Immunology 1988 **63** 513–520

Antibody-mediated erythrolysis and erythrophagocytosis by human monocytes, macrophages and activated macrophages. Evidence for distinction between involvement of high-affinity and low-affinity receptors for IgG by using different erythroid target cells

S. J. RÜEGG & T. W. JUNGI *Institute for Clinical and Experimental Cancer Research, University of Berne, Switzerland*

Accepted for publication 2 October 1987

SUMMARY

Antibody-dependent cellular cytotoxicity (ADCC) and Fc receptor-mediated phagocytosis were determined with human monocytes, monocyte-derived macrophages and activated macrophages, using rabbit IgG-covered sheep red blood cells (EA^s) and anti-D-treated human erythrocytes (EA^{hu}) as target cells. Monocyte and macrophage-mediated ADCC were distinguished by different kinetics, monocytes lysing either target more rapidly than macrophages. Macrophage activation by recombinant IFN-γ (rIFN-γ) led to a marked increase in ADCC activity against EA^{hu}. This manifested in increased lysis of optimally sensitized target cells, in a sustained lysis of target cells carrying low antibody densities, and as an enhanced resistance to lysis inhibition by competing fluid-phase IgG. All these effects were less striking when EA^s were the target cells. Phagocytosis of EA^s by rIFN-γ-treated cells was strongly suppressed, regardless of the amount of antibody on the target cells, and susceptibility to inhibition by fluid-phase IgG was slightly increased. By comparison, phagocytosis of EA^{hu} was depressed to a lesser degree, and susceptibility to inhibition by fluid-phase IgG was reduced when macrophages were rIFN-γ treated. These and other experiments suggested that the functional triggering of monocytes and macrophages by EA^s involved, at least in part, low-affinity Fc receptors (FcR), whereas EA^{hu} interacted with macrophages via high-affinity FcR. It is shown elsewhere that rIFN-γ treatment of macrophages increases the expression of high-affinity FcR, but not low-affinity FcR (Jungi, Lerch & Brcic, 1987). Differences in the rIFN-γ-induced functional alterations assessed with EA^{hu} or with EA^s are interpreted therefore as being a consequence of differential involvement of high-affinity FcR and of low-affinity FcR in mediating an effector function. For monitoring rIFN-γ-induced alterations in the effector capacity EA^s are more appropriate targets since up-regulation of high-affinity FcR has a smaller influence on the response to this type of target. Using metabolic inhibitors, ADCC could be dissociated from ingestion suggesting that ADCC is not a post-phagocytic event.

INTRODUCTION

ADCC is one of the effector functions by which a host can eliminate antibody-coated pathogens or tumours (Möller, 1965; reviewed by Perlmann, Perlmann & Wigzell, 1976; Pearson, 1978). It requires the binding to and specific triggering of cellular receptors for the Fc portion of IgG (Fc receptors, FcR). A variety of FcR-positive cells can engage in ADCC, including cells of the monocyte/macrophage series (Conkling, Klassen & Sagone, 1982; Katz *et al.*, 1980; Koren, Anderson & Adams, 1981; Ralph *et al.*, 1980), various types of granulocytes (Conkling *et al.*, 1982; Butterworth *et al.*, 1977), and cells belonging to the NK/K cell type of lymphocytes (De Lanzaduri *et al.*, 1979). The lytic processes set in motion are poorly understood and probably complex. However, there are good reasons to believe that the lytic mechanisms for different types of ADCC effectors are distinct (Podack, 1985; Russel, 1986). With the advent of monospecific and monoclonal antibodies against defined specificies on pathogens and tumours, ADCC is included in strategies aimed at eliminating these noxious entities.

Correspondence: Dr T. W. Jungi, Institute for Veterinary Virology, University of Berne, Länggassstr. 122, CH-3012 Berne, Switzerland.

