Clin. exp. Immunol. (1982) **48**, 685–692.

Reversal of immune complex inhibition of antibody-dependent cell-mediated cytotoxicity by normal human serum

M. A. ISTURIZ, SUSANA B. FINK & MARÍA M. DE E. DE BRACCO *Instituto de Investigaciones Médicas, Universidad de Buenos Aires, Buenos Aires, Argentina*

(Accepted for publication 24 December 1981)

SUMMARY

The results of this study demonstrate that inhibition of antibody-dependent cell-mediated cytotoxicity (ADCC) of human peripheral blood mononuclear cells (PBMC) by ovalbumin (OA)–IgG anti-OA immune complexes (IC) can be reversed by normal human serum (NHS) or serum from a patient with congenital deficiency of the second component of complement (C2 def-HS) lacking activity of the classical complement (C) pathway. On the other hand, NHS that had been inactivated by heating at 56°C for 30 min (HI-NHS) or NHS depleted of the alternative C pathway activity by absorption with zymosan (Zy-NHS) did not restore the ADCC of IC-blocked PBMC. These results suggest that the alternative pathway of C plays a very important role in the re-establishment of ADCC of PBMC blocked with IC. The recovered activity was susceptible to a new inhibition when re-exposed to IC, demonstrating that the NHS effect depends on the recovery of the functional activity of the receptor for the Fc fragment of IgG on PBMC and not on the induction of non-specific cytotoxicity. The ability of NHS to restore the cytolytic potential of IC-inhibited PBMC was dependent on the time of exposure of PBMC to IC before the addition of the unblocking agent (NHS). After prolonged reaction of PBMC with IC, the blocked cells were unable to recover their ADCC activity by incubation with NHS. Unblocking of the Fc receptor of PBMC by C may be a physiological way to prevent permanent impairment of the immune mechanisms that depend on its function in the free state.

INTRODUCTION

The receptor for the Fc fragment of IgG (FcγR) is present on the membranes of different cells. It has been described in B lymphocytes (Dickler & Kunkel, 1972; Dickler, 1974), T lymphocytes (Lee & Paraskevas, 1972; Ferrarini *et al.*, 1975), macrophages (Berken & Benacerraf, 1966), K cells (Fröland, Wislöff & Michaelsen, 1974), monocytes (Lawrence *et al.*, 1977) and polymorphonuclear leucocytes (Gale & Zighelboim, 1975).

This receptor is involved in different cell functions. Antibody-dependent cell-mediated cytotoxicity (ADCC), release of lymphokines (Thoman, Morgan & Weigle, 1981) and phagocytosis (Pearson, 1978; Kerbel & Davies, 1974) are well documented functions dependent on FcγR. In addition, polyclonal antibody synthesis (Thoman, Morgan & Weigle, 1980), blast transformation (Berman & Weigle, 1977) and the immune response to sheep red blood cells (Morgan *et al.*, 1980) are, at least in part, dependent on FcγR function.

Immune complexes (IC) are known to block effector cells in ADCC. Revillard *et al.* (1975) have

Correspondence: Dr Martín A. Isturiz, Instituto de Investigaciones Médicas, Av.D. Alvarez 3150, 1427 Buenos Aires, Argentina.

0009-9104/82/0600-0685$02.00

reported that the blockade of K cell function with heat aggregated IgG was not reversed by incubation of the cells at 37°C. In contrast, Kumagai *et al.* (1975) and Sugamura & Smith (1977) have demonstrated that cell bound aggregated IgG could be dissociated from peripheral blood lymphoid cells by short term culture with recovery of the ability of the cultured cells to bind aggregated IgG.

The purpose of this report was to look for a mechanism that would allow the FcγR to be operative in the presence of IC. We will show that when IgG-dependent ADCC is blocked by preformed IC, the function can be recovered if normal human serum (NHS) is added to the system. NHS and C2 deficient human serum (C2 def-HS) have the same effect, while zymosan treated NHS (Zy-NHS) has no activity in reversing the blockade. Our results suggest that the alternative pathway of C plays a preponderant role in the recovery of ADCC of cells blocked by IC.

