40 Milinski, M., and Heller, R., Influence of a predator on the optimal foraging behaviour of sticklebacks (*Gasterosteus aculeatus* L.). Nature *275* (1978) 642-644.
41 Milinski, M., and Löwenstein, C., On predator selection against abnormalities of movement - a test of a hypothesis. Z. Tierpsychol. *53* (1980) 325-340.
42 Milton, K., Factors influencing leaf choice by howler monkeys: a test of some hypotheses of food selection by generalist herbivores. Am. Nat. *114* (1979) 362-378.
43 Neill, S.R.St.J., and Cullen, J.M., Experiments on whether schooling by their prey affects the hunting behaviour of cephalopod and fish predators. J. Zool., Lond. *172* (1974) 549-569.
44 Ohguchi, O., Experiments on the selection against colour oddity of water fleas by three-spined sticklebacks. Z. Tierpsychol. *47* (1978) 254-267.
45 Ohguchi, O., Prey density and selection against oddity by three-spined sticklebacks. Advances in Ethology, Suppl. 23. 1981.
46 Orians, G.H., Some adaptations of marsh-nesting blackbirds. Monographs in population biology, vol. 14. Princeton Univ. Press, Princeton 1980.
47 Oster, G.F., and Wilson, E.O., Caste and ecology in the social insects. Princeton Univ. Press, Princeton, N.J., 1979.
48 Partridge, L., and Maclean, R., Effects of nutrition and peripheral stimuli on preferences for familiar foods in the bank vole. Anim. Behav. *29* (1981) 217-220.
49 Price, P.W., Evolutionary biology of parasites. Princeton Univ. Press, Princeton 1980.
50 Pulliam, H.R., Diet optimization with nutrient constraints. Am. Nat. *109* (1975) 765-768.
51 Pulliam, H.R., The principle of optimal behavior and the theory of communities; in: Perspectives in Ethology, vol. 2, pp. 311-332. Eds P.P.G. Bateson and P.H. Klopfer. Plenum Press, New York/London 1976.
52 Pulliam, H.R., Do chipping sparrows forage optimally? Ardea *68* (1980) 75-82.
53 Pyke, G.H., Pulliam, H.R., and Charnov, E.L., Optimal foraging: A selective review of theory and tests. Q. Rev. Biol. *52* (1977) 137-154.
54 Rapport, D.J., Optimal foraging for complementary resources. Am. Nat. *116* (1980) 324-346.
55 Schluter, D., Does the theory of optimal diets apply in complex environments? Am. Nat. *118* (1981) 139-147.
56 Sibly, R., and McFarland, D., On the fitness of behavior sequences. Am. Nat. *110* (1976) 601-617.
57 Stephen, D.W., The logic of risk-sensitive foraging preferences. Anim. Behav. *29* (1981) 628-629.
58 Völkel, P., and Zell, R.A., Beobachtungen und Versuche zum Mischschwarm- und Feindverhalten einiger neotropischer Singvögel. Thesis, Ruhr-University, Bochum 1980.
59 Werner, E.E., and Hall, D.J., Optimal foraging and the size selection of prey by the bluegill sunfish *(Lepomis macrochirus)*. Ecology *55* (1974) 1042-1052.
60 Westoby, M., What are the biological bases of varied diets? Am. Nat. *112* (1978) 627-631.
61 Whitford, W.G., and Bryant, M., Behavior of a predator and its prey: the horned lizard *(Phrynosoma cornutum)* and harvester ants (*Pogonomyrmex* spp.). Ecology *60* (1979) 686-694.

0014-4754/83/010025-10$1.50 + 0.20/0

Effects of mitogens on ADCC activity and Fc receptor bearing cells

A. Hügin[1] and A. Matter[1,2]

Pharmaceutical Research Division, F. Hoffmann-La Roche Ltd & Co, CH-4002 Basel (Switzerland), January 15, 1982

Summary. Murine spleen cells, when activated by various mitogens (Con A, PHA, LPS, BCG) or Con-A conditioned medium show altered behavior as effector cells of antibody-mediated cell cytotoxicity (ADCC) which does not correlate with the expression of Fc-receptors (FcR) at the effector cell surface.