In man, ADCC mediated by monocytes, polymorphonuclear leucocytes (PMN) and by K cells have been investigated intensively. Due to their limited availability, macrophages as ADCC effector cells have received less attention. In the present report, ADCC of human monocytes and macrophages were investigated. Monocytes were allowed to differentiate into macrophages *in vitro*, using hydrophobic teflon foil bags as culture vessels (Andreesen, Picht & Löhr, 1983; Jungi & Hafner, 1986). Erythrocytes (E), which are known to be lysed by mononuclear phagocytes, served as model targets. ADCC was determined in a novel spectrometric test, exploiting the pseudoperoxidase activity of haemoglobin released into the supernatant. It was found that rabbit IgG-sensitized sheep erythrocytes (EA^s) and human anti-D-sensitized erythrocytes (EA^{hu}) interact differently with ADCC effector macrophages. Evidence is provided that these differences root in the triggering of different FcR populations by the two types of model targets. Regardless of the differences in recognition, ADCC is increased upon effector-cell activation by rIFN-γ, while phagocytosis is depressed under these conditions.

MATERIALS AND METHODS

Chemicals

Cytochalasin B (CB) was obtained from Sigma (St Louis, MO). It was dissolved in dimethyl sulphoxide at 5 mg/ml and stored frozen until use. Hydroxyethyl piperazine ethane sulphonic acid (HEPES) and colchicine were purchased from Fluka, (Buchs). Iodoacetate, sodium dodecylsulphate (SDS), 2-desoxy glucose (2-DOG), ethylene diamino tetraacetic acid (EDTA) and inorganic chemicals were from Merck (Darmstadt, FRG). Tetramethyl benzidine and diaminobenzidine were from Sigma. Media (see below) were from Seromed (Fakola, Basel) and human serum albumin (HSA) was obtained from Behring (Hoechst, Zurich).

Effector-cell preparation

Mononuclear cells were obtained from buffy coats of whole blood donations as described elsewhere (Jungi & Hafner, 1986), using a modified Ficoll–Hypaque centrifugation procedure monocytes were purified by selective adherence to and subsequent detachment from polystyrene tissue culture flasks (Jungi & Hafner, 1986). Purified monocytes ($0{\cdot}5 \times 10^6$/ml) were then put into teflon bags in HEPES-buffered RPMI-1640 supplemented with glutamin (2 mM), non-essential amino acids (1%; Gibco, AG, Basel), MEM vitamins (1%; Gibco), sodium pyruvate (1%; Gibco), penicillin (100 IU/ml), streptomycin (100 μg/ml), fungizone (1%; Gibco) and 15% heat-inactivated human serum. Cells were kept for 7–10 days without change of the medium in humidified, gassed (5% CO_2 in air) chambers (Biotec, Gelterkinden) at 37°. Then, the bags were chilled, the cells harvested, washed once in phosphate-buffered (10 mM, pH 7·4) saline (PBS) at 4° and resuspended in HEPES-buffered (30 mM; pH 7·25) RPMI-1640 containing 0·5% (w/v) HSA (RHA). Cells used in phagocytosis tests were resuspended in HEPES-buffered minimal essential medium supplemented with 0·5% HSA (MHA).

In some experiments, macrophages were treated for 2 days with rIFN-γ. This was the kind gift of Biogen (Geneva). It had a specific activity of $1{\cdot}3 \times 10^7$ U/mg and the lyophilized material contained >99% rIFN-γ, HSA and salts. Onset of treatment (500 U/ml) was between Day 7 and Day 9 of culture.

Target-cell preparation

Human erythrocytes (E^{hu}) from one A/Rh^+ donor were used in all experiments. They were washed three times in PBS, sensitized with human anti-D-specific IgG (Central Laboratory of the Swiss Red Cross Blood Transfusion Service, Berne) in PBS–EDTA (5 mM), washed again in PBS and resuspended in Alsever's solution. Throughout the study, packed erythrocytes from the same sheep (E^s) were obtained from the Swiss Serum and Vaccine Institute, Berne. They were washed with PBS and sensitized with varying subagglutinating concentrations of affinity-purified rabbit-anti-E^s IgG (Diamedix, Miami, FL) (30 min 37°, 30 min on ice). After two washes in PBS–EDTA or PBS, respectively, they were resuspended in Alsever's solution. E suspensions were stored at 4° for up to 3 weeks.

Immediately prior to the assay, EA^{hu}, EA^s and control erythrocytes (E^{hu} and E^s, respectively) were washed, resuspended in PBS and admixed to the assay medium at the required concentration ($0{\cdot}5 \times 10^6$ E or EA in 50 μl for ADCC tests, 1% v/v in phagocytosis assays). Concentrations were adjusted by spectrophotometry at 412 nm, using experimentally determined conversion factors relating cell number per millilitre to optical density.

