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Characterization of mononuclear effector cells in human blood

P. HERSEY, ANNE EDWARDS & JANICE EDWARDS *Immunology Unit, Department of Bacteriology and Medicine, University of Sydney, Australia*

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SUMMARY

The effector cells responsible for cytotoxic activity induced by phytohaemagglutinin, (PHA) pokeweed mitogen (PWM) and target cells complexed with IgG antibody has been investigated using cell separation techniques based on rosette formation and separation through Hypaque–Ficoll mixtures. It was shown that PHA-induced cytotoxicity is predominantly a function of T cells and that Fc receptor-bearing cells are not involved to any major extent. Antibody-dependent killing is conversely a function of Fc receptor-bearing cells among which two subtypes can be distinguished. One of these has receptors for activated complement while the other bears Fc receptors only and has no detectable receptors for complement. PWM appears to induce cytotoxicity in both T- and non-T-cell populations but the major cell type involved appears to be Fc receptor-bearing cells similar to those mediating antibody-dependent killing. It is concluded that PHA and antibody-dependent killing are the two most useful assays for discriminating between the cytotoxic activity of T and non-T cells in clinical studies.

INTRODUCTION

Human blood mononuclear cells from normal subjects can be induced into cytotoxic activity by a number of different agents such as phytohaemagglutinin (PHA), staphylococcal filtrate (SF), purified protein derivative of tuberculin (PPD), mixed lymphocyte cultures (MLC) and by target cells sensitized with IgG antibody (Holm & Perlmann, 1967; Stejskal, Holm & Perlmann, 1973; Stejskal *et al.*, 1973; Stejskal *et al.*, 1974; MacLennan, Leowi & Howard 1969; MacLennan, 1972; Perlmann, Perlmann & Wigzell, 1972). The use of these agents as a means of determining the functional activity of the immune effector response of subjects with various clinical disorders is receiving some attention. Several studies have appeared indicating that selective defects can occur in the cytotoxic activity induced by these agents (Campbell *et al.*, 1973; Mellstedt & Holm, 1973; Holm *et al.*, 1974) and that dichotomy may occur between cytotoxic and proliferative responses (Stites, Carr & Fudenberg, 1972).

The nature of the effector cells in human blood responsible for cytotoxicity in the presence of these agents is, however, not well established. Different cell populations appear to

Correspondence: Dr P. Hersey, Immunology Unit, Department of Bacteriology, University of Sydney, Sydney, N.S.W. 2006, Australia.

be involved in antibody-dependent and PHA-induced killing as shown by studies on human lymph cells (Holm *et al.*, 1974). Thymus-dependent (T) cells appear to be the effector cells in MLC reactions and non-T cells have been implicated in the cytotoxic response to PWM (Stejskal *et al.*, 1974).

We have attempted a more precise definition of the effector cells responsible for cytotoxicity in the presence of PHA, PWM and sensitized target cells by use of cell separation methods based on red cell rosetting techniques. Our results confirm the heterogeneity of effector cells in human blood. PHA-induced killing appears to be predominantly a function of T cells, whereas antibody-dependent cell killing is a function of Fc receptor-bearing non-T cells of at least two cell types. PWM-induced killing also appears to induce killing by at least two cell types one of which appears to bear Fc receptors.

MATERIALS AND METHODS

Effector cells. Mononuclear cells were obtained from defibrinated blood samples from normal laboratory volunteers by separation on Hypaque–Ficoll mixtures (H–F) after the method of Boyum. The cells were washed twice in minimal essential medium (MEM) and resuspended in RPMI 1640 (Gibco) supplemented with heat-inactivated foetal bovine serum (FBS) (Australian Laboratory Services, Rockdale, N.S.W.). A small aliquot was left untreated and the remainder glass absorbed to remove macrophages.

Glass absorption. The mononuclear cells were suspended in RPMI plus 50% autologous serum at a concentration of 5×10^6/ml and incubated on columns of 2 mm glass beads at 37°C for 30 min. The cells were eluted with twice the original volume of MEM, washed once and resuspended in RPMI plus 10% FBS. This procedure removed all phagocytic cells as determined by their ability to take up sensitized sheep red blood cells. (SRBC) (Hersey, 1973). A small aliquot of the absorbed cells was kept for testing and the remainder submitted to rosetting procedures based on the presence of receptors for the Fc portion of IgG or for SRBC on their surface.