Antibody-mediated cell cytotoxicity (ADCC) represents one of the 4 cytotoxic immune activities, the others being a) the antibody plus complement-mediated lysis, b) the cytotoxic T cell mediated lysis, and c) lysis mediated via NK cells, or d) activated macrophages. These 4 immune activites are belived to be important for rejection of tissue grafts[3,4] and possibly of tumors[5,6].

ADCC activity is crucially dependent on antibody and a receptor linking the Fc portion of the antibody molecule to the effector cell surface. The antibody binding sites are then free to recognize tissue antigens. ADCC activity is usually measured in vitro using normal effector cells. All cells carrying Fc-receptors (FcR) are potential killer cells in ADCC and are operationally referred to as K cells. The heterogeneity of this K cell population is clearly recognized, but so far it has not been possible to attribute ADCC activities unequivocally to the various components in a mixed K cell population.

It was of interest, therefore, to stimulate spleen cells with various mitogenic principles in order to test whether a) activated cells were still effective in ADCC, b) subsets of FcR^+ activated cells were differentially active, and lastly, c) whether the degree of FcR^+ was the determining factor for K cells to be active in ADCC.

Materials and methods

Mice. C3H/HeJ, C3H/Tif and CBA/J mice were obtained from the Institut für Biologisch-Medizinische Forschung AG (Füllinsdorf, Switzerland). All experiments were done with 6-12-week-old mice.

Mitogens. Lipopolysaccharide (LPS) was purchased from GIBCO (Grand Island, NY). Both concanavalin A (Con A) and leucoagglutinin (PHA) were purchased from Pharmacia (Uppsala, Sweden). The BCG used was a phenol (2%)-treated BCG strain of the Pasteur Institute.

Antisera. Hyperimmune antisera were obtained from rabbits given 8 i.v. injections of 2 ml of a 10% solution of chicken red blood cells (CRBC) and sheep red blood cells (SRBC) respectively during an 18-day period. The animals were bled 10 days after the last injection, and the serum was heat inactivated (30 min at 56 °C). Antiserum against SRBC, used for EA-rosetting, was obtained in the same way.

Target cells. CRBC were obtained fresh from a fowl colony using heparin as an anticoagulant. SRBC were obtained from a sheep colony also using heparin as an anticoagulant. After thorough washing, they were stored in PBS at 4 °C.

Conditioned medium. Spleen cells at a concentration of 2×10^6 cells/ml were incubated in the presence of 2.5 µg/ml Con A for 6 h at 37 °C and subsequently washed thoroughly. Cells were resuspended at the same concentration in complete medium and 2-ml aliquots added to Costar tissue plates (No 3524, Costar, Cambridge, Mass.) and incubated overnight; the supernates were harvested after centrifugation.

Cell preparations. Spleen cells were prepared at room temperature under sterile conditions with the help of a loose-fitting glass potter homogenizer, washed once with BSS and subsequently incubated for 10 min in 0.83% NH_4Cl to eliminate erythrocytes. After 2 further washings with BSS, cells were resuspended in culture medium; usually RPMI 1640 medium (GIBCO, Grand Island, NY), supplemented with glutamin, penicillin, streptomycin, hepes buffer, β-mercaptoethanol and 10% fetal calf serum (GIBCO) if not indicated otherwise.

Preparation of adherent and non-adherent cells. Adherent cells were depleted as follows: 10×10^6 cells in 5 ml complete medium were incubated for 30 min at 37 °C in a 88-mm petri dish. The nonadherent cells were then transferred to a 2nd dish and incubated for further 30 min. Nonadherent cells were gently sucked of with a Pasteur pipette. Adherent cells were harvested after 15 min incubation in the presence of 1 mM EDTA in calcium- and magnesium-free phosphate buffered saline and washed twice subsequently.

Cell culture. Spleen cells were resuspended in complete medium at a concentration of 1.5×10^6 cells/ml. 2-ml aliqots were pipetted into Costar tissue plates. Mitogens were added to each well: 2 µg PHA, 10 µg Con A; 20 µg LPS, 200 µg BCG or nil, and cultures incubated at 37 °C with 5% CO_2 for 2–4 days. Cells were harvested and washed twice in complete medium.