In some experiments, one part packed E was labelled with one part $Na_2{}^{51}CrO_4$ (5 mCi/ml 0·9% NaCl; Amersham, Int. Amersham, Bucks, U.K.) for 30 min at 37°. E were then washed three times with PBS, and sensitized as described above.

ADCC assay

ADCC was determined in flat-bottomed microtitre plates for cell culture (Nunc, Roskilde, Denmark). Effector cells were allowed to adhere to the bottom. In experiments with monocytes, mononuclear cells containing $1{\cdot}25 \times 10^5$ monocytes were assayed per well, and non-adherent cells were washed away after a 30-min incubation at 37°. The remaining cells contained 80% of the monocytes added. Lymphocyte contamination was 5–10%. In macrophage experiments, 3×10^4 viable macrophages were allowed to adhere during 2 hr at 37°. Monolayers were then washed, covered with 50 μl of RHA, and 50 μl of target cells were distributed, resulting in an target-to-effector ratio of 5 (monocytes) or 17 (macrophages). In some experiments, 50 μl of an inhibitor or of medium, respectively, were also added. Unless otherwise noted, ADCC was allowed to proceed for 4 hr at 37°. Then, plates were spun (5 min at 1500 *g*) and 50 μl supernatant were transferred to a second plate. Also transferred were 50-μl aliquots from wells containing no target cells (calibration wells), and from wells containing no effectors (spontaneous lysis). Calibration wells received 50 μl sodium dodecyl sulphate (SDS) in sodium phosphate buffer (0·1 M, pH 5·5) (PB 5·5) in which known numbers of E were lysed. All other wells received 50 μl SDS solution without E lysate. Then, 150 μl of a peroxidase substrate solution were added. It contained five parts of a 0·2% tetramethyl benzidine solution in ethanol, 1·4 parts HCl (0·5 M), 0·4 parts H_2O_2 and 93·2 parts PB 5·5, and was prepared immediately before use. Pilot experiments showed that under these conditions and within a certain concentration range (2×10^3–100×10^3 E^s), substrate conversion was directly proportional to the OD, as determined in an automated ELISA-reader photometer (Dynatech Produkte, Embroch) at 405 nm (test

wave length) and 490 (reference wave length). The addition of SDS provided linearity over a wider range than would be obtained in the absence of SDS. Medium components such as phenol red did not interfere as long as serum-free media were used. In experiments with ^{51}Cr-labelled E, the ADCC lysates were measured in a gamma-counter after spectrophotometry.

Phagocytosis test

Monolayers of macrophages were prepared as described above in microtitre (MT) plates and covered with 50 μl MHA. Then, 50 μl sensitized E (1% v/v) were offered for phagocytosis. After 1 hr incubation at 37°, non-ingested E were removed by washing and hypotonic lysis. Monolayers containing ingested E were lysed in SDS solution, and the haemoglobin content in the wells determined by a spectrometric procedure similar to the one described above, except that diaminobenzidine was the substrate and PBS the buffer system. The assay is described in detail elsewhere (Jungi, 1985).

Data reduction and statistical analysis

All ADCC and phagocytosis experiments were performed in triplicate and were repeated at least once. In ADCC experiments, lysis was expressed as a percentage according to the formula:

$$\text{lysis (\%)} = \frac{\text{number of E lysed (test)} - \text{number of E lysed (spont.)}}{\text{number of E (added)} - \text{number of E lysed (spont.)}} \times 100.$$

No correction was made for specific lysis, since lysis of unsensitized E was insignificantly higher than spontaneous lysis. In addition, the number of lysed cells per monocyte or macrophage nucleus was calculated by counting the number of effector cell nuclei according to Nakagawara & Nathan (1983) (ADCC index). In phagocytosis tests, a phagocytic index (ingested cells per macrophage nucleus) was determined accordingly. These figures were corrected for background (ingestion of unsensitized E). For eliminating variation in the maximal effector capacity of different cell populations, lysis and ingestion values were normalized by expression as percentages of the maximal response obtained in a given assay; these relative figures were averaged. In assays containing an inhibitor, inhibition was expressed as a percentage of the uninhibited control.

In tests comparing two treatment modalities, the level of significance was calculated by the paired Student's *t*-test.