Fc receptor rosette formation (EA rosettes). These were formed after the methods described by Parish & Hayward (1974). SRBC (E) as a 5% suspension in MEM were trypsinized by incubation with 0·25% trypsin (Difco) (4 ml per/ml of 5% SRBC) for 1 hr. After washing three times in 25 ml MEM they were sensitized with the highest subagglutinating concentration of the 7S fraction of a rabbit anti-SRBC serum by incubation at 37°C for 30 min. The sensitized SRBC (EA) were washed twice in MEM and resuspended as a 5% suspension in RPMI plus 10% FBS buffered with Hepes at a concentration of 10 mM. Absorbed mononuclear cells (10^7/ml) were mixed with an equal volume of EA and rotated at 33 rev/min at 37°C for 20 min. The mixture was diluted five times with cold MEM and the percentage of EA rosettes estimated by counting under phase in a counting chamber.

Spontaneous SRBC rosettes (E rosettes). These were formed by the method of Jondal, Holm & Wigzell (1972). SRBC less than 7 days old as a 0·5% suspension in RPMI plus 10% FBS were mixed with an equal volume of glass-absorbed mononuclear cells at a concentration of 2×10^6/ml in RPMI plus 10% FBS. They were incubated at 37°C for 5 min, centrifuged at 400 *g* for 5 min, then placed on ice for 1–2 hr. They were then gently resuspended and counted as before in a counting chamber.

Complement receptor rosettes (EAC rosettes). Complement rosettes were formed by methods adapted from Bianco, Patrick & Nussenweig (1970). SRBC, trypsinized as above, were sensitized as a 5% suspension in RPMI plus 10% FBS by incubation with a subagglutinating concentration of the 19S fraction of a rabbit anti-SRBC serum for 30 min at 37°C. The sensitized SRBC (EA) were washed twice in MEM and incubated as a 5% suspension with fresh mouse serum at a dilution of 1:10 for 30 min. The EA plus complement suspension (EAC) was washed twice in MEM and resuspended as a 1% suspension in RPMI plus 10% FBS buffered with Hepes buffer at a concentration of 10 mM. EAC rosettes were than formed by mixing equal volumes of this suspension with the mononuclear cells at a concentration of 3×10^6/ml in RPMI plus 10% FBS and rotating the mixture at 33 rev/min for 20 min at 37°C.

Separation of rosetting from non-rosetting cells. Rosettes formed as above, were removed from the non-rosetting cells by centrifugation on H:F mixtures of specific gravity 1·078 at room temperature for 30 min at 400 *g* in 100×16 mm plastic tubes. Rosetted mononuclear cells passing to the bottom of the tubes were

recovered by lysis of the SRBC with a 35% solution of MEM for 45 sec. They were then washed twice in 25 ml of MEM and resuspended in RPMI plus 10% FBS. Cell fractions remaining at the top of the H–F mixtures were referred to as EA top, E top and EAC top respectively, while cell fractions passing through the H:F to the bottom of the tube were referred to as EA bottom, E bottom and EAC bottom respectively.

Target cells and ^{51}Cr labelling. Target cells were Chang human liver cells (Commonwealth Serum Laboratories, Melbourne) grown in continuous culture in glass bottles in MEM for suspension cultures supplemented with 10% FBS and gentamycin 50 μg/ml. They were harvested by agitation of the bottle, washed once in MEM and $1–2 \times 10^6$ cells resuspended in 1 ml of RPMI plus 10% FBS. Labelling with ^{51}Cr was carried out by adding 100 μCi of $Na_2\,^{51}Cr\,O_4$ (Amersham, Bucks, England) and incubating at 37°C for 2 hr. The labelled cells were washed twice in 25 ml of MEM and resuspended in RPMI plus 10% FBS at a concentration of 6×10^3/ml.