MATERIALS AND METHODS

Preparation of peripheral blood mononuclear cells. Peripheral blood mononuclear cells (PBMC) were isolated from normal human donors. Venous blood was defibrinated by shaking with glass beads and was centrifuged on Ficoll-Hypaque gradients (Böyum, 1968) (Ficoll, Pharmacia, Uppsala; Hypaque, kindly provided by Winthrop Products Inc., Farmasa Farmaceutica, Buenos Aires).

The resultant purified cells, composed of 95–98% mononuclear cells and 2–5% polymorphonuclear leucocytes (PMN), were submitted to an osmotic shock with distilled water for 30 sec to eliminate contaminating erythrocytes; isotonicity was restored with 10 × concentrated saline. The PBMC were then washed three times with TC199 medium (DIFCO Laboratories, Detroit, Michigan, USA) containing 50 μg/ml gentamicin (Schering Corporation, Essex, Argentina) and 2·5% heat-inactivated fetal calf serum (FCS). The same tissue culture medium (TC199-FCS) was used throughout the cytotoxicity experiments.

Antisera. Chromatographically purified ovalbumin (OA) (Cappel Laboratories, Detroit, Michigan) was passed through Sephadex G-150 (Pharmacia, Uppsala, Sweden) to remove high molecular weight aggregates. Antisera to OA were prepared by injecting rabbits with the chromatographed OA emulsified with Freund's complete adjuvant (FCA) three times at weekly intervals. The rabbits were bled 40 days after the first injection. IgG was obtained by precipitation of the serum with 50% ammonium sulphate, DE-52 cellulose (Whatman Paper Co.) chromatography in 0·01 M phosphate buffer, pH 8·0, and dialyzed against phosphate-buffered saline (PBS) (NaCl 0·13 M, Na_2HPO_4–NaH_2PO_4, 0·02 M, pH 7·2). The IgG fraction was then passed through an affinity column (Avrameas & Ternynck, 1969). The antibody was eluted with glycine-HCl 0·1 M, pH 2·8 buffer and dialyzed against PBS.

Antigen–antibody complexes. Insoluble antigen–antibody (IgG) complexes in antibody excess were preformed by incubation of 380 μl of IgG anti-OA (500 μg/ml) and 18 μl of OA (260 μg/ml) during 1 hr at 37°C and used in the cytotoxic experiments. The protein concentration was determined by OD_{280} nm considering an extinction coefficient of: $E^{1\%}_{1cm}$ OA = 7·3 and $E^{1\%}_{1cm}$ rabbit IgG = 1·6.

Sera. Normal human sera were obtained from healthy donors. When possible, we employed serum from the donor of the PBMC. Homozygous C2 deficient serum was obtained from a patient congenitally deficient in C2 (de Bracco *et al.*, 1979). C2 def-HS had no activity of the classical C pathway and 50% of the alternative C pathway of NHS. Zymosan was obtained as described by Lachman & Hobart (1979) and resuspended in veronal-buffered saline (VBS). Three millilitres of NHS were incubated 15 min at 37°C with 0·1 ml of the zymosan pellet and centrifuged. The same procedure was repeated two more times. Zymosan treated serum (Zy-NHS) had no alternative pathway activity but retained 25% of the classical pathway activity when compared to the original serum. C2 def-HS was used two-fold and Zy-NHS four-fold more concentrated than NHS to adjust the alternative or the classical pathway haemolytic activities to those of NHS. NHS was heated at 56 C for 30 min (HI-NHS) to inactivate C.

Preparation of $Na_2{}^{51}CrO_4$ labelled erythrocytes. A 0·1 ml aliquot of sheep, rabbit or chicken

blood obtained by venous puncture was drawn into syringes containing heparin or Alsever's solution. The samples were diluted 1:10 with TC199-FCS and then 100 μl of these suspensions were incubated with 50–100 μCi $Na_2\,^{51}CrO_4$ (^{51}Cr) (New England Nuclear, Boston, Massachussets) in a final volume of 0·2 ml. After 1 hr of incubation, the cells were washed four times and resuspended in TC199-FCS at the desired concentration.