RESULTS

Spectrometric and radiometric determination of monocyte and macrophage ADCC

The use of erythroid targets offers the possibility to use a spectrometric procedure, exploiting the pseudoperoxidase activity of haemoglobin in the lysate and circumventing radiometry. Pilot experiments revealed that peroxidase activity is of sufficient sensitivity to determine the number of lysed E in microtitre plates, and appropriate conditions were defined for use of this assay in ADCC tests. In order to validate this procedure further, ADCC lysates generated by monocytes and macrophages under a variety of conditions were assayed both ways, spectrophotometrically and radiometrically. An excellent correlation between the two methods was obtained (correlation coefficient $\geq 0{\cdot}98$). While in macrophage assays the degree of lysis was almost identical, the spectrometric figures in monocyte tests were systematically higher than the radiometric values (increase $\approx 40\%$). This could be due to a contribution of the endogenous peroxidase of monocytes which is absent from macrophages. For achieving the sensitivity of the spectrometric assay by radiometry, however, a relatively high ^{51}Cr concentration had to be used, approximating a concentration inhibiting various metabolic activities in E (Decré & Lewis, 1984). In subsequent tests, therefore, the spectrometric test was used.

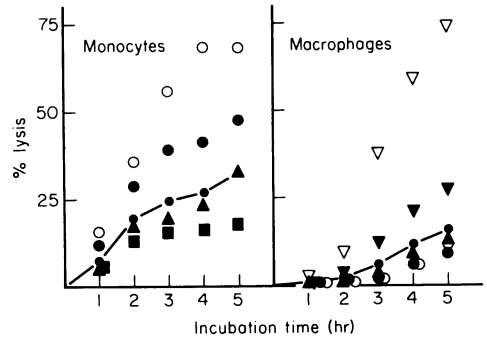

Figure 1. The kinetics of ADCC of EA^{s} (closed symbols) and EA^{hu} (open symbols) by human monocytes and macrophages. Symbols refer to individual experiments, the point-connecting lines refer to the mean of three experiments involving EA^{s}.

The influence of various effector and target cell numbers on ADCC

In preliminary tests, various effector-to-target ratios were used in 4 hr ADCC asays, using both monocytes and macrophages as effectors and both EA^{hu} and EA^{s} as targets. Lysis, expressed as a percentage, increased with increasing effector cell number, except that a macrophage number beyond 5×10^4/well showed decreasing efficiency in lysing EA^{s} targets. Otherwise, the number of lysed EA per effector remained constant but slightly increased with increasing target cell number. Using the conditions outlined in the Materials and Methods, the number of target cells lysed in 4 hr was between 0·5 and 1 per monocyte and between 1 and 2 per macrophage. These suboptimal conditions were chosen for further experiments such that alterations in the degree of cell triggering become expressed in a changed ADCC response.

Kinetics of monocyte and macrophage ADCC

ADCC was measured at different times after the onset of the assay (Fig. 1). Whereas monocyte ADCC showed the highest increase during the first 2 hr of incubation, macrophage ADCC was small during this period of time, but later on it increased progressively. This divergent behaviour was noted for either type of target cell and was also observed when ADCC was assessed radiometrically.

The influence of antibody density on monocyte and macrophage ADCC

Using target cells sensitized with different antibody dilutions,

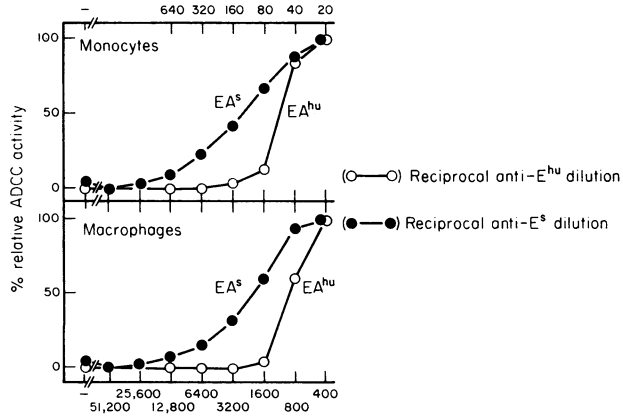

Figure 2. ADCC of EA by human monocytes and macrophages. EA^s and EA^{hu} were sensitized with varying amounts of antibody. Percentage lysis, calculated as described in the Materials and Methods, was normalized (maximal lysis in a given experiment = 100%), and values of two experiments were averaged.