^{51}Cr release cytotoxic assays. Effector cell populations were added to labelled target cells in duplicate $3 \times \frac{1}{2}$ in. round-bottomed plastic tubes (Disposable Products, Adelaide) to give effector cell:target cell ratios of 100:1, 30:1 and 10:1. To measure antibody-dependent cell-mediated cytotoxicity (ADCC), rabbit anti-Chang cell serum, produced by the methods described by MacLennan *et al.* (1969) was added to the Chang cell suspension to give a final dilution of 1 in 10^4.

PHA-induced cytotoxicity was carried out by adding PHA-P (Difco Laboratories Detroit Michigan) (batch no. 556513) at a final concentration of 25 μg/ml. This concentration was shown by prior tests to give optimal DNA synthesis and cytotoxicity.

PWM-induced cytotoxicity was carried out by adding PWM (Gibco Grand Island, New York) (batch number R 936404) to the Chang cell suspension to give a final dilution of 1 in 200. Again this dilution was that found to give optimal DNA synthesis and cytotoxicity in preliminary tests.

The cultures were set up in parallel, capped and incubated overnight for 16 hr in 5% CO_2. ^{51}Cr Released into the supernatant was assessed by centrifuging the tubes at 400 *g* for 7 min and removal of 0·5 ml of supernatant with an Eppendorf pipette. Percentage ^{51}Cr release was assessed according to the formula:

$$\frac{(a \times 2)}{(a+b)} \times 100,$$

where a = activity in supernatant corrected for background and b = activity in tube with cell sediment + remaining supernatant corrected for background.

Note: results of cytotoxic activity are expressed either in terms of percentage ^{51}Cr release or percentage ^{51}Cr release above baseline. This method of expression was chosen for convenience rather than the alternative index where the $\log_{10}$ number of cells required to kill 50% of the target cells is taken as the measure of cytotoxic activity (Campbell *et al.*, 1973). As there is a linear relationship between percentage ^{51}Cr release and the $\log_{10}$ number of effector cells (e.g. MacLennan, 1972) either parameter can be used.

Staining for surface immunoglobulin. The purified cell populations were stained for 20 min at room temperature with a 1 in 2 dilution of fluorescein-conjugated sheep anti-human immunoglobulin (Wellcome Reagents, batch no. K8078). (The reagent had been centrifuged at 100,000 *g* for 30 min to remove aggregates prior to use.) The cells were washed twice, mounted under a sealed coverslip and examined by dark ground fluorescent microscopy using a Leitz Orthoplan microscope with u.v. light source.

DNA synthesis. This was carried out in Cooke microtitre trays. Mononuclear cells 1×10^5 per well in 0·2 ml of RPMI plus 10% FBS were cultivated for 3 days in the presence of 0, 5 and 20 μg of PHA—P (Difco, as before). Sixteen hours prior to harvest, 1 μCi of tritiated thymidine ([^{3}H]T) was added (5 Ci/m mol Radiochemical Centre Amersham, Bucks) [^{3}H]T incorporation into the trichloracetic acid (TCA) precipitable fraction was assessed by transferring cell cultures into glass tubes, washing twice with 5% TCA, once with methanol and then dissolving precipitate in Insta-Gel (Packard) scintillation liquid. The vials were counted in a Beckmann liquid scintillation counter.

Identification of T cells. Anti-human lymphocyte serum was produced in goats by injection of human foetal thymus cells and absorbed against human red blood cells and platelets as described before (Sheils *et al.*, 1973). This was further absorbed three times with an equal volume of chronic lymphatic leukaemic cells (CLL) from two donors and once on chronic myeloid leukaemic cells. The CLL cells were known to bear immunoglobulin and Fc receptors for IgG. The absorbed serum was centrifuged at 100,000 *g* for 3 hr to remove aggregates and antigen–antibody complexes.

