

Contribution of lymphocytes bearing Fc γ receptors to PHA-induced cytotoxicity*

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Summary. Lymphocytes participating in PHA-induced lysis of chicken erythrocytes were characterized by means of cell fractionation methods. Selective depletion of, or enrichment in, E-rosetting cells indicated that the effector cell population was heterogenous, consisting of both T and non-T lymphocytes. Most effector cells, however, were shown to bear Fc γ receptors detected by the formation of erythrocyte-antibody (EA) rosettes, but to lack C3 receptors. The distribution of effector cells among tonsils, peripheral blood and thoracic duct lymph paralleled that of EA-rosette forming cells but not that of T or B cells. Addition of aggregated IgG resulted in a moderate decrease of PHA cytotoxicity. However, almost complete inhibition was achieved within a few hours of contact between effector cells and insoluble immune complexes. These results support the hypothesis that cytotoxic capacity is associated with the presence of Fc γ receptors on the cell surface.

INTRODUCTION

Non-immune effector cells have been described to mediate cellular cytotoxicity in the presence of phytomitogens or antibodies against target cell antigens (Perlmann & Holm, 1969). Application of

such *in vitro* models to the clinical investigation of immunological disorders would require a precise characterization of effector cells. In humans, the lymphocyte population that mediates antibody-induced lysis of heterologous erythrocytes was shown to bind aggregated or antigen-bound IgG via surface Fc γ receptors (Dickler, 1974) but to lack surface Ig or T-cell markers (Wislöff, Fröland & Michaelsen, 1974a; Cordier, Samarut, Brochier & Revillard, 1976). So far, the identity of effector cells mediating mitogen-induced cytotoxicity remains uncertain. In the mouse, PHA-induced lysis of chicken erythrocytes was first believed to be mediated by non-phagocytic thymus-dependent lymphocytes (Britton, Perlmann & Perlmann, 1973) until the demonstration that both macrophages and thymus-independent lymphocytes were endowed with cytotoxic potency in this reaction (Muchmore, Nelson, Kirchner & Blaese, 1975b; Nelson, Bundy, West & Strober, 1976b). In man as well, the nature of the effector cell in mitogen-induced cytotoxicity is still a matter of controversy, most of the activity being attributed either to T lymphocytes (Möller, Sjöberg & Andersson, 1972; Hersey, Edwards & Edwards, 1976; Wislöff, Fröland & Michaelsen, 1974b) or to non-T cells including adherent phagocytic cells (Silverman, Scheinberg, Yonkosky & Cathcart, 1976).

In an attempt to clarify these contradictory results in man, we investigated the nature of the PHA-stimulated effector lymphocytes in mitogen-induced lysis of chicken erythrocytes. We report that effector

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lymphocytes are distributed into two different subpopulations, one bearing and the other lacking T-cell markers. Both subpopulations have Fc γ but lack C3 receptors.

MATERIALS AND METHODS

Lymphocyte suspensions

Lymphocytes were isolated from the peripheral blood of healthy human donors by filtration of heparinized whole blood on a nylon column at 37° and further centrifugation on Ficoll-Isopaque gradient as already described (Cordier *et al.*, 1976b). Tonsillar lymphocytes were obtained from young adults undergoing surgery; contaminating adherent cells were removed by filtration on nylon column prior to Ficoll-Isopaque centrifugation. Cells were washed twice in Hanks's Balanced Salt Solution (HBSS) and resuspended in medium RPMI 1640 containing 5% foetal calf serum (FCS). Lymphoblastoid cell lines were obtained from Dr G. Lenoir (International Agency for Research on Cancer, Lyon), cells were washed twice in HBSS with 2% FCS before use. Cells were checked for viability by the Eosin Y exclusion dye test. All cell suspensions were shown to be contaminated by less than 1% monocytes identified by specific staining for endogenous peroxidase (Preud'homme & Flandrin, 1974) and less than 0.5% polymorphonuclear cells (Samarut, Brochier & Revillard, 1976).

