature for 1 h followed by washing three times in Media 199 with 5% fetal calf serum. The resulting EA reagent was brought to a 0.5% concentration and incubated with 2×10^5 lymphocytes in 0.1 cm³ at room temperature for 1 h. For E, EA, and EAC rosette determinations a lymphocyte having three or more adherent erythrocytes was counted as having formed a rosette.

Fractionation of Lymphocytes into T, B, and Null Subpopulations. Human peripheral blood mononuclear cells were isolated from normal volunteers by Ficoll-Hypaque density centrifugation. Whole populations of mononuclear cells were separated into SIg-positive (>98%) and SIg-negative (<2%) subpopulations by Sephadex G-200 antihuman Fab column immunoabsorbent chromatography as previously described (15). The nonimmunoglobulin-bearing lymphocyte population was further fractionated into E rosette-positive and E rosette-negative subsets by the formation and subsequent depletion of E rosette-forming cells over a Ficoll-Hypaque density gradient (12). The E rosette-depleted subpopulation (Null cells) contained less than 2% Ig-positive cells, less than 4% E rosette-positive cells, and varied between 30 and 60% EAC-positive cells. Similarly, highly purified T cells ($\geq 92\%$ E positive) were prepared by EAC depletion of the SIg-negative population (5, 12). All cell populations were washed four times and resuspended in final media (Media 199) with 10% fetal calf serum, 200 mM glutamine, and 1% penicillin-streptomycin (Microbiological Associates).

ADCC. The ADCC assay has been previously reported (5). In brief, purified mononuclear cells were coated with ALS (1/50 dilution) and used as target cells. 1.5×10^6 cells were labeled with 0.15 ml of [⁵¹Cr]sodium chromate (292 μ Ci/ml) (New England Nuclear, Boston, Mass.), for 30 min at 37°C with slow, continuing inversion. The antibody-labeled target cells and control cells (not treated with antibody) were washed twice to remove excess, nonbound antibody and brought to a final concentration of 10^5 cells/ml in final media. Labeled target cells were pipetted in triplicate

into 10 x 75 mm tubes. The appropriate suspension of killer cells (0.2 cm^3) was added and the tubes centrifuged at 400 *g* for 10 min and incubated at 37°C in a 5% CO_2 humid atmosphere overnight. After incubation, 1.7 ml of fresh media was added to each tube and the tubes were centrifuged at 400 *g* for 10 min and 1 ml of supernatant solution was removed and assayed in a gamma counter. Maximal ^{51}Cr released was determined after freeze-thawing target cells. Percent cytotoxicity was determined by the formula:

$$\text{Percent cytotoxicity} = \frac{{}^{51}\text{Cr released by experimental} - {}^{51}\text{Cr released from control}}{{}^{51}\text{Cr released by freeze-thaw} - {}^{51}\text{Cr released from control}}$$

Determination of Ig Production in Cell Culture. Polyvalent Ig synthesis was determined by a sandwich radioimmunoassay (13). Briefly, unfractionated and fractionated cells were incubated in final media at 37°C in a 5% CO_2 humid atmosphere for 6 days. Supernatant solutions were then analyzed for Ig content by reaction with antihuman Fab covalently coupled to Sephadex G-25. To 0.2 ml of the coupled Sephadex G-25 anti-Fab beads were added in triplicate either 0.5 ml of tissue culture supernates, 0.5 ml of final media or 0.5 ml of varying concentrations of human Ig (Miles Laboratories Inc., Miles Research Products, Elkhart, Ind.). After 90 min incubation at room temperature, the beads were washed three times and 0.1 ml of ^{125}I -labeled rabbit antihuman Fab was added. After an additional 1 h incubation, the tubes were washed three times and counted in a gamma counter. After subtraction of background counts (tubes with media alone) the amount of Ig was determined by reference to a standard curve. As previously reported, ^{125}I bound varied linearly with the log concentration of human Ig present in the range of Ig concentration between 10 and 500 ng (13).