The selectivity of the antiserum for T cells has been described in detail elsewhere (Hersey *et al.*, 1975b)

Two methods have been used to identify cells reacting with the antiserum. (1) Double labelling technique. The goat anti-T-cell serum was added at a final dilution of 1:30 for 20 min at room temperature followed (after two washes in MEM) by fluorescein-conjugated rabbit anti-goat serum (Wellcome Reagents) at a dilution of 1:20 for 20 min. The labelled cells were washed twice, mounted, sealed under a coverslip and read with fluorescent microscope as above. (2) Use of staphylococci as a marker. The Cowan I strain of *Staphylococcus aureus* (SPA) has affinity for the Fc portion of IgG and can therefore be used as a marker for IgG attached to cells (Ghetie, Nilsson & Sjöquist, 1974; Kearney, Chia & Basten, 1975). The cells were incubated in the anti-T-cell serum at a final concentration of 1:100 for 20 min at room temperature and then washed twice in MEM. Formalin-treated SPA was then added at a concentration of 10% for 5 min and any nonadherent SPA removed by washing three times in MEM at 100 *g*. The cell suspension was then mounted on a glass slide, dried, fixed in methanol and stained with Giemsa. Cells with SPA adhering to them were counted under light microscope at a magnification of 1000:1.

RESULTS

Identification of cells in fractions of blood mononuclear cells

Blood samples from seven normal individuals were submitted to the separation procedures described. In addition to carrying out cytotoxic assays on these fractions, the cells contained in them were identified by parallel studies using the cell markers outlined in Table 1 and

TABLE 1. Identification of cells in fractions of blood mononuclear cells

Cell fraction	Ig-positive 'B' cells*	Anti-T-positive 'T' cells*	Unidentified cells*	PHA response (ct/min)
Unseparated	23±5	72±6	5±7	14,900±7200
Glass absorbed	13±5	82±4	6±5	14,500±6200
EA top	3±2	90±9	7±8	19,000±13,000
EA bottom	37±15	29±17	30±11	7500±5000
E top	38±9	45±13	17±14	4100±2900
E bottom	1±1	93±6	6±5	18,100±7000

* Values indicated are mean percentages ± one standard deviation.

by assessment of their PHA responsiveness. The results indicated in this table are the mean values ± 1 s.d. from the mean. EA top and E bottom are seen to be enriched for T cells whereas EA bottom and E top are relatively heterogenous with approximately equal proportions of T, B and unidentified (null) cells. There were more unidentified cells in the EA bottom fractions which we cannot adequately account for but which may be a result of the hypotonic lysis step in the removal of SRBC. The glass absorption procedure regularly enriched for T cells and reduced the number of immunoglobulin-staining cells.

Cytotoxic activity of fractions of blood mononuclear cells

Fig. 1 illustrates a representative experiment where the fractions indicated were put in culture with Chang cells, Chang cells plus PHA and Chang cells plus sensitizing antibody as described before. (For clarity cytotoxicity in the presence of PWM is not illustrated.) Consistent findings in this type of experiment were as follows. (1) Spontaneous cytotoxicity against the Chang cell varied widely and was most marked for the glass absorbed and E

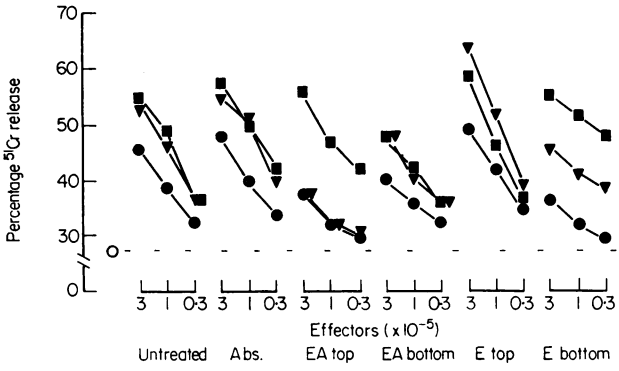

FIG. 1. Cell-mediated cytotoxicity against Chang cells by fractions of blood mononuclear cells. (●) Effector cells + Chang cells alone. (▼) Cytotoxicity in the presence of rabbit anti-Chang serum at a dilution of 1×10^4. (■) Cytotoxicity induced by PHA at a concentration of 25 μg/ml.