Surface markers of cell subpopulations

Lymphocytes bearing Fc γ receptors were identified by a rosette technique using EA complexes formed with human O Rh+ erythrocytes (Hu-E) coated with human anti-Rhesus IgG (serum LOR, Dr Eyquem, Pasteur Institute, Paris) at maximal subagglutinating concentration. T and B cells were identified by use of the markers already described (Samarut *et al.*, 1976). Briefly, T cells were detected by their ability to form rosettes with sheep erythrocytes (E) after centrifugation at 4°; cells bearing receptors for the third component of complement were detected by their ability to form rosettes with E coated with IgM antibodies and C56/B1 mouse complement (EAC rosettes).

Fractionation of RFC by gradient centrifugation

Cell suspensions depleted of or enriched in RFC were obtained by centrifugation of cell mixtures on Ficoll-Isopaque gradient as already described.

Cells were collected at the interface and in some cases from the pellet freed of erythrocytes by hypotonic shock. A slight modification was used to allow the recovery of cell suspension depleted of EAC-RFC without contamination by E-RFC; EAC complexes were prepared using chicken red blood cells (CRBC) coated with rabbit anti-CRBC-IgM antibodies and mouse complement (EcAC) as previously described (Cordier *et al.*, 1976b).

Target cells

Erythrocytes from young white Leghorn chickens were used as target cells. After three washes (HBSS, 2% FCS) CRBC were labelled (37°, 90 min) with 200 μ Ci of Na₂ ⁵¹CrO₄ (specific activity 150–300 mCi/mg; CEA Saclay) and resuspended in RPMI 1640 containing 5% FCS.

Cytotoxic assay

PHA-induced cytotoxic activity was measured in a microtest derived from Perlmann, Perlmann & Holm (1968). Equal volumes of labelled CRBC (150 \times 10³/ml) and lymphocyte suspensions (150 \times 10³ to 6 \times 10⁶/ml) were mixed and 0.18 ml of the mixture was distributed into the wells of a plastic plate (Linbro Plastics, MF 96) which contain 0.02 ml of diluted PHA. After incubation at 37° for 18 h the radioactivity was measured in supernatants and the percent release was calculated. Results were expressed as cytotoxic indices (C.I.) calculated according to the formula:

$$C.I. = \frac{E - S}{100 - S}$$

where *E* stands for the percent release in experimental wells and *S* for the spontaneous release in control wells. All measurements were performed in quadruplicate.

Aggregated IgG

Agg-IgG was prepared according to Dickler & Kunkel (1972) by heating normal human IgG at 63° for 15 min. The suspension was then homogenized and centrifuged for 15 min at 800 g to remove large aggregates.

RESULTS

Parameters of the cytotoxic reaction

Parameters of the lytic reaction had to be determined

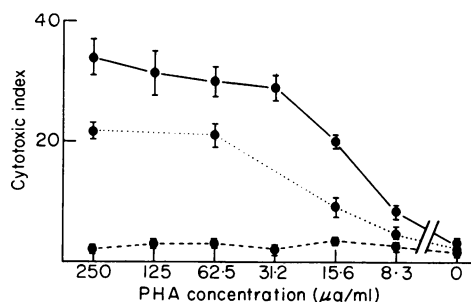


Figure 1. Dose dependency of PHA-induced cytotoxicity. Effect of different peripheral blood lymphocyte:target cell ratios. (—) 40:1; (····) 20:1; (---) 0:1.

in order to compare the efficiency of different effector cell populations. Maximum lysis was achieved in 18 h. The C.I. was found to depend on both PHA concentration and effector cell numbers (Fig. 1). With 31 µg/ml PHA, increasing effector to target cell ratios from 5:1 to 40:1 resulted in a linear increase of C.I. up to a plateau (Fig. 2). It was therefore assumed that differences between C.I. of two suspensions of PHA-activated cells reflect different numbers of effector cells rather than differences in activity per cell. Low effector to target cell ratios (5:1 to 20:1) were then most suitable to demonstrate an enrichment in effector cells whereas high ratios (40:1) were used to assess the depletion of effector-cells.