Assay for antibody-dependent cell cytotoxicity (ADCC). This assay was performed according to Perlmann and Perlmann[7] and modified to microtiter plates. Cells to be tested for ADCC activity were washed 3 times with BSS and concentration adjusted to 2.5×10^6 cells/ml in complete medium. Target CRBC were preincubated in 0.8 mCi $Na_2{}^{51}CrO_4$ (Radiochemical Center Amersham) in complete medium for 4 h at 37 °C, washed 3 times and resuspended in complete medium at a concentration of 10^5 CRBC/ml. Antiserum dilutions in PBS were added to CRBC immediately before the test. 100-µl aliquots of spleen cells and CRBC-antiserum mixtures were added in triplicate to each well of microtiter plates (PS Microplatte 96 K, Greiner Labortechnik). Incubation was done at 37 °C for 24 h. After 5 min centrifugation at $400 \times g$, 100 µl of the supernate of each well was counted in a gamma-counter (Searle, Nuclear Chicago Division, Model 1185). Background levels were determined, replacing lymphocytes by adding 10^6 unlabeled CRBC/well. Maximal release was determined after either 3 cycles of freezing and thawing or addition of 2% sodium dodecyl sulphate. Where necessary, background levels were also determined by adding mitogens or conditioned medium to tubes and measuring background release of CRBC. Lysis was calculated as follows: specific percent ^{51}Cr release:

$$\frac{\text{experimental cpm} - \text{background cpm}}{\text{maximal release cpm} - \text{background cpm}} \times 100$$

A minimum of 3 separate experiments was performed for each parameter tested. Figures depict single representative experiments with the curve points showing the arithmetic mean of triplicate values. For clarity, standard errors, which never exceeded ±5%, are not shown.

^{3}H-TdR incorporation. Lymphocytes at concentrations used for cultures were tested for ^{3}H-TdR (Radiochemical Center, Amersham) incorporation by a 2-h pulse label with 0.2 mCi ^{3}H-TdR/well. Cells were harvested by a cell harvester (Microtiter, Dynatech Automash 2) on a glass fiber filter and ^{3}H-TdR incorporation measured by scintillation counting (Packard Liquid Scintillation Spectrometer).

EA-Rosetting. Aliquots of 10^8 SRBC were washed 3 times, resuspended in decreasing dilutions of anti SRBC-antiserum and incubated for 2 h at 37 °C. After 2 washings in BSS, 10^7 lymphocytes in 0.5 ml were added to each aliquot, followed by centrifugation for 5 min at $500 \times g$ and overnight incubation at 4 °C. Counting of rosetting cells was done in the presence of 0.2% trypan blue after gentle resuspension of cells. The number of Fc rosettes (cells binding 3 SRBC or more) was determined in the microscope. Duplicates were counted for each antiserum dilution.

Results

1. *ADCC activity of normal mouse spleen cells in the presence of various mitogens.* a) *Con A.* In a typical micro-hemagglutination assay, Con A was able to agglutinate CRBC at a concentration of 5 µg/ml. The presence of Con A affected ADCC activity at much lower concentrations: 0.25 and 2.5 µg/ml markedly increased cytotoxic activity, whereas an agglutinating concentration (25 µg/ml) did not have this effect, and was even slightly suppressive (fig. 1A). Background lysis and lysis in the absence of antibody but presence of lymphocytes was unaffected except at 25 µg/ml where a slight increase was noted.

b) *PHA.* Compared to Con A, PHA was somewhat better able to agglutinate CRBC, 2 µg/ml being the minimal concentration for a ±-reaction. PHA strongly increased lytic activity in the absence as well as in the presence of Ab (fig. 1B). This effect was present at concentrations below agglutinating doses (0.5 µg/ml) and still more pronounced at agglutinating concentrations (5 µg/ml). Since the presence of PHA increases the values in the absence as well as in the presence of antibody to the same extent, the probable conclusion is that PHA only affects activity which is independent from antibody, i.e. has no effect on ADCC. This increase of macrophage-like cytolysis is, however, not linked to increased spontaneous lysis of CRBC (data not shown), as was also the case with Con A.