Haemolytic assay to measure complement activity. Activity of the classical C pathway was measured with 100 μl ^{51}Cr labelled sheep red blood cells (^{51}Cr-SRBC) ($4{\cdot}5 \times 10^7$/ml) sensitized with anti-sheep haemolysin (Instituto Malbran, Buenos Aires). Two hundred microlitres of the different sera dilutions in TC199-FCS were added to the ^{51}Cr-SRBC in a final volume of 0·3 ml. After incubation for 1 hr at 37°C, the tubes were centrifuged at 400 ***g*** (5 min, 4°C) then 0·15 ml aliquots were removed from the supernatant and the radioactivity was measured in a gamma counter (Nuclear Chicago AEC-320-5).

The percentage of lysis was calculated by the following formula:

$$\%\ \text{lysis} = \frac{^{51}\text{Cr released to the supernatant} \times 2}{\text{Total radioactivity}} \times 100$$

This value was correcting by subtracting the percentage of ^{51}Cr released in the absence of antibody. The alternative pathway activity of C was measured essentially as described by Aguado *et al.* (1980). One hundred microlitres of ^{51}Cr labelled rabbit red blood cells (^{51}Cr-RRBC) at $4{\cdot}5 \times 10^7$ cells/ml and 200 μl of serum dilutions in TC199-FCS. EGTA (ethylene-glycol-bis [β-aminoethyl-ether] *N,N'*-tetraacetic acid, Baker Chemical Co., New Jersey) at 10 mM (final concentration) was used in the reaction. After 1 hr incubation at 37°C, the tubes were centrifuged, 0·15 ml of the supernatant was removed and the radioactivity measured. The percentage of lysis was calculated as indicated above.

Antibody-dependent cell-mediated cytotoxicity. ADCC was assayed as previously described (Perlmann & Perlmann, 1970) utilizing the chicken red blood cell (CRBC)–anti-CRBC system. Rabbit anti-CRBC was prepared as described elsewhere (Isturiz, de Bracco & Manni, 1975). Cytotoxic assays were performed by reacting $1{\cdot}0 \times 10^6$ PBMC with $1{\cdot}0 \times 10^5$ ^{51}Cr-CRBC and subagglutinant amounts of rabbit anti-CRBC serum. The reaction was done in TC199-FCS in a final volume of 0·2 ml. The tubes were centrifuged at 180 ***g*** for 5 min to insure contact of the reacting cells and resuspended carefully. After incubation at 37°C for 2·5 hr, 0·2 ml of TC199-FCS was added, the tubes were centrifuged at 400 ***g*** for 10 min and 0·2 ml of the supernatant was removed and transferred to a test tube. Radioactivity of the supernatant and residual cells was measured in a gamma counter. The cytotoxicity was calculated as follows:

$$\%\ \text{ADCC} = \frac{^{51}\text{Cr released to the supernatant} \times 2}{\text{Total radioactivity}} \times 100$$

This value was corrected by subtracting the percentage of ^{51}Cr released in the absence of antibody (1–3%). Duplicate or triplicate tubes were set up for each reaction. Experiments were performed at least three times (3–12 times) and a representative one will be shown under Results to avoid dispersion due to differences in the ADCC activity from different PBMC donors.

Inhibition of cytotoxicity by immune complexes and its reversal by serum factors. For the ADCC inhibition tests, 5 μl of IC were added to 0·1 ml of TC199-FCS containing 10^6 PBMC. The mixtures were incubated for 2 hr at 37°C unless otherwise stated. After the inhibition, 0·1 ml of the different dilutions of sera was added and the reaction mixture was incubated for 18 hr at 37°C. The cells were washed three times with TC199-FCS and ADCC was measured.