Fractionation of EA-RFC

Peripheral blood lymphocytes were mixed with EA

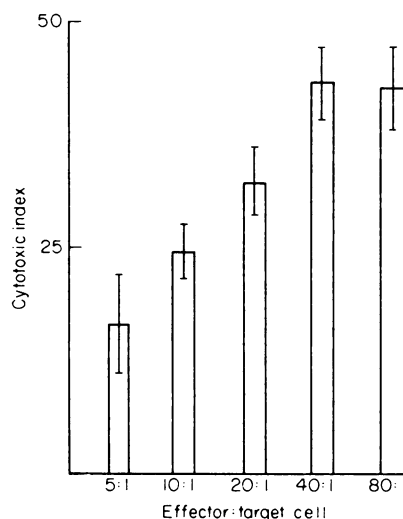


Figure 2. Effector:target cell ratio dependency of PHA (31 µg/ml)-induced cytotoxicity.

complexes to form EA rosettes and then fractionated on Ficoll-Isopaque gradient. Non-rosetting lymphocytes collected at the interface showed much lower C.I. than unseparated cells ($P < 0.025$, Student's *t*-test on paired values). Conversely, rosetting lymphocytes recovered from the pellet after hypotonic lysis of erythrocytes showed greater cytotoxic activity than unseparated ($P < 0.025$) or EA-RFC depleted ($P < 0.01$) suspensions (Table 1). The plateau of C.I. obtained with effector to target cell ratios greater than 5:1 was considered as further evidence that precursors of PHA cytotoxic

Table 1. Effect of EA-RFC fractionation on PHA-induced cytotoxicity

Cell suspension	RFC		Cytotoxic index			
	E	EA	40:1	20:1	10:1	5:1
(1) Untreated	74 ± 3.7	13.9 ± 1.7	33.0 ± 5.7	26.3 ± 4.5	21.7 ± 4.2	16.3 ± 3.0
(2) EA-RFC depleted	73 ± 5.4	0 ± 0.1	14.6 ± 3.3	10.5 ± 1.9	10.0 ± 1.8	5.5 ± 1.1
(3) EA-RFC enriched	54 ± 5.6	48.5 ± 4.3	46.6 ± 7.8	45.8 ± 8.0	40.0 ± 7.6	36.9 ± 6.9
<i>P</i> values*						
(2/1)			0.01	0.025	0.025	0.025
(3/1)			0.025	0.01	0.01	0.01
(3/2)			0.01	0.01	0.01	0.01

Cell suspensions were depleted of EA-RFC by centrifugation on Ficoll-Isopaque gradient. Enriched cell suspensions were obtained from the pellet after lysis of erythrocytes. The cytotoxic index was measured for different effector:target cell ratios after incubation at 37° for 18 h (PHA: 31 µg/ml).

Mean ± s.e.m. from seven experiments.

* Student's *t*-test on paired values.

Table 2. Enrichment in PHA-induced cytotoxicity after fractionation of EAC-RFC

Cell suspension	Rosette-forming cells			Cytotoxic index		
	EAC	EA	E	40 : 1	20 : 1	10 : 1
Untreated	11 ± 2.5	19 ± 1.8	67 ± 9.4	66.5 ± 7.2	55.8 ± 5.1	46.4 ± 4.2
EAC-RFC depleted	2 ± 0.6	25 ± 4.4	75 ± 9.7	80.1 ± 3.9	74.4 ± 3.8	61.5 ± 3.2
<i>P</i> values*				0.01	0.01	0.01

Cell suspensions were depleted of EAC-RFC by centrifugation of EcAC rosette mixture on Ficoll-Isopaque gradient. Percentage of rosette-forming cells ± s.e.m., cytotoxic index ± s.e.m.

* Student's *t*-test on paired values.