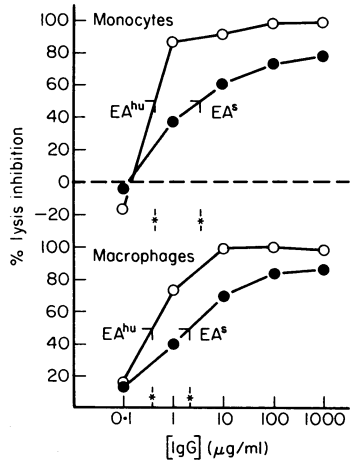

Figure 3. The inhibition of ADCC by varying amounts of competing fluid-phase IgG. Target cells were optimally sensitized EA^s and EA^{hu}. Values of two similar experiments were averaged.* denotes LD_{50}.

the influence of antibody density on ADCC was determined (Fig. 2). The number of antibodies per EA^s was known from earlier studies (Jungi & Barandun, 1985). Regardless of whether monocytes or macrophages were the effector cells, 600 IgG molecules per EA^s sufficed to induce small but significant lysis of such targets (12,800-fold antibody dilution). With increasing antibody density, the ADCC slowly increased, following a sigmoid dose–response curve. By comparison, a much steeper dose–response curve was obtained with EA^{hu}.

The influence of fluid-phase IgG on monocyte and macrophage ADCC

Lysis of optimally sensitized EA is inhibitable by fluid-phase IgG in a dose-dependent manner (Fig. 3). The dose-inhibition curve for EA^{hu} and EA^s differed reproducibly in that inhibition of EA^s increased more slowly with increasing IgG concentration, and did not achieve 100% within the tested range. Moreover, the ID_{50} was lower for EA^{hu} than for EA^s.

The influence of IFN-γ pretreatment on macrophage ADCC

Macrophages exposed to rIFN-γ for 2 days were compared with control macrophages with respect to their ADCC activity (Table 1). The pretreatment induced a considerable and highly significant increase in the lysis of EA^{hu}, whereas the lysis of EA^s did not increase to the same extent. Since the number of cell nuclei harvested at the end of the assay were significantly and reproducibly higher with rIFN-γ-treated macrophages, the observed ADCC increase was somewhat smaller but still significant when comparing the number of lysed E per effector cell nucleus. It is unknown whether the increase in nucleus number is due to a higher proportion of multinucleated cells generated under the influence of rIFN-γ (Weinberg, Hobbs & Misukonis, 1984) or due to a lower proportion of rIFN-γ-treated cells lost from the monolayers during washing.

Using target cells carrying varying amounts of antibody, rIFN-γ-treated macrophages outperformed their control counterparts at all antibody densities in their ADCC activity, but the relative increase was more pronounced for low antibody densities (not shown). Another difference between rIFN-γ-treated and control macrophages was manifested when fluid-phase IgG was present during the ADCC assay (Fig. 4). The ADCC of rIFN-γ-treated cells was more resistant to the presence of fluid-phase IgG than that of untreated counterparts. This increased resistance was more pronounced when EA^{hu} were the target cells than with EA^s.

Comparison of ADCC and phagocytosis by human macrophages

Adherent macrophages were assessed for their capacity to ingest the same targets as used in the ADCC assay. In contrast to ADCC, pretreatment with rIFN-γ decreased phagocytosis, and this decrease was more pronounced with EA^s (five-fold) targets than with EA^{hu} (2·7-fold). Compared with phagocytosis of optimally sensitized EA by a given effector cell type, decreasing antibody densities on target cells influenced EA^s phagocytosis by rIFN-γ-treated and control macrophages similarly, whereas phagocytosis of EA^{hu} decreased more slowly with activated effector cells (Fig. 5). Susceptibility of ingestion to fluid-phase IgG was also different for these two targets (Fig. 5). Pretreatment of macrophages with rIFN-γ made them slightly more susceptible to inhibition of EA^s ingestion by fluid-phase IgG, although this difference was not significant. In contrast, rIFN-γ pretreatment lightly increased their resistance to fluid-phase IgG.