Controls consisted of PBMC incubated with TC199-FCS; PBMC incubated with TC199-FCS and with serum thereafter and PBMC incubated with IC and then with TC199-FCS. The percentage of the recovery of ADCC was calculated as follows:

$$\%\ \text{Recovery} = \frac{\%\ \text{ADCC of (PBMC + IC + serum)} - \%\ \text{ADCC of (PBMC + IC)}}{\%\ \text{ADCC of PBMC} - \%\ \text{ADCC of (PBMC + IC)}} \times 100.$$

RESULTS

Effect of NHS on the reversion of ADCC

ADCC was blocked by incubating the effector cells with IC formed by IgG antibodies during 2 hr at 37°C, and the inhibition was not reversed by prolonged incubation of these cells in the same medium. To investigate if serum factors could help the IC-blocked PBMC to regain their ADCC activity, the cells were incubated for 18 hr with NHS after exposure to the IC. A significant recovery of ADCC was observed (Fig. 1) indicating that NHS could revert part of the ADCC inhibition caused by IC.

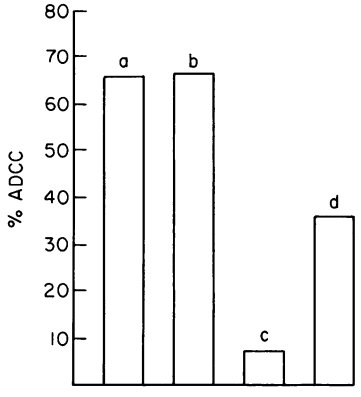

Fig. 1. PBMC (0·1 ml) at a concentration of 10×10^6 cells/ml was incubated with: (a) 5 μl PBS; (b) 5 μl PBS; (c) 5 μl IC and (d) 5 μl IC. After 2 hr at 37°C, 0·1 ml TC199-FCS (a); 0·1 ml NHS (b); 0·1 ml TC199-FCS (c) and 0·1 ml NHS (d) was added and the reaction tubes kept for 18 hr at 37°C. The cells were then washed three times with TC199-FCS, resuspended in 0·1 ml TC199-FCS and ADCC assayed. IC were prepared and ADCC was performed as described in Materials and Methods.

It is known that NHS has the capacity of increasing non-specific cytotoxicity against non-sensitized xenogeneic target cells (Muchmore, Decker & Blaese, 1979). This cytotoxicity was not inhibited by IC (Muchmore *et al.*, 1979; Isturiz, unpublished results). In order to determine whether NHS acted by aiding the recovery of ADCC of the blocked cells or by inducing non-specific cytotoxicity, the following experiment was performed: PBMC were incubated with IC as in Fig. 1 and NHS was added to revert the inhibition of ADCC. After recovery of ADCC with NHS, a new addition of IC completely inhibited the cytotoxicity showing that the first possibility was correct. Besides, the non-specific cytotoxic activity of lymphocytes that had been previously treated with

Table 1. Effect of a new addition of immune complexes to mononuclear cells after recovery of ADCC

IC inhibition (2hr, 37°C)	Incubation with (18 hr, 37°C)	IC addition	%ADCC
PBMC	TC199-FCS	TC199-FCS	68·5
PBMC+IC	TC199-FCS	TC199-FCS	1·2
PBMC+IC	NHS	TC199-FCS	29·4
PBMC+IC	NHS	TC199-FCS+IC	0·4

Inhibition of ADCC by IC (2 hr, 37°C) and incubation with either culture medium or NHS (18 hr, 37°C) were done as described in Materials and Methods. After re-establishment of ADCC with NHS, the cells were washed three times and either culture medium (TC199-FCS) or IC in excess (20 μl) was added. ADCC was assayed immediately as described in Materials and Methods.

