Brief Definitive Report

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY BY ALLOSENSITIZED HUMAN T CELLS*

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Present evidence indicates that the immune response to foreign tissues may involve several types of cellular cytotoxicity, including two mechanisms of lymphocyte-mediated killing which have been extensively studied in vitro. (*a*) Cell-mediated lympholysis (CML) is effected by cytotoxic T lymphocytes which interact with targets via specific receptors for cellular antigen. (*b*) Antibody-dependent cellular cytotoxicity (ADCC) is effected by a small subpopulation of non-T lymphocytes (1–5), termed K cells, that interact with antibody (Ab)-bound targets via receptors for the Fc portion of IgG (6, 7). In contrast to CML, K-cell ADCC does not require sensitization, and may therefore be considered to be "spontaneous".

While this and other laboratories have reported that thymus-dependent lymphocytes do not effect ADCC (1–5), we have found that peripheral human T cells develop this activity when sensitized to alloantigen. Specifically, sheep erythrocyte (SRBC)-rosetting (T) cells have ADCC potential when stimulated in mixed lymphocyte culture (MLC), but not when freshly isolated or when unstimulated in culture. Like "spontaneous" ADCC, this activity is dependent on the interaction of T-cell Fc receptors (FcR) with Ab on the target cell.

Materials and Methods

Isolation of Human T Lymphocytes from Unfractionated Peripheral Blood. T cells were purified from unfractionated mononuclear cells by passage over nylon wool columns, followed by formation and subsequent sedimentation of SRBC-rosetting cells over Ficoll-Hypaque to separate E^+ (T) from E^- (K) cells (8). Cells recovered from the rosetted pellet by treatment with 0.155 M NH_4Cl were <2% EAC-rosette$^+$, and >95% E-rosette$^+$ as determined by methods previously described (8).

Preparation of the Antisera. The anti-human lymphocyte serum (ALS) used to coat mononuclear target cells for the ADCC reaction was prepared as described elsewhere (5). A rabbit anti-chicken erythrocyte serum (N. L. Cappel Laboratories, Inc., Cochranville, Pa.) was absorbed with cultured human EBV-transformed lymphoblasts until cross-reactivity with normal human lymphocytes was not detectable by indirect immunofluorescence on a Fluorescence Activated Cell Sorter (FACS I).

Sensitization Cultures. Standard one-way MLCs were established as previously described (9). Killer cells for the ADCC assay were recovered on day 6.

ADCC Assay. The assay for ADCC has been previously reported (5). In brief, target cells for ADCC were either autologous peripheral blood mononuclear cells purified on the day of assay, or chicken erythrocytes (Spafas, Inc., Norwich, Conn.). 1.5×10^6 cells of each target population were

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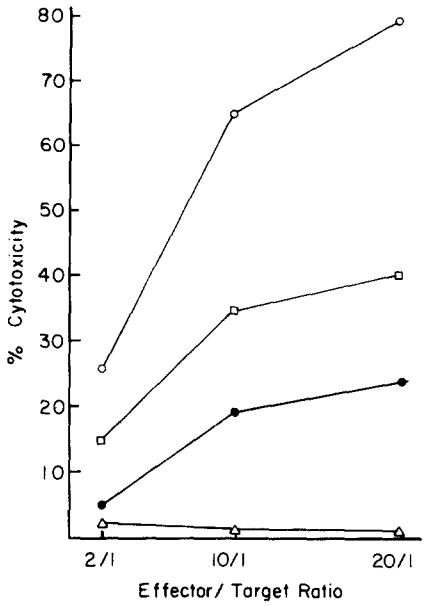

FIG. 1. Cytotoxicity against autologous Ab-coated lymphocytes by unfractionated peripheral blood mononuclear cells stimulated in a standard one-way MLC by either allogeneic ○, or autologous lymphocytes ●. Killing by fresh unfractionated mononuclear cells □. Target cells were labeled with a rabbit anti-human lymphocyte serum in a 6-h ^{51}Cr release assay. Cytotoxicity against autologous lymphocytes not labeled with Ab by allosensitized mononuclear cells △.

labeled with 0.15 ml of (^{51}Cr) sodium chromate (292 μCi/ml) (New England Nuclear Corp., Boston, Mass.) for 1 h at 37°C. Human mononuclear targets were then labeled with the ALS (1/50 dilution) while chicken erythrocytes were labeled with a rabbit anti-chicken erythrocytes (CRBC) serum (1/100 dilution) for ½ h at 4°C. 1×10^4 mononuclear and 2×10^4 CRBC targets were incubated in triplicate tubes for 6 h at 37°C with the appropriate concentration of killer cells. Target cell lysis was determined as described elsewhere (5).