c) *BCG.* Phenol-treated BCG was used to examine its effect on ADCC of untreated normal spleen cells (fig. 1C) and of normal spleen cells enriched in or deprived of adherent cells (data not shown). BCG did not agglutinate CRBC at the concentrations used (0.05–100 µg/ml, data not shown) but was a true mitogen for spleen cells at relatively high concentrations (table 1 and 2). It is apparent from figure 1C that BCG not only promoted strongly antibody-independent activity but was even able to abrogate completely any antibody-dependent activity in a dose dependent manner. BCG, as well as Con A and PHA did not increase background lysis of CRBC.

d) *LPS.* No effect was observed at any dose used from 0.5 to 100 µg/ml (data not shown) either in a LPS-nonresponder strain (C_3H/HeJ) or in LPS-responder strains (C_3H/Tif, CBA/J).

e) *CM.* Supernates of Con A stimulated spleen cells were used undiluted (fig. 4) during the time of the ADCC assay. A significantly enhanced ADCC activity could be observed without elevated activity in the absence of antibody.

2. *ADCC activity of normal mouse spleen cells after culture with various mitogens.* a) *Con A.* Con A-stimulated spleen cells showed a markedly decreased ADCC and also nil activity (absence of antibody) was remarkably low (figs 2A and 3A). In experiments where both ADCC activity and EA-rosettes were

Table 1. ^{3}H-TdR incorporation of C3H/HeJ spleen cells after a 24-h incubation in the presence of various amounts of BCG

	Mean	± SD
Nil	18,972	1785
100 µg BCG/ml	113,719	5079
10 µg BCG/ml	51,391	8984
1 µg BCG/ml	29,474	1614

Table 2. Stimulation indices as a function of culture time in the presence of 100 µg BCG/ml

	24 h	48 h	72 h	96 h
$\frac{^3\text{H-TdR BCG}}{^3\text{H-TdR nil}}$	6.0	3.4	3.8	2.0

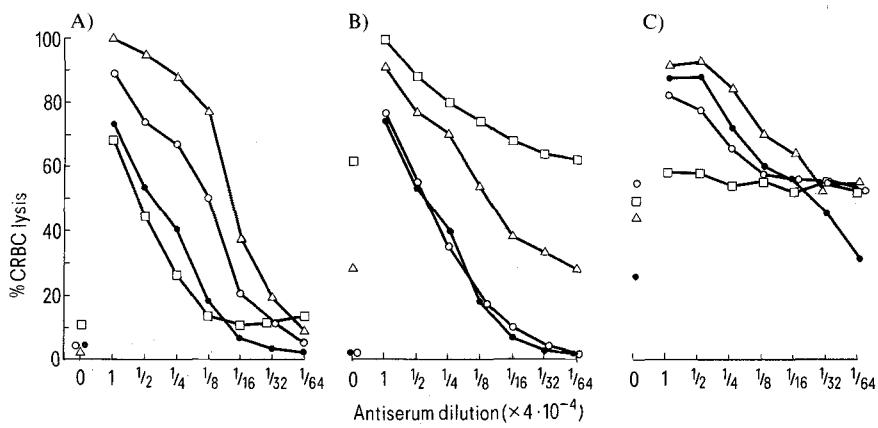

Figure 1. *A* ADCC activity of normal C3H/HeJ (nsc) spleen cells in the presence of various concentrations of Con A. ●, nsc; ○, nsc + 0.25 µg/ml Con A; △, nsc + 2.5 µg/ml Con A; □, nsc + 25 µg/ml Con A. *B* ADCC activity of normal C3H/HeJ (nsc) in the presence of various concentrations of PHA. ●, nsc; ○, nsc + 0.05 µg/ml PHA; △, nsc + 0.5 µg/ml PHA; □, nsc + 5 µg/ml PHA. *C* ADCC activity of normal C3H/HeJ spleen cells (nsc) in the presence of various concentrations of BCG. ●, nsc; △, nsc + 0.5 µg/ml BCG; ○, nsc + 5.0 µg/ml BCG; □, nsc + 50 µg/ml BCG.