Physical Depletion of Cells on Goat Antirabbit Fab Cellular Immunoabsorbents. Purified goat antirabbit Fab (G/R Fab) was prepared, passed through a human Ig-Sepharose column to remove cross-reacting antibodies to human Ig, and covalently linked to Sephadex G-200 by methods identical to those used for the preparation of antihuman Fab and antimouse Fab columns (15, 16). The G/R Fab cellular immunoabsorbent was used to deplete human lymphocyte subpopulations which had specific rabbit antibodies bound to their surface. Thus, 60×10^6 cells were incubated with either heat-inactivated normal rabbit serum (NRS) (dilution 1/20) or rabbit anti-p23,30 (dilution 1/20) for 1 h at room temperature. Cells were washed four times, in final media containing 2.5 mM EDTA and applied to the G/R Fab columns using a flow rate of 5 ml/min. Control cells incubated in the presence of NRS and then washed were not retained.

Results

The Effector Cells and Quantitative Parameters of ADCC. The quantitative parameters and effector lymphocyte populations important in ADCC are illustrated in Fig. 1. Using optimal concentrations of antilymphocyte sera (1:50 dilution) added to ^{51}Cr -labeled human lymphocyte target cells, maximal cell lysis is usually seen with an effector to target cell ratio of 40:1. Significant lysis is usually present at ratios as low as five effector cells to one target cells. In previous studies, it was shown that most of the ADCC activity within the peripheral lymphocyte population is due to the subset of lymphocytes which are E rosette negative and do not bear immunoglobulins on their surface. A representative experiment showing these points is depicted in Fig. 1. As can be seen, experimental procedures which deplete either the E rosette-positive cells (T cells) or the SIg-bearing cells from an unfractionated lymphocyte population increases ADCC activity. The predominant ADCC effector resides in the SIg-negative, E rosette-negative subset. In contrast, purified populations of either Ig-positive B cells or E rosette-positive T cells are relatively inefficient or inactive in this ADCC system (5).

Depletion of ADCC Effector Activity by Removal of p23,30-Bearing Lymphocytes. Since previous studies have shown that approximately 15–30% of cells

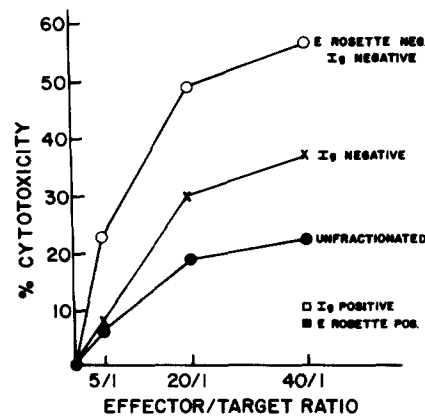


FIG. 1. Antibody-mediated cellular cytotoxic activity of isolated human lymphocyte subpopulations. Effector cells were incubated with 10^4 ^{51}Cr -labeled antibody-coated lymphocytes for 16 h and the results expressed as percent cytotoxicity of triplicate cultures at each effector to target ratio. The freeze-thaw value was 313 ± 16 and the spontaneous release was 56 ± 13 . The ^{51}Cr released from target cells not incubated with antibody was virtually identical to the spontaneous release.

within the Ig-negative, E rosette-negative fraction of human lymphocytes bear p23,30 on their surface, it was important to know whether it was those cells which were the effector cells of ADCC. In initial experiments we asked whether treatment of effector cell populations with antibody p23,30 in the presence of C could deplete ADCC activity. Lymphocyte subsets were incubated in the presence of media or of NRS (dilution 1:20) or of anti-p23,30 serum each with C for 2 h at room temperature. All cell populations were washed exhaustively and tested for their ability to trigger the ADCC reaction. The results of a number of such experiments on a variety of effector lymphocyte populations is shown in Table I. In every experiment, a single treatment of cells with anti-p23,30 serum and C diminished and in some cases totally eliminated ADCC reactivity. As can be seen, NRS had minimal to no effect relative to the media and C controls.

Whether the p23,30 antigen was important for the triggering of the ADCC reaction was addressed in the next series of experiments in which cells were incubated without a C source either in the presence of NRS or anti-p23,30 serum and tested for ADCC activity. As shown in Table II, the presence of anti-p23,30 serum but not NRS during the effector phase abrogated ADCC reactivity. These results suggested that not only is p23,30 antigen present on ADCC effector cells, but this antigen may also be important for the initiation of ADCC.