Aggregated Ig. Aggregates of Ig were formed by heating a solution of human Ig 15 mg/ml (Fraction II, Miles Laboratories, Inc., Elkhart, Ind.) at 63°C for 30 min.

Results and Discussion

Initially we observed that sensitization to alloantigen augmented the ADCC activity of unfractionated peripheral blood lymphocytes. As shown in Fig. 1, lymphocytes responding in a standard one-way MLC were markedly more cytotoxic to autologous lymphocytes labeled with a rabbit ALS than either an unstimulated control or lymphocytes taken freshly from the same normal donor. This suggested that allosensitization either expanded the population of K cells responsible for ADCC or recruited this activity from a different subpopulation of cells. Since human K cells belong to a subpopulation of null, (E-rosette$^-$, SmIg$^-$), lymphocytes that do not respond in MLC (5, 9), we investigated the possibility that T cells were triggered by alloantigen to express ADCC.

Highly purified T lymphocytes were prepared by passing peripheral blood mononuclear cells over nylon wool and rosetting the nonadherent population with SRBCs to separate E^+(T) cells from E^-(K) cells by density centrifugation.

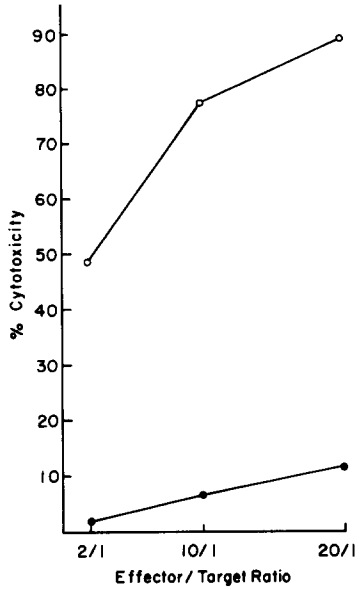

FIG. 2. Cytotoxicity against autologous antibody-coated lymphocytes by T cells stimulated in MLC by allogeneic ○, or autologous ●, lymphocytes.

T cells isolated in this manner were not appreciably cytotoxic to antibody coated target cells either immediately after purification, or after a 6-day incubation with autologous lymphocytes (Fig. 2). However, the same T cells stimulated by allogeneic lymphocytes in MLC exhibited a high level of antibody-dependent killing. When responder T cells were recovered from 6 day MLC and again fractionated into E^+ and E^- populations, E^+ cells were found to contain most of the lytic activity and to comprise greater than 90% of the cells subjected to rosetting. Moreover, ongoing studies indicate that alloactivated T cells responsible for ADCC express other T-cell markers, including a recently-defined thymus-dependent differentiation antigen, T_{H2}.[1]

The very high lytic potential of activated T cells suggested that killing did not arise from artifact introduced by the ALS, but rather a specific cell-cell interaction dependent on antibody. The obvious model for such an interaction occurs in "spontaneous" ADCC which is dependent on the binding of K-cell FcR to the Fc portion of Ab on the target cell (6, 7). If lysis of Ab-coated targets by activated T cells occurred by an analogous or identical mechanism, it should similarly be inhibited by immunoglobulin. We therefore assayed the ADCC activity of cytotoxic T cells against Ab-coated CRBC in the presence or absence of aggregated human Ig. The use of CRBC also permitted assay of this activity against targets labeled with Ab that did not cross-react with human effector cells. As shown in Table I, lysis of Ab-coated CRBCs by MLC-primed (killer) T cells was markedly inhibited by the presence of aggregated Ig. Again, unacti-

[1] R. L. Evans, H. Lazarus, A. C. Penta, and S. F. Schlossman. 1977. Two functionally distinct subpopulations of human T cells that collaborate in the generation of cytotoxic cells responsible for cell mediated lympholysis. Manuscript submitted for publication.