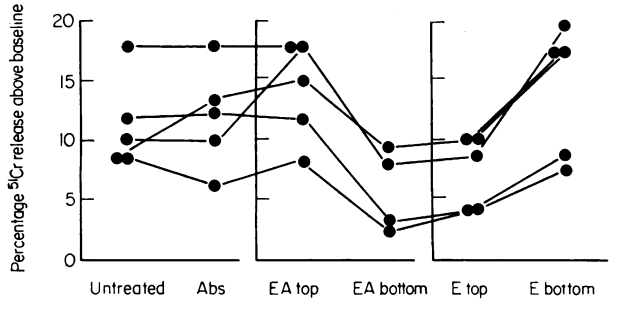

FIG. 2. Individual results of PHA-induced cytotoxicity for five experiments at an effector: target cell ratio of 30:1. Baseline taken as ^{51}Cr release from Chang cells in presence of effector cells alone.

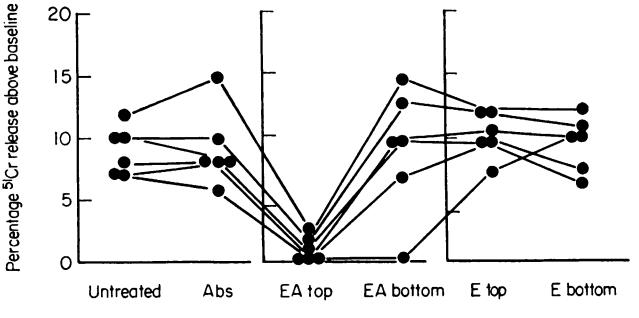

FIG. 3. Antibody-induced cell mediated cytotoxicity against Chang cells at an effector: target cell ratio of 30:1. Anti-Chang serum dilution 1×10^4. (Baseline as for Fig. 2.)

top fractions. It was lowest for the EA top and E bottom fractions. Further analysis of this spontaneous cytotoxicity has been described in assays against melanoma cells. (Hersey *et al.*, 1975a). (2) PHA-induced cytotoxicity was most marked in the T cell-enriched EA top and E bottom fractions and lowest in the T cell-depleted fractions, EA bottom and E top. This is further shown in Fig. 2 where the data from five experiments is shown at a ratio of 30 : 1, effector to target cells in terms of percentage ^{51}Cr release above the baseline formed by Chang cells plus effector cells in the absence of PHA or antibody. Mean values plus or minus one standard deviation for this data are shown in Table 2. (3) Antibody-dependent cell-mediated cytotoxicity (ADCC) was seen almost equally in each fraction except the EA top fraction depleted of Fc receptor-bearing cells. This is further illustrated

TABLE 2. Cytotoxic activity of fractions of mononuclear cells

Cytotoxicity induced by:	Unseparated	Glass absorbed	EA top	EA bottom	E top	E bottom
PHA	10·8±3	12·6±4	14·3±4	5·0±3	7·0±3	14·0±5
Antibody	8·6±2	8·8±4	0·9±1	8·0±4	9·2±2	8·0±4
PWM	17·0±6	19·0±6	5·0±5·0	12·0	17·0±6·0	14·0±4·0

Values indicated are mean ^{51}Cr release percentage ±1 s.d. about baseline formed by Chang cells + effector cells alone. 30:1 effector:target cell ratio.

TABLE 3. Identification of subfractions of E top cell population

Cell fraction	Ig-positive B cells	Anti-T-positive T cells	Unidentified cells	PHA response
E top	38±6	52±8	10±4	3480±1200
E top EAC top	18±6	66±8	16±6	7750±6100
E top EAC bottom	48±11	25±4	27±10	2150±780
E top EA top	20±4	77±10	3±15	3960±3700
E top EA bottom	58±9	27	15	2400±2100

Values indicated are mean percentages plus or minus one standard deviation.

in Fig. 3 where results at an effector target cell ratio of 30 : 1 are shown for six experiments. Mean values plus or minus one standard deviation for this data are shown in Table 2.

Cytotoxicity induced by PWM has in general been higher than with PHA or antibody, mean values plus or minus 1 s.d. for four studies at the same ratio as above is given in Table 2. The pattern of cytotoxicity is seen to resemble that induced by antibody rather than PHA.