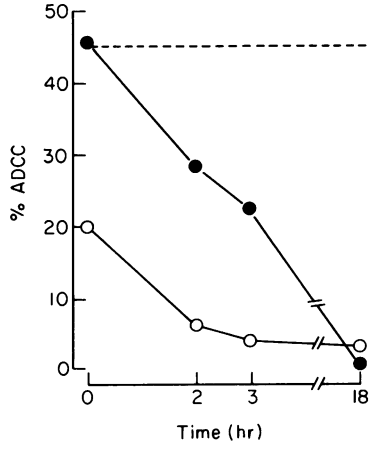

Fig. 2. PBMC (0·1 ml) at a concentration of 10×10^6 cells/ml were incubated with 5 μl of IC during the times indicated in the graph. Then the cells were washed three times, resuspended in 0·1 ml of TC199-FCS and ADCC was assayed (O——O). In another set of tubes (●——●) 0·1 ml of NHS was added at those times. After 18 hr incubation at 37°C, the cells were washed and ADCC tested. Dotted lines represent control ADCC for that experiment. IC were prepared and ADCC was assayed as described in Materials and Methods.

NHS was not significantly different from that of the controls (PBMC + TC199-FCS) after 18 hr of incubation at 37°C (Table 1).

Effect of incubation of PBMC with IC on ADCC recovery

It is known that IC inhibit ADCC by competing with the sensitized target cells for the FcγR. Besides, it has been shown (Ziegler & Henney, 1977) that the inhibition process is an active phenomenon dependent on the time of incubation of the effector cells with the IC.

Taking these data into account, we studied the effect of NHS on the recovery of ADCC when effector cells had been exposed to the IC for variable incubation times. The results shown in Fig. 2 indicate that reversal of IC inhibition of ADCC was maximum when PBMC were reacted simultaneously with the IC and NHS and was nil when PBMC were incubated with IC for 18 hr before the addition of NHS. In most experiments, inhibition of PBMC with IC lasted for 2 hr before the addition of NHS. Therefore, the recovery was not total, ranging from 40 to 60% of the original values.

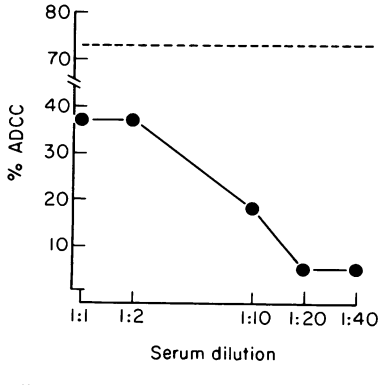

Fig. 3. PBMC (0·1 ml) at 10×10^6 cells/ml was incubated with 5 μl IC during 2 hr. After this period, 0·1 ml of NHS at different dilutions was added and the tubes were kept for 18 hr at 37°C. The cells were washed three times with TC199-FCS, resuspended in 0·1 ml of TC199-FCS and ADCC was assayed. Dotted lines represent control ADCC for that experiment. IC were prepared and ADCC was performed as described in Materials and Methods.

Effect of NHS dilution

Fig. 3 shows that the effect of NHS on the recovery of ADCC depended on its concentration. Maximal restoration was observed at a 1/2 dilution of NHS. Increasing the amount of NHS had no further effect on ADCC. The ability of NHS to re-establish ADCC was lost upon dilution of the serum and at 1/20 recovery was nil. The fact that relatively high amounts of serum were required for activity is remindful of processes that depend on activation of the alternative pathway of C, and this possibility will be discussed in the next section.

Effect of heat-inactivated NHS, C2 deficient human serum and zymosan treated NHS on the recovery of ADCC

Miller, Saluk & Nussenzweig (1973) reported that when IC reacted with complement they could be fixed to the C3 receptor on the cell surface and that the release of these IC from cell membranes was mainly a function of the alternative pathway of C. To investigate if the C system was involved in the recovery of ADCC by IC-blocked cells, different sources of sera deficient either in the alternative or the classical pathways and heat inactivated NHS (HI-NHS) were compared to fresh NHS. Heat inactivation (56°C, 30 min) and zymosan treatment of NHS abolished the recovery effect. On the contrary, C2 def-HS was as active as NHS in the re-establishment of ADCC (Table 2) showing that the activity of the alternative pathway of C was essential for reversal of IC inhibition.