Further evidence that the ADCC effector cell bore p23,30 antigen was obtained in studies in which lymphocyte effector cell populations were incubated with anti-p23,30 serum and then depleted by passage through Sephadex G-200 antirabbit Fab immunoabsorbent columns. In these experiments, the immunoglobulin-negative, E rosette-negative cell population (Null cell) was incubated either in the presence of anti-p23,30 serum or NRS at dilutions of 1-20 for 1 h and passed through an antirabbit Fab immunoabsorbent column. Approximately 30% of the cells treated with anti-p23,30 serum were retained on the column. By contrast only 7% of cells incubated in NRS were retained. More important, as

TABLE I
Depletion of ADCC Effector Activity by C-Mediated Lysis of p23,30-Bearing Cells

Isolated lymphocyte population*	Effector/target ratio	% Cytotoxicity†		
		Media + C	NRS + C	Anti-p23,30 + C‡
Unfractionated lymphocytes	40/1	30.0 ± 2.6	28.7 ± 2.3	12.5 ± 1.1
	20/1	17.3 ± 1.8	16.5 ± 3.1	3.0 ± 0.2
Ig negative (T + Null)	40/1	29.3 ± 2.7	25.2 ± 6.1	6.1 ± 7.2
	20/1	15.7 ± 1.3	18.6 ± 1.3	0.5 ± 0.2
Ig negative (T + Null)	40/1	56.3 ± 2.3	48.6 ± 3.1	1.7 ± 0.6
	20/1	18.5 ± 2.7	15.3 ± 2.7	0.3 ± 0.1
Ig negative (T + Null)	40/1	20.3 ± 3.5	23.5 ± 2.6	3.4 ± 2.1
	20/1	13.6 ± 4.1	12.8 ± 1.7	2.9 ± 1.7
Ig negative, E rosette negative (Null)	40/1	57.4 ± 3.2	59.6 ± 2.1	18.9 ± 4.3
	5/1	23.4 ± 5.6	20.6 ± 1.0	3.2 ± 1.7
Ig negative, E rosette negative (Null)	40/1	33.8 ± 6.2	29.6 ± 3.2	2.6 ± 1.4
	5/1	21.7 ± 2.5	18.7 ± 4.1	0.7 ± 1.2

* Effector lymphocyte populations in these six experiments were either Ficoll-Hypaque-purified lymphocytes (unfractionated), lymphocytes not retained on anti-Fab columns (T + Null), or cells not retained on anti-Fab columns and subsequently E depleted (Null).

† 4×10^5 or 2×10^5 effector cells were added to the 10^4 ^{51}Cr -labeled antibody-coated target lymphocytes and ^{51}Cr released into the supernatant solutions from triplicate cultures was assayed. The results are expressed as mean percent cytotoxicity \pm SEM. The freeze-thaw value averaged 323.5 ± 12.6 and the spontaneous release was 45 ± 2.7 .

‡ Effector lymphocytes were incubated with either media, NRS, or anti-p23,30 serum at room temperature for 1 h and treated with rabbit C for 1 h at 37°C.

shown in Fig. 2, the nonretained anti-p23,30-treated cells were inactive in ADCC, while the nonretained NRS-treated cells were fully active.

Antibody to p23,30 Antigen Does not Inhibit EA Rosette Formation. One interpretation for the above results is that anti-p23,30 serum exhibits ADCC by binding to the Fc receptor. In order to explore this possibility, we reasoned that if p23,30 is reacting with the Fc receptor on the ADCC effector cell, then incubation of this antibody with the effector cells should inhibit binding to EA cells, a function which is known to depend on Fc receptors. Thus, lymphocytes were incubated with anti-p23,30 serum or NRS at dilutions of 1:20. Afterwards, these cells were reacted with EA cells and the percentage of cells forming EA rosettes was enumerated. As shown in Table III, the percentage of EA-rosetting cells with either no treatment, NRS, or anti-p23,30 serum treatment remained constant. These results indicate that anti-p23,30 serum does not bind to the Fc receptor.