Subfractions of the non-rosetting (E top) population

The heterogeneous population of cells left behind after removal of E rosetting (T cells) implies that a greater differential effect should be apparent by further subfractionation of this population. Table 3 indicates the partition of cells reacting with anti-Ig and the anti-T-

cell serum after the E top fraction had been submitted to EA and EAC rosetting and separation through H:F as described. The PHA responsiveness of these fractions is also indicated (data from three experiments). Enrichment for T cells was seen after removal of complement receptor-bearing cells and Fc receptor-bearing cells and *vice versa* for B cells. Percentages of EAC rosettes obtained for the three experiments was 40, 36 and 45 and EA rosettes 50, 42 and 48.

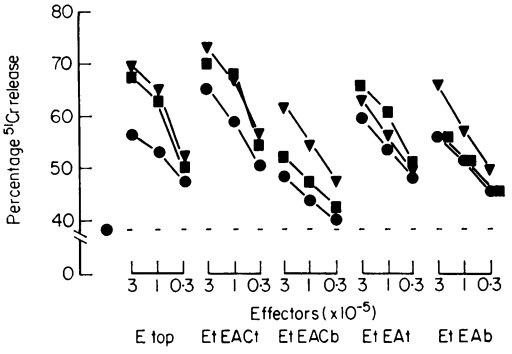

FIG. 4. Antibody and PHA-induced cytotoxicity by subfractions of E top population. (●) Effector cells + Chang cells alone. (▼) Cytotoxicity in presence of rabbit anti-Chang serum at dilution of 1×10^4. (■) Cytotoxicity in presence of PHA. (Et EACt = E top fraction depleted of EAC rosettes. Et EACb = EAC rosetting cells of E top fraction. Et EAt and Et EAb are equivalent fractions obtained after formation of EA rosettes in E top fraction.)

TABLE 4. Cytotoxic activity of E top cell subfractions

Cytotoxicity induced by:	E top	E top, EAC top	E top, EAC bottom	E top, EA top	E top, EA bottom
PHA	7·5±4	9·0±2	5·5±3	7·5±1	1±1·4
Antibody	13·0±3	9·0±1	16·5±8	2·0±1	10·5±6
PWM	15·0	12·0	14·0	3·0	6·0

Values indicated are mean percentage ^{51}Cr release above baseline release from Chang cells + effector cells alone, 30:1 effector:target cell ratio.

Cytotoxic activity by subfractions of the E top population

Fig. 4 illustrates a study in which the subfractions above were studied for their cytotoxic activity in the presence of PHA, PWM and sensitizing Chang cell antibody. The mean values for three experiments of this nature at a ratio of 30:1 are shown in Table 4. As before a wide variation in baseline ^{51}Cr release due to spontaneous cytotoxicity by the cell fractions was noted. PHA-induced cytotoxicity was maximal in the T cell-enriched populations and correlated reasonably well with the PHA mitogenic response except for the E top, EA bottom subfraction which had very low cytotoxic activity in the presence of PHA but a mitogenic response equivalent to the E top, EAC bottom subfraction. (The PHA response is, however, so low that the significance of this difference is doubtful.)

ADCC was most marked in the EAC rosette-enriched population but significant cyto-

toxicity was still seen in the EAC rosette depleted population. PWM-induced cytotoxicity (not shown in the figure) was still seen in the EAC rosette-depleted population. PWM-induced cytotoxicity again approximated the pattern seen with ADCC by the cell fractions.

Identity of antibody-dependent cytotoxic cell in the E bottom fraction

EAC and EA rosetting was carried out on the E bottom population in three experiments and the rosetted cells separated on H–F as before. No complement rosettes were seen and the ADCC was not reduced after the EAC plus E bottom mixture had been centrifuged on H–F. 1-4% EA rosettes were formed in the E bottom population and their removal on H–F resulted in marked reduction in ADCC. The percentage of anti-T reacting cells approached 100% and no immunoglobulin-positive cells were seen after EA rosette removal.