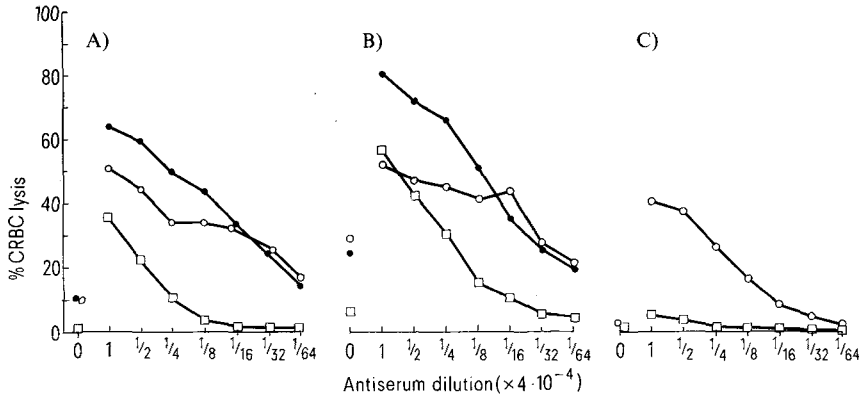

Figure 2. ADCC activity of normal C3H/HeJ spleen cells (nsc) after 65 h culture in the presence or absence of mitogens. *A* ●, fresh untreated nsc; ○, nsc cultured without mitogen; □, nsc cultured with 5 µg/ml Con A. *B* ●, fresh untreated nsc; ○, nsc cultured without mitogen; □, nsc cultured with 1 µg/ml PHA. *C* ○, nsc cultured without mitogen; □, nsc cultured with 100 µg/ml BCG.

measured (fig. 3), this suppression coincided with a very low count in EA-rosettes (fig. 3B). It should be mentioned, however, that Con A-stimulated cells were generally able (5 separate experiments) to exhibit ADCC activity at high antibody concentrations.

b) *PHA.* PHA-stimulated spleen cells showed, similarly to Con A-stimulated cells, depressed ADCC activity and a low activity in the absence of antibody (figs 2B and 3A). Interestingly, this low activity did not correlate with the EA-rosette count, which was nearly the same as for nil-cultured cells (fig. 3B). On the other hand, nil-cultured cells showed a somewhat variable, but generally good ADCC activity correlating with an EA-rosette count in the neighbourhood of the one observed with fresh, untreated spleen cells (figs 3A and 3B).

c) *BCG.* BCG-stimulated cells showed by far the most pronounced suppression of ADCC activity (fig. 2C). In contrast to results obtained when BCG was present (fig. 1C) nil activity was low. The low nil and ADCC activity of these cultures cannot be explained on the basis of low viability since a) the assay was set up with a corrected viable cell number, and b) viability of these cultures was as good as that of PHA or Con A cultures with recoveries after culture times ranging between 74 and 98%.

d) *LPS.* Cultures maintained in the presence of mitogenic concentrations of LPS showed, as did T mitogens, depressed ADCC activity. In contrast, EA-rosette counts were high for these cultures, and even higher than for fresh, untreated cells.

e) *CM.* As seen with fresh, untreated cells (fig. 4), CM markedly increased ADCC activity. This increase only concerned ADCC activity and left nil-activity unaltered. No good correlations existed with the presence of Fc receptors as measured by the number of EA-rosettes: untreated fresh cells showed similar EA-counts to cells treated with CM during the assay time. However, cells cultured in the presence of CM plus FCS had a somewhat lower EA-count than cells cultured with FCS only or cells cultured in the absence of both CM and FCS.

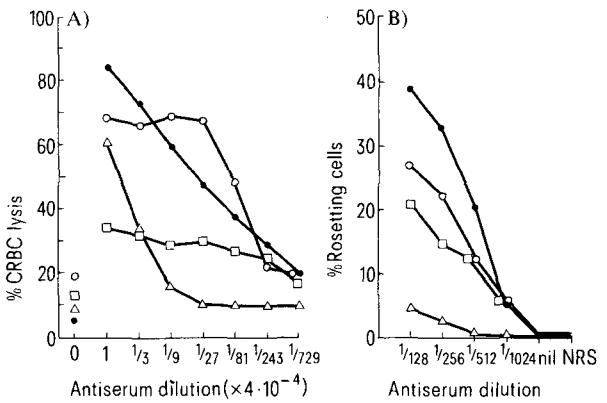

Figure 3. Effect of Con A and PHA on ADCC activity (A) and EA-rosette formation (B). C3H/HeJ cells (nsc) were cultured for 96 h in the presence of: ○, nil; △, 5 µg/ml Con A; □, 1 µg/ml PHA; ●, fresh untreated nsc served as controls.