Table 2. Effect of normal or C deficient sera on the recovery of ADCC of mononuclear cells that had been blocked with IC

Incubation with (18 hr, 37 C)	% Recovery of ADCC
NHS	75
C2 def-HS	71
Zy-NHS	4
HI-NHS	0

PBMC (0·1 ml) at 10×10^6 cells/ml were incubated with 5 μl of IC during 2 hr at 37°C. After this period, an aliquot of each serum was added and the reaction mixtures were kept for 18 hr at 37°C. Cells were then washed three times, resuspended in TC199-FCS and ADCC was assayed. The concentrations of C2 deficient serum (C2 def-HS) and zymosan adsorbed serum (Zy-NHS) were adjusted to give the same alternative or classical C pathway activities as NHS respectively. ADCC of PBMC that had been reacted with IC 2 hr at 37°C and remained at 37°C with no addition of serum was 28%. ADCC of PBMC without IC was 63%. Percentage recovery was calculated as described in Materials and Methods.

DISCUSSION

The results presented in this paper indicate that the inhibition of ADCC by IC is a phenomenon that can be reversed by autologous as well as by allogeneic NHS (Fig. 1). The proof that NHS affects the recovery of the function of the FcγR and does not produce a non-specific cytotoxic effect (Muchmore *et al.*, 1979), is shown in the experiments in which the recovered cytotoxic activity was

susceptible to a new inhibition by IC (Table 1). Non-specific cytotoxicity induced by NHS could not be inhibited by IC (Muchmore *et al.*, 1979).

Since NHS as well as C2 def-HS were able to restore the ADCC of PBMC (Table 2) while HI-NHS or Zy-NHS were ineffective, the alternative pathway of C appears to have a crucial role in the reversal of IC-induced inhibition of ADCC. The fact that a relatively high concentration of NHS was required to re-establish the cytotoxic activity of PBMC (Fig. 3) is also in agreement with the involvement of the alternative pathway of C in the unblocking process. Whether the integrity of the alternative pathway is needed or the activation of some of its components is sufficient to induce the recovery of ADCC remains to be established.

The recovery of ADCC by NHS correlated with the extent of the contact period of PBMC and IC (Fig. 2), becoming nil after prolonged incubation. This effect could be due to the fact that the inhibition of ADCC by IC may not be simply a phenomenon of blockade of the receptors by the IC (Ziegler & Henney, 1977).

An important question on the recovery of ADCC was to determine whether NHS induced the appearance of new $Fc\gamma R$ on the effector cells or it clears up the receptors that had been previously blocked by IC. Both mechanisms could be possible. Birch, Fanger & Bernier (1979) showed that β_2 microglobulin could act on lymphocyte membranes inducing the appearance of receptors for the Fc fragment of IgG. On the other hand, Miller, Saluk & Nussenzweig (1973) demonstrated that C activation, mainly through the alternative pathway, was effective in the release of IC when these were bound to cell membranes through the C3 receptor. In addition, Miller & Nussenzweig (1975) reported that C solubilized IC essentially through activation of the alternative pathway of C.

Since NHS induced the recovery of ADCC only during the early stages of inhibition and ADCC did not increase with further incubation in the presence of serum, the restoration of $Fc\gamma R$ dependent cytolytic activity of PBMC could be due to functional recovery of existing receptors on the cell membrane.

Whenever IC persist in circulation or the normal depuration mechanisms are overcome, the biological functions that depend on the maintenance of free $Fc\gamma R$ may be impaired. These functions are of different nature and they may be relevant to: the regulation of the immune response (Morgan *et al.*, 1980), efficient defence mechanisms such as ADCC and phagocytosis or the release of lymphokines from immunocompetent lymphocytes (Thoman *et al.*, 1981). IC may not only be fixed by receptors on the cells of the reticuloendothelial system and circulating leucocytes but on $Fc\gamma R$ present in other tissues. As an example, the reaction of IC with $Fc\gamma R$ present on the renal glomeruli (Mizoguchi *et al.*, 1978) could have immunopathological consequences and might justify the presence of IC in the kidney of patients with diseases of different aetiology. Therefore, the existence of a physiological way for the recovery of $Fc\gamma R$ activity on different cells would provide means for the maintenance of the different mechanisms dependent on their function.