DISCUSSION

The identity of cells mediating antibody-dependent (AD) killing has aroused considerable interest since the description of this cytotoxic mechanism. A number of studies have shown that phagocytic cells can be removed without effecting the degree of ADCC (Perlmann *et al.*, 1972; MacLennan, 1972; Wunderlich, Rosenberg & Connolly, 1971). This is not to say that phagocytic cells cannot mediate ADCC and several reports have indicated that phagocytic mononuclear cells can mediate this type of killing (Perlmann & Perlmann, 1970; Gelfand, Resch & Prester, 1972). It is also generally conceded that the cells do not bear immunoglobulin on their surface and therefore are not typical B lymphocytes (Wisløff & Frøland, 1973; Greenberg *et al.*, 1973; Kedar, De Landazuri & Fahey, 1974). Some authors have preferred to retain the name lymphocyte for the cell type involved (MacLennan, 1972; Perlmann *et al.*, 1972) whilst others have suggested the cells belong more correctly to the macrophage series (Allison, 1972; Greenberg *et al.*, 1973).

The results in the present study using cell-surface receptors to identify and separate cells seems to clearly indicate that at least two mononuclear cell types can be defined in human blood which can mediate this type of killing. One cell type bears receptors for both complement and the Fc portion of IgG, whereas another bears only Fc receptors and is without identifiable complement receptors. This appeared evident from the experiments in which significant numbers of AD effector cells were left after removal of complement receptor-bearing cells from the non-SRBC-rosetting fraction. In addition, no complement-receptor rosettes could be identified in the E-rosetting fraction even though this fraction contained significant numbers of AD effector cells. The finding of AD effector cells without complement receptors is at variance with previous reports suggesting these cells bear such receptors (Van Boxel *et al.*, 1972; Perlmann *et al.*, 1972). Our findings are, however, supported by those of I. A. Ramshaw and C. R. Parish (personal communication) who were also able to identify AD effector cells without complement receptors in rat spleen cells using rosette separation techniques.

The finding of AD effector cells in the E-rosetting fraction is also of interest. They presumably belong to the unidentified 6% of cells in this fraction, as removal of Fc receptor-bearing cells removed AD effector cells and left practically a pure population of T cells. We cannot yet exclude with certainty that they represent a subpopulation of T cells with Fc receptors and AD effector cell function. This possibility is being examined more closely at the present time.

PHA-induced cytotoxicity appears to be predominantly a function of T cells. Fractions enriched for T cells had maximum cytotoxicity and conversely those depleted of T cells had low cytotoxicity. This result is perhaps not surprising in that several authors have previously shown that the mitogenic response to PHA is also a function of T cells (Geha & Merler, 1974; Greaves, Roitt & Rose, 1968).

Several recent reports have shown that B lymphocytes in man can also respond to PHA (Phillips & Roitt, 1973). Whether the same applies to PHA-induced cytotoxicity cannot be excluded entirely from these studies as it has not been possible to obtain a pure population of B lymphocytes. What is evident from the data, however, is that pure T cells alone can respond and that the cytotoxic response corresponds to the number of T cells present rather than the number of B cells or unidentified cells. The cell fraction with an almost absent PHA-induced cytotoxicity was the Fc rosetting cells obtained from the non-SRBC-rosetting fraction. This fraction had a high percentage of B lymphocytes and unidentified cells which argues strongly against these cell types having a cytotoxic role in the presence of PHA.

It is also clear from these studies that cells bearing Fc receptors are not required for PHA-induced cytotoxicity. This result again is perhaps not surprising but somewhat at variance with a recent report to the contrary (Hallberg, 1974).

PWM has been shown to induce a mitogenic response in both T and B cells in man (Geha & Merler, 1974) and rodents (Stockman *et al.*, 1971). A cytotoxic response by T cells in the presence of PWM can be demonstrated but is much weaker than the cytotoxic response in the presence of PHA. The major cell type involved appears instead to be similar to the effector cells mediating AD killing and includes the two cell types with and without complement receptors. We, like Stejskal *et al.* (1974), are unable to say from our data whether immunoglobulin-bearing cells are involved or whether it is precisely the same effector cell populations as those involved in AD killing.

Our findings suggest that for practical assessment of the immune cytotoxic activity in clinical studies the two most useful assays for discriminating between T- and non-T-cell cytotoxic function are PHA and antibody-induced cytotoxicity. The role of PWM-induced cytotoxicity in this context is less certain as there appears to be poor discrimination between T- and non-T-cell cytotoxic function with this agent.

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