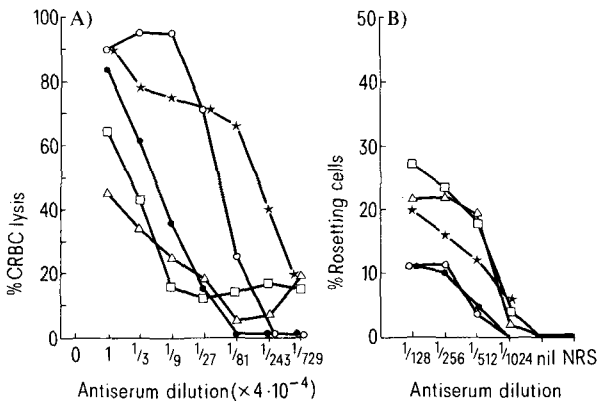

Figure 4. Effect of CM on ADCC activity (A) and EA-rosette formation (B). C3H/HeJ spleen cells (nsc) were cultured 48 h in the presence of: △, no FCS, no CM; □, 10% FCS, no CM; *, 10% FCS, plus CM, undiluted. ●, fresh untreated nsc; ○, fresh nsc, kept in undiluted CM during the assay time.

Discussion

Cytolytic effector cells such as those involved in ADCC and NK activity are studied almost by definition using normal unprimed effector cell populations. It was of interest, therefore, to stimulate cells by means of various mitogens or growth factors and to compare lytic capabilities. In contrast to normal cells this allows us to deal with activated cell populations and to correlate lytic capability with FcR expression. Such data should provide further insight into the complexity of the ADCC effector cells of normal lymphoid tissue[8].

This study had to deal with several potential pitfalls:

a) Mitogens might have been used under non-optimal conditions. The degree of blast cell formation was therefore checked by measuring ^{3}H-TdR incorporation. Routinely, stimulation indices of 5 or more were achieved in 3-day cultures. Adherent cells were lost in the culture trays. Microscopically cell populations contained between 70% and 90% large lymphocytes.

b) Mitogens, sticking to the cell surface could have affected target cell lysis via nonspecific attachment of effector cells to target cells. No rosettes with CRBC or SRBC were found, however, in the absence of antibody.

c) Mitogens remaining on the cell surface after washing might have affected the lytic potential of the cell. ADCC tests were therefore performed with normal cells in the presence of non-agglutinating mitogen concentrations. These conditions should lead to a large fraction of mitogen receptors being occupied, and thus in a state similar to that after 3-day culture in the presence of mitogen. If such binding did result in an altered lytic potential of the K cell one might assume that the effect would be identical in both cases, i.e. with K cells pretreated with mitogen and with K cells assayed in the presence of mitogen. This was, however, not the case. Effects of mitogens were quite different according to the protocol used.

d) Blast cells might have lysed target cells via soluble lymphotoxins[9,10]. No lysis was seen over background levels obtained with normal cells in the absence of antibody.

Mitogens used for cell activation during 3-day cultures were Con A, PHA, BCG and LPS, and growth factors contained in Con A-conditioned media.

Con A: The general suppression of ADCC activity disappeared at high antibody concentrations, an observation which might be explained by low affinity receptors. This interpretation does not fit, however, with the observation that EA-rosettes were low at all antibody concentrations. On the other hand, it is possible that FcR were directly affected by Con A since subagglutinating doses of Con A, when present during the assay time, already suppressed ADCC activity to a great deal. This suppression might therefore represent a blocking of receptors caused by ligand-induced redistribution of the receptor sites.

PHA: The basic finding with this mitogen were a low ADCC activity contrasted by EA-rosette count nearly as high as in the controls. PHA present in the assay increased nil activity only and neither decreased nor increased ADCC activity proper.