Briefly, this work demonstrates that NHS, through activation of the alternative C pathway, allows the recovery of ADCC of PBMC that had been previously blocked by interaction of IC with surface $Fc\gamma R$. This activity of NHS could provide a widespread mechanism for the maintenance of operative $Fc\gamma R$ in physiopathological processes characterized by the presence of circulating IC.

The authors gratefully acknowledge the generous aid of Dr R. Castañé and R. Bayo of the Blood Bank, Instituto de Investigaciones Médicas. We thank Drs Elda Giavedoni and Rita L. Cardoni for helpful discussion and valuable advice.

This work was supported by CONICET grants 7617/80 and 81 and 9466/80 and by a grant of Cherny Foundation, Argentina.

REFERENCES

AGUADO, M.T., PERRIN, L.H., RAMIREZ, R., MIESCHER, P.A. & LAMBERT, P.H. (1980) Evaluation of alternative pathway and factor B haemolytic activities in patients with systemic lupus erythematosus: correlations with the alternative pathway regulatory proteins. *Clin. exp. Immunol.* **42,** 495.

AVRAMEAS, S. & TERNYNCK, T.H. (1969) The cross linking of proteins with gluteraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry*, **6,** 53.

BERKEN, A. & BENACERRAF, B. (1966) Properties of antibodies cytophilic for macrophages. *J. exp. Med.* **123,** 119.

BERMAN, M.A. & WEIGLE, W.O. (1977) B-lymphocyte

activation by the Fc region of IgG. *J. exp. Med.* **146,** 241.

BIRCH, R.E., FANGER, M.W. & BERNIER, G.M. (1979) β_2 microglobulin enhances human lymphocyte surface receptor expression for IgG. *J. Immunol.* **122,** 997.

BÖYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. Lab. Invest.* **21** (Suppl. 97), 77.

BRACCO, M.M.DE E.DE, BIANCHI, O. & STRINGA, S.G. (1979) Hereditary complement (C2) deficiency with discoid lupus erythematosus and idiopathic atrophoderma. *Intl. J. Dermatology.* **18,** 713.

DICKLER, H.B. (1974) Studies of the human lymphocyte receptor for heat aggregated or antigen-complexed immunoglobulin. *J. exp. Med.* **140,** 508.

DICKLER, H.B. & KUNKEL, H.G. (1972) Interaction of aggregated γ-globulin with B lymphocytes. *J. exp. Med.* **136,** 191.

FERRARINI, M., MORETTA, L., ABRILE, R. & DURANTE, M.L. (1975) Receptors for IgG molecules on human lymphocytes forming spontaneous rosettes with sheep red blood cells. *Eur. J. Immunol.* **5,** 70.

FRÖLAND, S.S., WISLÖFF, F. & MICHAELSEN, T.E. (1974) Human lymphocytes with receptors for IgG. A population of cells distinct from T and B lymphocytes. *Int. Arch. Allergy.* **47,** 124.

GALE, R.P. & ZIGHELBOIM, J. (1975) Polymorphonuclear leukocytes in antibody-dependent cellular cytotoxicity. *J. Immunol.* **114,** 1047.

ISTURIZ, M.A., DE BRACCO, M.M.E. & MANNI, J.A. (1975) Reaction of human lymphocytes with aggregated IgG columns. Effect on antibody-dependent cytotoxicity and EAC rosette formation. *Cell. Immunol.* **16,** 82.

KERBEL, R.S. & DAVIES, J.S. (1974) The possible biological significance of FC receptors on mammalian lymphocytes and tumor cells. *Cell,* **3,** 105.

KUMAGAI, K., ABO, T., SEKIZAWA, T. & SASAKI, M. (1975) Studies of surface immunoglobulins on human B lymphocytes. I. Dissociation of cell-bound immunoglobulins with acid pH or at 37 C. *J. Immunol.* **115,** 982.