Table 3. PHA-induced cytotoxic activity after fractionation of E-RFC

Cell suspension	RFC		Cytotoxic index		
	E	EA	40 : 1	20 : 1	10 : 1
Untreated	74 ± 3.7	14 ± 1.7	36.6 ± 6.9	23.8 ± 4.4	14.3 ± 4.8
E-RFC depleted	7 ± 2.7	22 ± 1.2	n.d.	25.5 ± 5.2	17.8 ± 3.6
E-RFC enriched	95 ± 5.3	7 ± 2.0	28.0 ± 5.3	15.2 ± 2.4	10.0 ± 3.8

Cell suspensions were depleted of E-RFC by centrifugation on Ficoll-Isopaque gradient. Enriched cell suspensions were obtained from the pellet after lysis of erythrocytes. Percentage of rosette-forming cells (RFC) cytotoxic index, mean values ± s.e.m. from eight experiments. n.d. not done.

cells belong to the subpopulation of EA rosetting cells.

Fractionation of EAC-RFC

Peripheral blood lymphocytes were mixed with chicken erythrocytes coated with IgM antibodies and mouse complement to form EAC rosettes. After centrifugation on Ficoll-Isopaque, non-rosetting cells collected at the interface were indeed depleted of EAC-RFC, slightly enriched in E- and EA-RFC, and significantly more efficient in PHA-induced cytotoxicity than unseparated cells ($P < 0.01$). It was therefore concluded that effector cells were unlikely to bear C3 receptors (Table 2).

Fractionation of E-RFC

Peripheral blood lymphocytes were mixed with sheep erythrocytes (100 : 1 giving maximum rosetting) and fractionated by centrifugation on Ficoll-Isopaque. Rosetting lymphocytes obtained from the pellet were freed of erythrocytes by hypotonic

lysis. PHA-induced cytotoxic activity appeared equally distributed among rosetting and non-rosetting cells (Table 3). The activity of both populations was similar to that of unseparated cells indicating that no significant loss of effector cells had occurred during the separation procedure.

Fractionation of E-RFC bearing Fc γ receptors (E-EA-RFC)

Lymphocyte suspensions were first enriched in E-RFC by gradient centrifugation, then mixed with EA complexes and centrifuged on Ficoll Isopaque. Lymphocytes collected at the interface were shown to form E but not EA rosettes. Conversely the lymphocyte suspension recovered from the pellet was found to bind both sheep erythrocytes and EA complexes, after it had been freed from erythrocytes by hypotonic lysis. Among E-RFC those bearing Fc γ receptors demonstrated by EA rosette formation were more potent effectors of PHA-induced cytotoxicity than T cells lacking Fc γ receptors (Table 4).

Table 4. PHA-induced cytotoxic activity of T cells bearing Fc γ Receptors (Fc γ R)

Cell suspension	RFC		Cytotoxic index		
	E	EA	40 : 1	20 : 1	10 : 1
(1) E-RFC enriched	91 \pm 0.4	6 \pm 2.6	50.3 \pm 9.4	24.2 \pm 4.5	14.7 \pm 2.9
(2) E-EA-RFC depleted	96 \pm 0.9	1 \pm 0.7	21.3 \pm 4.0	12.5 \pm 2.3	12.9 \pm 2.4
(3) E-EA-RFC enriched	83 \pm 4.4	40 \pm 1.7	n.d.	38.4 \pm 7.2	18.5 \pm 3.6
<i>P</i> values*					
(2/1)			0.05	0.05	n.s.
(3/1)				0.05	0.05

Cell suspension obtained from a first enrichment in E-RFC were subjected to a second rosetting with HuEA complexes then centrifuged on Ficoll-Isopaque gradient. Cells at the interface were depleted of E-RFC bearing Fc γ R (E-EA-RFC depleted); cells obtained from the pellet after lysis of erythrocytes were enriched in E-RFC bearing Fc γ R (E-EA-RFC enriched). Percentage of RFC \pm s.e.m.; cytotoxic index \pm s.e.m.; results from five experiments.