These results could mean that FcR were functional but that the lytic potential of the ADCC effector cells was hindered, possibly via lymphotoxins produced by tte blast cells[9,10].

BCG: The BCG preparation at our disposal was mitogenic at relatively high doses and non-agglutinating at all tested concentrations. BCG, at sufficiently high doses suppressed ADCC activity completely. When present during the ADCC assay, BCG generated a high nil activity which was absent in cells cultured in the presence of BCG. One interpretation of these data could be that BCG-activated macrophages suppressed K cells during the 24-h assay but that such macrophages were lost in cultured cells after harvesting due to their adherence. Another interpretation would be that K cells were driven by BCG to a differentiated (macrophage) state which was devoid of ADCC activity. At present, it is not possible to settle this point. No explanation can be offered, either, for the fact that BCG in vivo seems to augment ADCC activity markedly[11-13].

LPS: The increase in FcR^+ cells is in accordance with results of Forman and Möller[14] who tested LPS stimulated cells in a tumor cell- ADCC system. Their non-mitogen stimulated cells expressed a remarkable ADCC activity, but LPS cultured cells, after a 72-h stimulation, did not. Since most B cells are FcR^+[15] but are inactive in ADCC against CRBC[16-18], a LPS driven culture contains FcR^+, but ADCC inactive B-blasts and ADCC active cells of non-B origin would seem to be diluted out or lost, thus explaining the discrpancy between ADCC and the expression of FcR.

CM: Con A induced spleen cell culture supernates have been reported to contain T cell growth factor. Since such supernates may contain a variety of factors, besides TCGF (IL-2), all or part of them affecting K cell activity, the term 'conditioned medium' (CM) was retained.

CM increased both in the assay and in culture ADCC activity, without affecting noticeably the presence or affinity of FcR. This result points most probably to an increase in killing efficiency per cell, since the assay time (24 h) did not allow proliferation of K cells. This increase is not due to the presence of Con A in the supernates since CM was produced after mitogen stimulation and subsequent washings.

It is interesting to note that TCGF (the major product of Con A conditioned medium) is able to stimulate NK cells[19] which points to possible parenthood of NK

and ADCC cells such as described for human cells[20]. On the other hand, it must be noted that immune interferon, which is also present in Con A-conditioned medium[21], stimulates ADCC activity of polymorphs[22]. In conclusion, it appeared that the complexity of K cell subpopulations was readily apparent with the mitogens used as probes. Each mitogen had its particular effect. In particular, ADCC activity and FcR expression did not seem to correlate, as is readily apparent from table 3, which summarizes results obtained with cultured cells. FcR expression is probably a necessary but not a sufficient condition for cells to perform ADCC activity.

Table 3. Summary of results with mitogen or CM activated cells

	Con A	PHA	LPS	BCG	CM
FcR	↓	±	↑	n.d.	↓
ADCC	↓	↓	↓	↓	↑

1 Acknowledgments. We would like to express our thanks to the excellent technical assistance of Mrs J. Higelin.
2 Present address: UNICET/URD Laboratory, Faculté de Médecine René Descartes, 45, rue des Saints-Pères, F-75006 Paris, France, to whom reprint requests and correspondence should be sent.