TABLE I
The Effect of Aggregated Human Ig on Killing of Ab-Coated Chicken Erythrocytes by Allosensitized T Cells

Effectors	Percent cytotoxicity	
	20:1	5:1
MLC-Primed T cells	71	35
+ Ig 2 mg/ml	12	0
+ Ig 0.4 mg/ml	29	4
+ Ig 0.08 mg/ml	45	12
+ Albumin 2 mg/ml	68	28
Unactivated cultured T cells	2	0
MLC-Primed T cells against unlabeled CRBC	0	0

vated cultured T cells were not cytotoxic, nor were unlabeled CRBCs susceptible to lysis by allosensitized T cells. These experiments therefore suggest that T-cell ADCC is dependent on the interaction for FcR with Ab on the target cell. Although it is possible that inhibition by aggregated Ig is not due to competitive binding to FcR, we have found that $F(ab')_2$ fragments of the ALS, prepared by pepsin digestion and fractionation on G150, induce markedly less target cell lysis than undigested Ab. Taken together, these results strongly implicate T-cell FcR in the lytic process.

Although our data indicates that T-cell ADCC occurs by an effector-target interaction distinct from that occurring in CML, this cannot be taken as proof that each activity is effected by a different subpopulation of T cells. Stout et al. have shown that murine T cells responsible for CML develop FcR for IgG during sensitization in MLC (10). It is therefore possible that a common cytotoxic T cell can effect target cell destruction via receptors for membrane antigen or via receptors for Fc, with both cell-cell interactions potentiating an identical lytic process.

Other investigators have reported that IgM antibodies induce cytolysis of sarcoma virus-induced tumor cells by mouse lymphocytes including T cells (11) or of bovine erythrocytes by human T cells (12). Prior sensitization was not required to induce IgM-dependent T-cell killing, whereas sensitization was clearly required to generate killing in our system. Moreover, preliminary evidence indicates that induction of ADCC by allosensitized T cells is dependent on 7S and not 19S antibody. Given the conflicting reports regarding the capacity of IgM antibodies to induce cellular cytotoxicity (13, 14), and the relatively restricted in vitro conditions under which this activity occurs, the relationship between IgM-dependent T-cell killing and ADCC by allosensitized T cells is presently unclear. Each cytotoxic mechanism may reflect a distinct T-cell subpopulation bearing receptors for either IgM or IgG as described by Moretta et al. (15). Alternatively T cells responsible for IgM-dependent lysis may be triggered by alloantigen to differentiate into IgG-dependent killers.

In view of the recent evidence that K cells are FcR-bearing lymphocytes that rosette with SRBC under optimal conditions at 4°C (16), we would emphasize the following two points in regard to our own findings: (*a*) K cell or "spontaneous" ADCC is enriched by depletion of lymphocytes rosetting at 25°C (5). (*b*) Lymphocytes rosetting under these conditions (T cells) do not effect ADCC in either of the systems described here unless activated by antigen. Although this

does not argue against a thymic origin of the K cell per se, evidence in murine systems indicates that K cells do not bear thymus-dependent surface markers (2). This does not, however, exclude the possibility that K and T cells share a prethymic ancestry distinct from that of B cells. In fact, our results would conform to this view, suggesting that T cells may be similarly activated to express surface antigens present on K, but not on resting T cells. Ongoing studies indicate that a human Ia-like determinant, p23,30 (17), represents one such antigen, since it is expressed by K but not T cells (18), unless activated by antigen.

Summary

Peripheral human T cells, isolated by sheep erythrocyte-rosette formation and density centrifugation, were highly cytotoxic to both Ab-coated autologous lymphocytes and antibody (Ab)-coated chicken erythrocytes when stimulated in mixed lymphocyte culture, but were not lytic when freshly purified, or when unstimulated in 6-day culture. Allosensitized T cells were shown to effect this activity by a specific effector-target cell interaction dependent on Ab, as indicated by: (*a*) induction of killing by Ab to target cells not lysed in the absence of Ab. (*b*) inhibition of Ab-dependent killing by aggregated Ig. The mechanism by which allosensitized T cells effect antibody-dependent cellular cytotoxicity is discussed.

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