The influence of metabolic inhibitors on macrophage ADCC and phagocytosis

ADCC of EA has been shown to depend or to be influenced by phagocytosis (Hershey, 1973; Katz *et al.*, 1980; Beelen & Walker, 1985). In an effort to dissociate ADCC and phagocytosis, both ADCC and phagocytosis were assessed in the presence of a variety of metabolic inhibitors. Iodoacetate (Table 2) and NaF (not shown), which inhibit glycolysis, blocked both effector functions to a similar degree. 2-DOG, which interferes with glycolysis and/or glycoprotein synthesis (Michl, Ohlbaum

Table 1. The effect of rIFN-γ on macrophage ADCC

	Target cells	Control cells	rIFN-γ-treated cells	Ratio	*n*	Statistical significance
Lysis (%)	EA^{hu}	6·30±2·00*	12·67±4·35	2·32±0·70	10	<0·001
	EA^{s}	5·86±2·75	9·39±4·27	1·74±0·81	17	<0·001
Lysed target cells per effector nucleus	EA^{hu}	1·17±0·44	2·12±0·87	1·81±0·45	10	<0·001
	EA^{s}	1·29±0·64	1·80±1·08	1·48±0·69	15	0·05

* Means±SD.

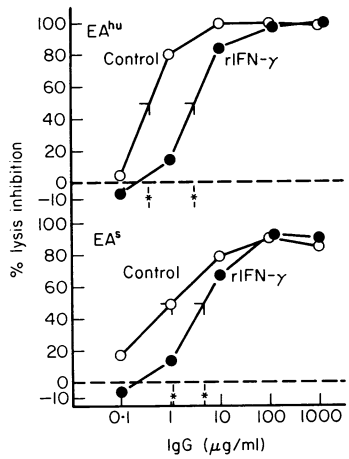

Figure 4. The effect of competing fluid-phase IgG on the ADCC by rIFN-γ-treated and by control macrophages. Values of two similar experiments were averaged.* denotes LD_{50}.

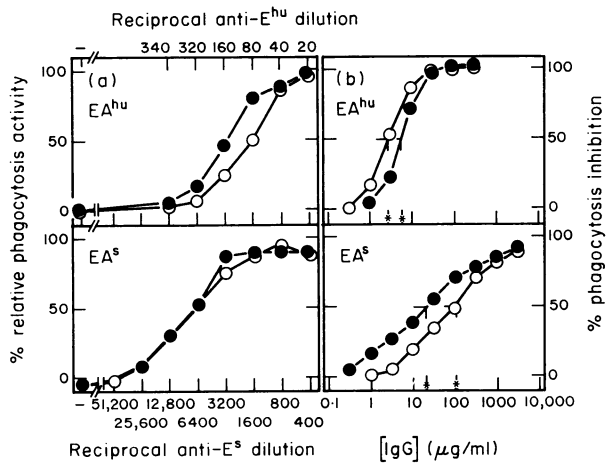

Figure 5. (a) Phagocytosis of EA sensitized by varying amounts of antibodies by rIFN-γ-treated macrophages (●) and control macrophages (○). Maximal phagocytosis obtained in a given experiment and with a given cell type was defined as 100%, and values of six (EA^{hu}) or nine (EA^{s}) experiments were averaged. (b) EA ingestion by rIFN-γ-treated (●) and by control (○) macrophages is inhibited by varying doses of competing fluid-phase IgG. Values of three similar experiments were averaged. * denotes LD_{50}.

Table 2. The effect of various metabolic inhibitors on ADCC and phagocytosis of EA by human macrophages

Inhibitor	Target cells	Relative activity (% of control) ADCC	Phagocytosis
		Control macrophages	
0·3 mM Iodoacetate	EA^{s}	25	1
1 mM Iodoacetate	EA^{s}	13	1
1 mM Colchicine	EA^{s}	156	40
8 μM Cytochalasine B	EA^{s}	62	5
	EA^{hu}	128	6
		rIFN-γ-treated macrophages	
0·3 mM Iodoacetate	EA^{s}	5	0
1 mM Iodoacetate	EA^{s}	3	0
1 mM Colchicine	EA^{s}	159	48
8 μm Cytochalasine B	EA^{s}	31*	ND
	EA^{hu}	43*	ND

* Significantly different from respective control macrophage group ($P<0·001$).
ND, not done.