* Student's *t*-test on paired values; n.s., not significant; n.d., not done.

Table 5. PHA-induced cytotoxicity of lymphoid cells from different sources

Cell suspension	Rosette-forming cells				Cytotoxic index		
	<i>n</i>	E	EA	EAC	<i>n</i>	40 : 1	20 : 1
Peripheral blood lymphocytes	≥ 20	74.0 \pm 3.7	13.9 \pm 1.7	16.0 \pm 3.4	83	43.1 \pm 4.0	31.7 \pm 3.3
Tonsillar lymphocytes	≥ 20	41.7 \pm 2.2	0.5 \pm 0.3	53.7 \pm 3.0	7	11.0 \pm 1.7	7.7 \pm 2.2
Lymph lymphocytes	8	75.5 \pm 3.4	0.4 \pm 0.3	10.7 \pm 2.3	28	15.4 \pm 1.9	n.d.*
Cell lines							
Raji		< 1	6	98.5		1.8	2.9
Ly 38		< 1	1.8	85.6		9.1	11.1
Ly 11		< 1	1.7	85.9		7.8	9.0
Ly 2		< 1	1.7	93.2		13.9	10.2

* Not done.

PHA-induced cytotoxicity of lymphoid cells from different sources

The percentage of lymphocytes forming E, EA and EAC rosettes and the C.I. generated from the same populations were determined on cells obtained from various sources (Table 5). In tonsils where an equal proportion of T and B cells was found, the percentage of EA-RFC was very small and PHA-induced cytotoxicity much lower than in peripheral blood. Thoracic duct lymph, obtained from uraemic patients was compared with peripheral blood collected from the same patients. The percentages of T and B cells were comparable in lymph and blood but thoracic duct lymph contained fewer EA-RFC and PHA-

cytotoxic effector cells than did peripheral blood (Table 5). Finally four lymphoblastoid cell lines bearing C3 receptors but virtually no Fc γ receptors, mediated only minimal target cell lysis in the presence of PHA. In agreement with the results of fractionation experiments reported above, these data suggested a parallelism between numbers of EA-RFC and PHA-cytotoxic activity. No such parallelism was found with E-RFC nor with EAC-RFC.

Involvement of Fc γ receptor in PHA-induced target cell lysis

The finding that PHA-induced cytotoxicity was

Table 6. PHA-induced cytotoxicity following interaction between Fc γ R and aggregated IgG or EA complexes

	EA-RFC	PHA-induced cytotoxicity		
		Effector : target cell	Cytotoxic index	P values
(1) Unfractionated lymphocytes				
0	22.1	40 : 1	26.6 \pm 5.6	
Aggregated IgG 20 μ g/ml	12.8	40 : 1	21.4 \pm 7.1	0.05
200 μ g/ml	0.9	40 : 1	18.6 \pm 8.0	0.05
(2) EA-RFC enriched suspension treated by hypotonic lysis and				
Culture for 18 h at 4 $^{\circ}$	35.0	10 : 1	23.0 \pm 6.0	
Culture for 18 h at 37 $^{\circ}$	2.0	10 : 1	2.6 \pm 1.0	0.01

(1) Unfractionated lymphocytes were incubated with aggregated IgG (0, 20, 200 μ g/ml) for 15 min at 37 $^{\circ}$ and 30 min at 4 $^{\circ}$, then washed twice. They were then tested for their capacity to form EA rosettes or to act as effector cells in PHA-induced cytotoxicity.