Depletion of p23,30-Positive Cells Eliminates Antibody-Forming Precursor Cells. We then determined whether the p23,30-positive cells within the Ig-negative, E rosette-negative subset was the same subpopulation of cells which could mature in cell culture into Ig-positive, immunoglobulin-secreting cells. Unfractionated lymphocytes, T cells, Ig-positive cells, and Null cells were

TABLE II
Specific Binding of Anti-p23,30 to Effector Lymphocytes Inhibits ADCC

Isolated effector population*	Effector/target ratio	% Cytotoxicity	
		NRS	Anti-p23,30‡
Unfractionated lymphocytes	40/1	32.2 ± 3.5	12.6 ± 2.1
	20/1	25.9 ± 1.8	7.2 ± 0.7
Ig negative (T + Null)	40/1	20.3 ± 3.5	3.4 ± 1.5
	20/1	13.6 ± 2.6	2.9 ± 0.6
Ig negative (T + Null)	40/1	29.0 ± 2.6	6.1 ± 3.7
	20/1	10.0 ± 3.5	5.7 ± 2.1
Ig negative (T + Null)	40/1	32.3 ± 5.7	0.8 ± 1.2
	20/1	5.7 ± 1.3	0.6 ± 0.7
Ig negative, E rosette negative (Null)	40/1	75.9 ± 3.2	12.6 ± 1.3
	20/1	43.5 ± 6.5	1.7 ± 5.8

* Effector lymphocyte populations were isolated in separate experiments as indicated in the Materials and Methods and in Table I.

‡ Cells were incubated with NRS (1/20 dilution) or anti-p23,30 serum (1/20 dilution), washed three times, and assayed for ADCC on ⁵¹Cr-labeled, antibody-coated target cells.

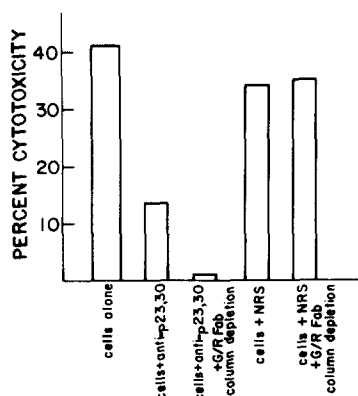


FIG. 2. Specific depletion of p23,30-bearing cells on G/R Fab immunoabsorbent columns eliminates ADCC activity.

incubated with either NRS or anti-p23,30 serum in the presence of C, washed, and placed in cell culture for 6 days in a 5% CO₂ atmosphere at 37°C. Supernates were analyzed for Ig. As seen in Table IV, both Ig-positive cells and a subset of cells within the Ig-negative, E rosette-negative subset of human lymphocytes (Null cells) do have the capacity to secrete immunoglobulin during cell culture (Table IV). By contrast, isolated T-cell populations do not secrete Ig. More important, prior treatment of the Ig-secreting cell populations with anti-p23,30 and C completely abrogated the ability to secrete immunoglobulin in cell culture. Thus, the p23,30-positive, E rosette-negative, Ig-negative subset of lymphocytes is important both with respect to ADCC function and with respect to Ig synthesis.

TABLE III
Effect of Anti-p23,30 on EA Rosette Formation

Cell population	% EA rosette-forming cells	
	Control NRS	Anti-p23,30*
Unfractionated	23 ± 2	17 ± 3
Unfractionated	25 ± 1	16 ± 2
Unfractionated	34 ± 4	30 ± 5
Unfractionated	20 ± 3	27 ± 5
Ig-positive lymphocytes (B cells)	89 ± 3	82 ± 6
Ig-negative lymphocytes (T + Null cells)	12 ± 2	9 ± 1
Ig-negative, E rosette-negative lymphocytes (Null cells)	72 ± 3	63 ± 7

* Unfractionated lymphocytes and isolated populations were treated with either NRS (1/20 dilution) or anti-p23,30 serum (1/20 dilution) for 1 h at room temperature and subsequently assayed for the percent EA-rosetting cells.

TABLE IV
Inhibition of Ig Synthesis In Vitro by Pretreatment with Anti-p23,30*

	Cell population	Media	NRS	Anti-p23,30
Exp. 1	Unfractionated cells	200	172	<10
	T cells	<10	<10	<10
	Ig ⁺ B cells	430	385	26
	Null cells (Ig ⁻ E ⁻)	360	300	<10
Exp. 2	Unfractionated cells	137	143	<10
	T cells	<10	<10	<10
	B cells	230	265	33
	Null cells	195	185	22

* Unfractionated lymphocytes and isolated populations were treated with media, NRS, or anti-p23,30 serum and then with C, as in Table I. Cells were then incubated in final media for 6 days at 37°C in a 5% CO₂ humid environment and supernatant solutions were analyzed for nanograms of secreted Ig by ¹²⁵I-labeled anti-Fab radioimmunoassay.