& Silverstein, 1976), also inhibited both functions similarly (not shown), suggesting similar energy requirements for the two effector functions. Colchicine, an agent disrupting microtubuli, decreased phagocytosis at relatively high concentration but enhanced ADCC (Table 2). Cytochalasin B inhibited phagocytosis in a dose-dependent manner, phagocytosis of EA^{hu} being slightly more sensitive than that of EA^{s}. In contrast, the effect of CB on ADCC was relatively variable (Table 2). A dose of 4 μg/ml, which inhibits phagocytosis of either target to >95%, strongly inhibits monocyte-mediated ADCC, but showed little inhibition (or even enhancement) of macrophage-mediated ADCC. ADCC by macrophages exposed to rIFN-γ, however, became sensitive again to cytochalasin B. In all instances, ADCC of EA^{s} was affected more than that of EA^{hu}.

DISCUSSION

In the present report, antigen-dependent cytotoxicity against erythroid target cells by human monocytes and monocyte-

derived macrophages has been investigated. Quantitative differences in ADCC were noted between monocytes and macrophages, between rIFN-γ-activated and control macrophages, and between different types of target EA. Below, the implications from these differences will be discussed.

A difference in ADCC between monocytes and macrophages was manifested in different kinetics, targets being lysed much more rapidly by monocytes than by macrophages. These differences probably do not stem from different signal perceptions upon stimulation by IgG, since varying the densities of IgG on target cells and varying the amounts of fluid-phase IgG competing antibody on target cells, affected ADCC of monocytes and macrophages similarly. Rather, the kinetic differences between monocyte and macrophage ADCC may be due to different effector mechanisms involved. These include oxygen-independent and oxygen-dependent processes (Katz *et al.*, 1980; Klassen & Sagone, 1980; Koller & LoBuglio, 1981). Macrophages contain significantly less myeloperoxidase than monocytes. However, there is little evidence for the involvement of this enzyme in ADCC, for azide, a myeloperoxidase inhibitor, does not impair the degree of ADCC (Koller & LoBuglio, 1981; Clark & Klebanoff, 1977).

Activated human macrophages displayed enhanced ADCC when compared with control cells. It has been shown previously that murine (Koren *et al.*, 1981) and human macrophages (Randazzo, Hirschberg & Hirschberg, 1979) expressed enhanced ADCC upon treatment with activating agents. We now show that a defined activating agent, rIFN-γ, exerts this effect, thus extending observations made with PMN (Hockland & Berg, 1981) to macrophages. Moreover, we show that this biological response modifier exerts its ADCC-enhancing effect in three ways. Firstly, the lysis of optimally sensitized targets is enhanced. Secondly, the capacity to lyse targets is extended towards targets carrying a lower antibody density on their surface. Thirdly, the resistance to ADCC inhibition by fluid phase IgG is considerably increased. All three effects are relevant for increasing the lytic activity *in vivo* against a given tumour target.

Regardless of the effector cell type and pretreatment, ADCC of rabbit IgG-sensitized E^s was characteristically different from that of anti-D- sensitized E^{hu}. With EA^{hu}, a much steeper decline of ADCC was noted when the surface antibody density was decreased than with EA^s, and a much smaller amount of fluid-phase IgG could inhibit ADCC of EA^{hu} than that of EA^s. This suggests that high-affinity FcR alone are involved in EA^{hu} ADCC, while FcR of lower affinity participate in ADCC of EA^s. This is consistent with a suggestion put forward by Kurlander *et al.* (1984) and by findings that anti-D-coated EA^{hu} are poorly bound and lysed by PMN which do not possess high-affinity FcR (LoBuglio, Cotran & Jandl, 1967; Klassen & Sagone; 1980). The failure of such targets to be recognized by low-affinity FcR is parallelled by a failure to be lysed by complement, and may be related to the configuration of the antigenic determinants on human cells, being positioned relatively far apart from each other and buried in the erythrocyte membrane (Nydegger & Kazatchkine, 1983). The concept of a differential triggering of distinct FcR populations by the two model targets used is further supported by our findings with rIFN-γ-activated macrophages. rIFN-γ upregulates high-affinity FcR (Guyre, Morganelli & Miller, 1983; Perussia *et al.*, 1983) but not low-affinity FcR of mononuclear phagocytes (Jungi *et al.*, 1987; T.W. Jungi *et al.*, manuscript submitted for publication). The significantly larger rIFN-γ-mediated increase in EA^{hu} lysis, as opposed to EA^s lysis (Table 1), coincides with a larger dependence on recognition by high-affinity FcR, and the same is true for the increased resistance to lysis inhibition by fluid-phase IgG (Fig. 4). Conversely, FcR-mediated phagocytosis was impaired after rIFN-γ treatment, and this decrease was smaller for EA^{hu} than for EA^s as targets. Again this is compatible with a selective up-regulation of high-affinity FcR involved primarily in EA^{hu} ingestion, as this up-regulation partly antagonized a reduced phagocytosis effector capacity of rIFN-γ-treated cells. In keeping with this hypothesis, activated macrophages show a decreased or an increased susceptibility to IgG-mediated phagocytosis inhibition of EA^{hu} or EA^s, respectively (Fig. 5). Viewed in this way, the use of EA^s as model targets more closely reflects a rIFN-γ modified effector capacity than an altered receptor function, whereas the use of anti-D-treated EA^{hu} mirrors an altered receptor function rather than rIFN-γ-induced changes in the effector capacity. Following this interpretation, rIFN-γ influences macrophage ADCC by increasing the receptor function rather than the effector capacity. In contrast, phagocytosis effector function is significantly decreased, and this decrease cannot be compensated by an increased receptor function.