(2) EA-RFC enriched suspension was obtained as described in Table 1. Following the lysis of erythrocytes, cells were incubated for 18 h at 4 $^{\circ}$ or 37 $^{\circ}$; the PHA-induced cytotoxicity was then measured.

associated with the presence of EA-RFC raised the possibility that Fc γ receptor may contribute to the triggering, or control the expression, of the lytic process as it does with K cells mediating antibody-dependent cell-cytotoxicity. Addition of heat-aggregated human IgG at concentrations sufficient to fully inhibit EA-rosette formation (200 μ g/ml) resulted in a significant but slight decrease of PHA cytotoxicity (Table 6). In contrast, antibody-dependent cell-cytotoxicity was fully inhibited at the same aggregated IgG concentration (data not shown). These experiments suggested that binding of aggregated IgG to their Fc γ receptors did not abolish the cytotoxic capacity of PHA-stimulated lymphocytes unlike that of antibody-dependent cytotoxic lymphocytes.

By fractionation of EA-RFC, positive selection of PHA cytotoxic effector cells could be achieved. In the experiments depicted in Table 1, EA-RFC were freed of erythrocytes by hypotonic lysis and PHA added immediately thereafter. If, however, dissociated EA-RFC were left 18 h at 37 $^{\circ}$ prior to addition of PHA and target cells, cytotoxicity was suppressed and cells were no longer capable of forming EA rosettes (Table 6). Conversely, dissociated EA-RFC kept at 4 $^{\circ}$ retained their capacity to form EA-rosettes and to lyse chicken erythrocytes in the presence of PHA. Therefore, after a first binding to insoluble (EA) complexes, Fc γ receptors became undetectable within a few hours at 37 $^{\circ}$ and their

modulation was accompanied by a marked decrease in the cytotoxic effect.

DISCUSSION

Results reported in this study only concern human lymphocytes and care was taken to remove non-lymphoid cells without selective loss of a lymphocyte subpopulation (Samarut *et al.*, 1976). PHA-induced lysis of chicken erythrocytes can be mediated by a variety of cell types including, in addition to lymphocytes, polymorphonuclear cells (Simchowicz & Schur, 1976), adherent phagocytic cells susceptible to inhibition by hydrocortisone (Silverman *et al.*, 1976) or carrageenan (Muchmore *et al.*, 1975b). Even certain tumour cells have been shown to induce a significant lysis of target cells in the presence of PHA (Muchmore *et al.* 1975a).

The contribution of T lymphocytes from human peripheral blood in PHA-induced cytotoxicity is supported by fractionation experiments in which purified E-RFC were found to be as potent effectors as unseparated cells. This result is in agreement with previous reports in which CRBC were used as targets and human lymphocytes (Wislöf *et al.*, 1974b; Nelson, Bundy, Pitchon, Blaese & Strober, 1976a) or mouse lymphocytes (Britton *et al.*, 1973; Nelson *et al.*, 1976b) as effector cells. Similarly T cells were shown to mediate PHA-induced cytotoxicity on tumour cell targets (Hersey *et al.*, 1976;

Nelson *et al.*, 1976a; Bonavida, Robins & Saxon, 1977; Muchmore *et al.*, 1975b). Conversely, Dawkins & Zilko (1975) and Silverman *et al.* (1976) failed to obtain significant cytotoxicity with cell suspensions enriched in E-RFC. The discrepancy between these results might be accounted for by minor differences in the E rosette technique. Hence T cells bearing Fcy receptors have a low binding avidity to sheep erythrocytes and may not be detected as such if a SRBC : lymphocyte ratio of less than 100 : 1 is used (Samarut *et al.*, 1976). Most of the cytotoxic capacity of T cells is supported by a subpopulation bearing Fcy receptors which was selected by a two-step fractionation involving first an enrichment in E-RFC then in EA-RFC. This observation is in agreement with the decreased cytotoxic effect of populations depleted of Fcy receptor-bearing cells after elimination of EA-RFC (Wisloff *et al.*, 1974b; Hallberg, 1974) or by adherence to insolubilized antigen-antibody complexes (Waller *et al.*, 1976; Bonavida *et al.*, 1977). Fractionated blood T cells lacking Fcy receptors appear to contribute very little to PHA-induced cytotoxicity. Furthermore, fractionated cell suspensions prepared from human tonsils or thoracic duct lymph from which the T-cell subset with Fcy receptors is absent are quite poor effectors. T cells undergoing proliferation under PHA stimulation can be distinguished from those mediating PHA-induced cytotoxicity. The dichotomy between cytotoxic and proliferative responses was demonstrated by their different ontogeny in human foetuses (Stites, Carr & Fudenberg, 1972) and by the differential effect of lymphocyte depletion by thoracic duct drainage which reduces the proliferative response without affecting PHA-induced cytotoxicity (Cordier, Brochier & Revillard, 1976). Cells mediating these two responses cannot, however, be distinguished according to the presence of Fcy receptors since both EA-rosetting and non-rosetting cells proliferate in the presence of PHA (Moretta, Ferrarini, Mingari, Moretta & Webb, 1976; Samarut & Cordier, 1977) whereas most of the cytotoxic activity is confined to the EA-RFC subset. Finally, it is noteworthy that precursors of effectors of cell-mediated lympholysis (Eijsvoogel, Schellekens, DuBois & Zeijlemaker, 1976) and a part of activated T cells with H2 specific killer activity (Kramer, Elliott & von Boehmer, 1976) were shown to bear Fcy receptors like T cells mediating PHA cytotoxicity.