LAWRENCE, E.C., POPLACK, D.G., HOLIMAN, B.J., DOOLEY, N.D., KOSKI, I.R. & BLAESE, R.M. (1977) Studies of monocyte Fc receptor avidity and monocyte mediated antibody dependent cellular cytotoxicity in man. In *Regulatory mechanisms in lymphocyte activation* (ed. by D. O. Lucas) pp 521–523. Acad. Press Inc., London.

LACHMANN, P.J. & HOBART, M.J. (1979) Complement technology. In *Handbook of Experimental Immunology.* Vol. 1 (ed. by D. M. Weir) pp 5.A1–5.A23. Blackwell Scientific Publications, Oxford.

LEE, S.T. & PARASKEVAS, F. (1972) Cell surface-associated gamma globulin in lymphocytes. IV. Lack of detection of surface globulin on B-cells and acquisition of surface G globulin by T-cells during primary response. *J. Immunol.* **109,** 1262.

MILLER, G.W. & NUSSENZWEIG, V. (1975) A new complement function: solubilization of antigen–antibody complexes. *Proc. Natl. Acad. Sci. USA.* **72,** 418.

MILLER, G.W., SALUK, P.H. & NUSSENZWEIG, V. (1973) Complement dependent release of immune complexes from the lymphocyte membrane. *J. exp. Med.* **138,** 495.

MIZOGUCHI, V., TANIMOTO, K., MITAMURA, T., NAKAI, H., HORIUCHI, Y. & UMEDA, T. (1978) Detection of Fc receptor on human renal glomerulus. *Clin. Immunol. Immunopathol.* **10,** 129.

MORGAN, E.L., WALKER, S.M., THOMAN, M.L. & WEIGLE, W.O. (1980) Regulation of the immune response. I. The potentiation of *in vivo* and *in vitro* immune responses by Fc fragments. *J. exp. Med.* **152,** 113.

MUCHMORE, A.V., DECKER, J.M. & BLAESE, R.M. (1979) Synergistic cytotoxicity. I. Characterization of a heat labile plasma fraction that induces nonspecific cytotoxicity by human mononuclear cells. *J. Immunol.* **122,** 1146.

PEARSON, G.R. (1978) *In vitro* and *in vivo* investigations on antibody-dependent cellular cytotoxicity. In *Current Topics in Microbiology and Immunology.* Vol. 80, p. 65. Springer-Verlag Ed.

PERLMANN, P. & PERLMANN, H. (1970) Contactual lysis of antibody coated chicken erythrocytes by purified lymphocytes. *Cell. Immunol.* **1,** 300.

REVILLARD, J.P., SAMARUT, C., CORDIER, G. & BROCHIER, J. (1975) Characterization of human lymphocytes bearing Fc receptors with special reference to cytotoxic (K) cells. In *Membrane Receptors of Lymphocytes.* (ed. by M. Seligman, J. L. Preud'homme and F. M. Kourilsky) p. 161. North Holland, Amsterdam.

SUGAMURA, K. & SMITH, B.J. (1977) Reversible blocking of antibody-dependent cell mediated cytotoxicity. *Cell. Immunol.* **30,** 353.

THOMAN, M.L., MORGAN, E.L. & WEIGLE, W.O. (1980) Polyclonal activation of murine B lymphocytes by Fc fragments. II. Replacement of T cells by a soluble helper T cell-replacing factor (TRF). *J. Immunol.* **125,** 1630.

THOMAN, M.L., MORGAN, E.L. & WEIGLE, W.O. (1981) Fc fragment activation of T lymphocytes. I. Fc fragments trigger Lyt 1^+23^- T lymphocytes to release a helper T cell-replacing activity. *J. Immunol.* **126,** 632.

ZIEGLER, H.K. & HENNEY, C.S. (1977) Studies on the cytotoxic activity of human lymphocytes. II. Interactions between IgG and Fc receptors leading to inhibition of K cell function. *J. Immunol.* **122,** 997.