The opposite effect of rIFN-γ on macrophage phagocytosis and ADCC, respectively, deserves another comment, for ADCC, at least in part, interferes with or requires phagocytosis (Hershey, 1973; Katz *et al.*, 1980; Beelen & Walker, 1985). The human monocyte-derived macrophage has a strong bias for ingestion (Jungi *et al.*, 1987) as opposed to lysis, and rIFN-γ pretreatment only partly corrects this disbalance. Attempts to dissociate lysis from ingestion included the use of selective inhibitors (Ralph & Nakoinz, 1980; Koren *et al.*, 1981) and the physical prevention of ingestion (Ralph & Nakoinz, 1980; Katz *et al.*, 1980). In the ADCC assay described here, extracellular lysis could be clearly dissociated from phagocytosis. Thus, ingestion is abolished by 4 μg/ml cytochalasin B and reduced to less than half by 1 mM colchicine. Depending on the target cell type, ADCC is either enhanced or slightly reduced by CB, and enhanced by colchicine (Table 2). Our results differ significantly from those of Fleer *et al.* (1978), who used monocytes as effector cells and much longer assay times, but conforms to studies of Ralph & Nakoinz (1980). The significantly and consistently increased susceptibility to CB of ADCC by activated macrophages cannot be explained at present, since both rIFN-γ and CB exert multiple effects on macrophages, and the actual effector mechanism(s) involved remain(s) to be determined.

Given the opposite effect of rIFN-γ on two closely related effector functions, ingestion and lysis, the question arises whether different FcR populations are connected with different effector functions. In man, at least three FcR types are found on human leucocytes, distinct with respect to antigenicity, molecular size, binding affinity and isotype specificity (Anderson & Looney, 1986; Fleit, Wright & Unkeless, 1982; Kurlander, Haney & Gartrell, 1984); at least two, possibly three FcR populations are expressed on human macrophages. The above considerations suggest that more than one FcR type are involved in both ADCC and phagocytosis, respectively, of a given EA target, an assumption supported by data from mouse

macrophages (Ralph *et al.*, 1980). In the case of a joint involvement of high-affinity and low-affinity FcR (EA^s targets), it cannot be decided whether the low-affinity FcR merely facilitates mediation of the effector function by high-affinity FcR or mediates effector functions on its own right. Studies in which individual effector functions could be blocked by monoclonal antibodies against defined FcR types provided evidence that triggering either the high-affinity 72,000 MW monocyte/macrophage FcR or the low-affinity 40,000 MW monocyte FcR mediates ADCC (Graziano & Fanger, 1987). Moreover, the medium-affinity 55,000–70,000 MW FcR of macrophages contributes to phagocytosis (Fleit *et al.*, 1982; Clarcson *et al.*, 1986), probably together with the high-affinity 72,000 MW FcR, whereas the triggering of the low-affinity 40,000 MW FcR induces a respiratory burst (Anderson *et al.*, 1986). Obviously, the knowledge on signal–response coupling for individual human macrophage FcR populations is far from being complete.

ACKNOWLEDGMENTS

This work was supported by the Central Laboratory of the Swiss Red Cross Blood Transfusion Service, Berne, Switzerland, and the Swiss National Science Fund, grant No. 3.858-85. The technical assistance of Mrs M. Brčić is gratefully acknowledged.

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