In addition to T cells a second population of

non-T cells can destroy CRBC in the presence of PHA. In agreement with previous reports in the mouse (Nelson *et al.*, 1976b) and in man (Wisloff *et al.*, 1974b; Hallberg, 1974; Nelson *et al.*, 1976a; Bonavida *et al.*, 1977) this second population of effectors is characterized by the presence of surface receptor for Fcy. These cells cannot be regarded as mature monocytes on the basis of their morphology, their lack of adherence and phagocytic capacities and the absence of endogenous peroxidase activity. Furthermore we have shown that unlike most B lymphocytes these cells did not fractionate with EAC-RFC indicating that receptors for C3b and C3d are either not expressed or of very low avidity. Therefore the non-T effectors of PHA cytotoxicity share most of their characteristics with K cells defined as effectors of antibody-dependent cell-cytotoxicity. K cells have been characterized as non-adherent non-phagocytic lymphoid cells lacking B- and T-cell markers and bearing 'high avidity' Fcy receptors (Cordier *et al.*, 1976b).

The mechanisms of mitogen-induced cytotoxicity are still poorly understood. The possibility that polyclonal activators induce the production of anti-target antibodies triggering K cells via their Fcy receptors may be considered. It appears, however, unlikely in view of the following results: (1) purified T cells are potent effectors; and (2) blocking of Fcy receptors with Agg-IgG inhibits antibody-dependent cell-cytotoxicity without major reduction of PHA-induced cytotoxicity.

Finally, cytotoxic capacity is associated with the presence of receptors for Fcy on different lymphocyte subsets as well as on monocytes and polymorphonuclear cells. It may therefore be assumed that antigen-antibody complexes can interfere with the cytotoxic reactions mediated by these different cells. If the lytic process is triggered by target-bound antibodies, as with K cells or polymorphonuclears, unrelated immune complexes can inhibit the cytotoxic reaction by the mere blocking of Fcy receptors on effector cells. If Fcy receptors are not involved in the triggering of the cytotoxic reaction, as is the case in PHA-induced cytotoxicity, immune complexes may still interfere with the lytic process. After first binding to insoluble immune complexes or aggregated IgG, cytotoxic cells loose both their Fcy receptors and their cytotoxic capacity within a few hours. The phenomenon is temperature dependent, faster at 37° than at 4°. It has been termed 'modulation' of Fcy receptors since the cells were shown to be alive and metabolically active

(Cordier, Samarut & Revillard, 1978). It may represent a new pathway of interaction between immune complexes and cytotoxic cells which could be considered in clinical situations associating circulating immune complexes with a decreased cytotoxic response to PHA, as for instance, in rheumatoid arthritis (Wisłöf *et al.*, 1975).

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