3 Jeannet, M., Vasalli, P., and Botella, F., Lymphocyte-dependent cytotoxic antibody (LDA) in kidney transplantation. Transplant. Proc. *7* (1975) 631–633.
4 Thomas, J.M., Thomas, F.T., Kaplan, A.M., and Lee, H.M., Antibody-dependent cellular cytotoxicity and chronic renal allograft rejection. Transplantation *22* (1974) 94–100.
5 Pollack, S.B., Heppner, G., Brawn, R.J., and Nelson, K., Specific killing of tumor cells in vitro in the presence of normal lymphoid cells and sera from hosts immune to the tumor antigens. Int. J. Cancer *9* (1972) 316–323.
6 Hellström, I., Hellström, K.E., and Warner, G.A., Increase of lymphocyte mediated tumor-cell destruction by certain patient sera. Int. J. Cancer *12* (1973) 348–353.
7 Perlmann, P., and Perlmann, H., Contactual lysis of antibody-coated chicken erythrocytes by purified lymphocytes. Cell. Immun. *1* (1970) 300–315.
8 Perlmann, P., Cellular immunity: Antibody-dependent cytotoxicity (K-cell activity), in: Clinical Immunology, vol. 3, pp. 107–132. Eds F.H. Bach and R.A. Good. Academic Press, New York 1971.
9 Granger, G.A., and Williams, T.W., Lymphocyte effector molecules: Mechanism of human lymphotoxin induced in vitro target cell destruction and role of PHA-induced lymphocyte-target cell cytolysis, in: Progress in Immunology, pp. 438–445. Ed. B. Amos. Academic Press, New York 1971.
10 Henney, C.S., Gaffney, J., and Bloom, B.R., On the relation of products of activated lymphocytes to cell-mediated cytolysis. J. exp. Med. *140* (1974) 837–852.
11 Pollack, S.B., Effector cells for antibody-dependent cell-mediated cytotoxicity. Immunology *29* (1977) 373–381.
12 Tagliabue, A., Mantovani, A., Polentarutti, N., Vecchi, A., and Spreafico, F., Effect of immunomodulators on effector cells involved in antibody-dependent cellular cytotoxicity. J. natl Cancer Inst. *59* (1977) 1019–1022.
13 Richman, S.P., Chism, V.T., and Murry, S.G., Augmentation of ADCC in mice by microbial fractions. Cancer Immun. Immunother. *11* (1981) 233–238.
14 Forman, J., and Moller, G., The effector cell in antibody-induced cell mediated immunity. Transpl. Rev. *17* (1973) 108–149.
15 Warner, N.L., and McKenzie, J.F.C., Surface antigens and receptors of normal and neoplastic lymphocytes: Nature and distribution, in: The Lymphocyte, pp. 434–492. Ed. J.J. Marchalo is. Marcel Dekker Inc., New York 1977.
16 Greenberg, A.H., Hudson, L., Shen, L., and Roitt, I.M., Antibody-dependent cell-mediated cytotoxicity due to a 'Null' lymphoid cell. Nature New Biol. *242* (1973) 111–113.
17 Greenberg, A.H., Shen, L., and Roitt, I.M., Characterisation of the antibody dependent cytotoxic cell. Clin. exp. Immun. *15* (1973) 251–259.
18 Nelson, D.L., Bundy, B.M., West, T.D., and Strober, W., The nature of effector cells mediating mitogen-induced cellular cytotoxicity (MICC) and antibody-dependent cellular cytotoxicity (ADCC). Cell. Immun. *23* (1976) 89–98.
19 Henney, C.H., Kuribayashi, K., Kern, D.E., and Gillis, S., Interleukin-2 augments natural killer cell activity. Nature *291* (1981) 335–338.
20 Ojo, E., and Wigzell, H., Natural killer cells may be the only cells in normal mouse lymphoid cell populations endowed with cytolytic ability for antibody-coated tumor target cells. Scand. J. Immun. *7* (1981) 297–306.
21 Marcucci, F., Aller, M., Kirchner, H., and Krammer, P., Production of immune interferon by murine T-cell clones from long-term cultures. Nature *291* (1981) 79–81.
22 Wardley, R., Babiuk, L.A., and Rouse, B.T., Polymorph-mediated antibody-dependent cytotoxicity - modulation of activity by drugs and immune interferon. Can. J. Microbiol. *32* (1976) 1222–1228.

0014-4754/83/010034-06$1.50 + 0.20/0

Studies on chemically induced tumors in rats: I. Heterogeneity of tumor cells and establishment of syngeneic, tumor-specific cytotoxic T cell clones*

H. Binz, M. Fenner and H. Wigzell

Institute for Immunology and Virology, University of Zürich, Gloriastrasse 30, CH-8028 Zürich (Switzerland), and Institute for Immunology, Biomedicum, Uppsala University, Uppsala (Sweden), April 23, 1982

Summary. Sarcoma P1 was induced in DA rats by DMBA. Anti-P1 antibodies were produced in DA rats, purified via fixed tumor cells and used to induce anti-idiotypic antibodies in syngeneic rats. The anti-idiotypic antibodies were used to generate cytotoxic, P1 specific DA T cells in vitro. These cytotoxic T cells and P1 tumor