Discussion

The anti-p23,30 serum is valuable for the analysis of human lymphocyte functions both because it possesses a unique cellular specificity (i.e., reactivity with 100% of Ig-positive B cells and approximately 20% of Ig negative, E rosette-negative [Null] cells and not with T cells) and because of its similarity to murine anti-Ia sera (14).

In the present study we utilized anti-p23,30 serum to explore further the functional properties of the surface Ig-negative, E rosette-negative (Null cell) subset of human lymphocytes. In earlier studies it has been shown that this population contains most of the ADCC activity present in human peripheral blood (5). Moreover, a subset of this population has been shown to develop surface immunoglobulins in cell culture and to develop the capacity to secrete immunoglobulins in vitro (13). The question of whether the two functional properties of the Ig-negative, E rosette-negative (Null) subset of lymphocytes, that is, ADCC and Ig synthesis, resides within the same subset of cells were addressed in the experiments presented above.

The following points emerge from these studies: (a) The human lymphocyte which functions as the effector cell in ADCC bears the p23,30 antigen. Depletion of cells bearing p23,30 from either the Ig-negative, E rosette-negative (Null) subset or alternatively, from unfractionated human lymphocytes by either C-mediated lysis or by depletion over immunoabsorbent columns, eliminates ADCC reactivity. (b) Exposure of the p23,30 antigen is required for ADCC function since binding of anti-p23,30 serum to the effector cell surface inhibits ADCC. That this effect was not due to binding of anti-p23,30 serum to the Fc receptor was shown in studies which demonstrates that anti-p23,30 serum did not inhibit EA rosette formation. Moreover, in previous studies it was shown that the anti-p23,30 serum does not inhibit the binding of aggregated gamma globulin to the cell surface, another function dependent on Fc receptors (14). Since Fc receptors are known to be necessary for ADCC, these results suggest that at least two distinct sites, Fc and p23,30, are involved in this form of cell-mediated lysis. (c) Depletion of cells bearing p23,30 from an unfractionated population of cells, from Ig-positive B cells and from purified populations of Ig-negative, E rosette-negative (Null) cells completely abrogates the ability of these cells to secrete immunoglobulin during cell culture. Thus, all Ig-secreting peripheral lymphocytes bear p23,30 antigen. Taken together, these data provide evidence that the ADCC and Ig-synthetic functions present within the Ig-negative, E rosette-negative (Null) subset of human lymphocytes reside in the same population of cells bearing the antigen p23,30.

The simplest interpretation of these results is that the ADCC effector lymphocyte and the Ig-synthesizing cell have differentiated along B-cell pathways. Consistent with this view is the evidence that the ADCC effector cell, in addition to bearing p23,30 is known to have other surface determinants in common with B cells such as the Fc and C receptors (9, 11). Moreover, the ADCC effector lymphocyte is nonphagocytic and does not bear antigens or receptors in common with T cells. We cannot exclude the possibility that another as yet undefined receptor on the surface of the ADCC effector cell may distinguish this subset from other B cells. The fact that Ig-positive B cells which are retained on anti-Fab immunoabsorbent columns, are relatively inefficient in ADCC suggest that this ADCC function defines a distinct small subset of B cells. Whether this subset of B cells is unique with respect to either the control of immunoglobulin synthesis or with respect to the type of immunoglobulin produced, or in fact with respect to other biologic functions, remains to be determined. However, it is clear that all ADCC effector subsets of lymphocytes bear the p23,30 antigen.

The precise role of p23,30 in either immunoglobulin synthesis or ADCC is unknown. The structural and functional similarities of p23,30 to murine Ia antigens as documented in the accompanying manuscript are of interest with respect to recent evidence that Ia antigens and Fc receptors appear to be closely associated on the cell surface (17). One can thus envision a model in which the triggering of ADCC is dependent on two signals: one being recognition of the antibody-coated target cell by the Fc receptor and the other, the activation of the nearby p23,30 antigen by either the target cell or by the activated Fc receptor itself. This mechanism would of course be analogous to the triggering of B cells towards their differentiation into antibody-secreting cells, a function thought to

require at least two signals. Since the Ia antigens are thought to be important in the facilitation of cell-cell interactions as for example, during T, B cooperation, (18), it is possible that these antigens may have a broader biologic function in other cell-cell interaction involving B cells such as occurs in ADCC.

Summary

Rabbit antisera to the human B-cell-specific surface antigen complex, p23,30, was used to define further the functional heterogeneity of isolated human lymphocyte subpopulations. Specific depletion of p23,30-bearing cells from Ig-negative cell populations and Ig-negative, E rosette-negative (Null) populations by either complement-mediated lysis or by physical separation on goat antirabbit Fab immunoabsorbent columns, eliminates the antibody-dependent cellular cytotoxic (ADCC) function. Furthermore, binding of anti-p23,30 serum to the effector cell surface inhibits ADCC but does not interfere with EA rosette formation. Apparently p23,30 represents a cell surface site which is distinct from the Fc receptor but which is important in the triggering of ADCC.

In addition, depletion of p23,30-bearing cells from unfractionated cell populations, Ig-positive B-cell populations and Ig-negative, E rosette-negative (Null) populations eliminates the capacity of these populations to secrete immunoglobulin during subsequent culturing. Thus both the Ig-secreting cells and the ADCC effector cells within the Ig-negative, E rosette-negative (Null) population reside in the same population of cells which bears the p23,30 antigen.

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References

1. Harding, B., D. J. Pudifin, K. Gotch, and I. C. M. MacLennan. 1971. Cytotoxic lymphocytes from rats depleted of thymus processed cells. *Nat. New Biol.* 232:80.
2. Van Boxel, J. A., J. D. Stobo, W. E. Paul, and I. Green. 1972. Antibody dependent lymphocyte cell mediated cytotoxicity: no requirement for thymus derived lymphocytes. *Science (Wash. D. C.)*. 175:194.
3. 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.
4. Perlmann, P., H. Wigzell, P. Goldstein, F. W. Lanun, C. Larsson, H. 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.
5. Brier, A. M., L. Chess, and S. F. Schlossman. 1975. Human antibody dependent cellular cytotoxicity: isolation and identification of a subpopulation of peripheral blood lymphocytes which kill antibody-coated autologous target cells. *J. Clin. Invest.* 56:1580.
6. Dennert, G., and E. S. Lennox. 1973. Phagocytic cells as effectors in a cell mediated immunity system. *J. Immunol.* 111:1844.
7. Scornik, J. C., and H. Cosenza. 1974. Antibody dependent cell mediated cytotoxicity. III. Two functionally different effector cells. *J. Immunol.* 113:1527.
8. Forman, J., and G. Möller. 1973. The effector cell in antibody induced cell mediated immunity. *Transplant. Rev.* 17:108.
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.

10. MacLennan, I. C. M. 1969. Competition for receptors on cytotoxic lymphocytes. *Clin. Exp. Immunol.* 10:275.
11. Perlmann, P., H. Perlmann, and H. J. Müller-Eberhard. 1975. Cytolytic lymphocytic cells with complement receptor in human blood. Induction of cytolysis by IgG antibody but not by target cell-bound C₃. *J. Exp. Med.* 141:287.
12. MacDermott, R. P., L. Chess, and S. F. Schlossman. 1975. Immunologic functions of isolated human lymphocyte subpopulations. V. Isolation and functional analysis of a surface Ig negative, E rosette negative subset. *Clin. Immunol. Immunopathol.* 4:415.
13. Chess, L., H. Levine, R. P. MacDermott, and S. F. Schlossman. 1975. Immunologic functions of isolated human lymphocyte subpopulations. VI. Further characterization of the surface Ig negative, E rosette negative (Null cell) subset. *J. Immunol.* 115:1483.
14. Humphreys, R. E., L. Chess, S. F. Schlossman, and J. Strominger. 1976. Isolation and immunologic characterization of a human, B-lymphocyte-specific, cell surface antigen. *J. Exp. Med.* 144:98.
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.
16. Schlossman, S. F., and L. Hudson. 1973. Specific purification of lymphocyte populations on a digestible immunoabsorbent. *J. Immunol.* 110:313.
17. Dickler, H. B., and D. H. Sachs. 1974. Evidence for identity or close association of the Fc receptor of B lymphocytes and alloantigens determined by the *Ir* region of the *H-2* complex. *J. Exp. Med.* 140:779.
18. Katz, D. H., and B. Benacerraf. 1975. Hypothesis: the function and interrelationships of T cell receptors, *Ir* genes and other histocompatibility gene products. *Transplant. Rev.* 22:175.