*Immunology* 1981 **43** 635

# Functional studies of rabbit T lymphocytes

JANET M. McNICHOLAS*, J. R. WATKINS, AUDREY D. JOHNSON & KATHERINE L. KNIGHT *Department of Microbiology and Immunology, University of Illinois at the Medical Center, Chicago, Illinois, U.S.A.*

*Accepted for publication 5 February 1981*

**Summary.** Rabbit spleen and mesenteric lymph node cells were treated with a monoclonal anti-rabbit T-lymphocyte antibody (MAb) and complement and the effect of the treatment on various lymphocyte functions was determined. Lysis of spleen and mesenteric lymph node cells reactive with this MAb, 9AE10, essentially eliminated their proliferative responsiveness to allogeneic lymphocytes in the mixed lymphocyte reaction and to the T-cell mitogens, concanavalin A and phytohaemagglutinin; responsiveness to the B-cell mitogen, anti-immunoglobulin (Ig) was not decreased by lysis of 9AE10$^+$ cells. In addition, the 9AE10$^+$ cells were found to be necessary for the secondary *in vitro* antibody response to the T-dependent antigen sheep red blood cells (SRBC), as removal of 9AE10$^+$ cells blocked the generation of plaque forming cells (PFC) in culture. The PFC's themselves were not sensitive to lysis by 9AE10 MAb and complement. Thus, the 9AE10 MAb appears to recognize cells which have functions characteristic of T lymphocytes and this monoclonal antibody will be useful in further studies of the rabbit cellular immune system.

Abbreviations: MLN, mesenteric lymph node; SRBC, sheep red blood cells; FCS, foetal calf serum; Ig, immunoglobulin; Con-A, concanavalin A; PHA, phytohemagglutinin; PWM, pokeweed mitogen; PFC, plaque forming cell; MLC, mixed lymphocyte culture; MAb, monoclonal antibody; NRS, normal rabbit serum; PBL, peripheral blood lymphocytes; MLR, mixed lymphocyte reaction; c.p.m., counts per minute.

* Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305, U.S.A.

Correspondence: Dr K. L. Knight, Department of Microbiology and Immunology, University of Illinois, Medical Centre, P.O. Box 6998, Chicago, Illinois 60680, U.S.A.

0019-2805/81/0800-0635$02.00


## INTRODUCTION

Rabbit T lymphocytes have been identified as peripheral lymphocytes which pass through a nylon wool column (Redelman, Scott, Sheppard & Sell, 1976), rosette with papain-treated rabbit erythrocytes (Wilson, Gurner & Coombs, 1975), and react with a heterologous antiserum to rabbit thymocytes (Redelman *et al.*, 1976; Sheppard, Redelman & Sell, 1976; Fanger, Pelley & Reese, 1972; Fradelizi, Chou, Cinader & Dubiski, 1973; Zimmerman, Okumura, Rabkin & Kern, 1974; Wilson, Teodorescu & Dray, 1976), but not with antibody to immunoglobulin (Ig) (Redelman *et al.*, 1976). These lymphocytes possess functional properties similar to those identified for mouse and human T lymphocytes. For example, they are able to provide helper activity for secondary antibody responses (Stavitsky & Cook, 1974; Metzger, Hendricks & Teodorescu, 1977; Ozer & Waksman, 1972), to proliferate in response to allogeneic lymphocytes (Lancki, Tissot & Cohen, 1979; Sheppard, Sell, Poler & Redelman, 1977; Milthrop & Richter, 1979), and to the mitogens concanavalin A (Con-A) and phytohaemagglutinin (PHA; Teodorescu, Mayer, Reiter &

Dray, 1976; Ozer & Waksman, 1974; Shek, Chou, Dubiski & Cinader, 1974).

We have previously described the preparation and characterization of a mouse monoclonal antibody (MAb) which identifies rabbit T lymphocytes (McNicholas, Raffeld, Loken, Reiter & Knight, 1981). This MAb designated 9AE10, reacts with essentially all thymocytes, with T lymphocytes in spleen, mesenteric lymph node (MLN) and peripheral blood and with approximately 30% of bone marrow cells. The antigen recognized by this MAb appears to be a cell-surface glycoprotein with a mol. wt of approximately 25,000. The MAb is of the IgM class and can be used with complement to specifically lyse rabbit T lymphocytes. In the present study, we have investigated the functional capabilities of rabbit lymphocytes using 9AE10 MAb as a reagent to separate spleen and/or MLN subpopulations.

## MATERIALS AND METHODS

*Rabbits*

Rabbits were obtained from the closed colony maintained by Drs S. Dray and K. L. Knight and from the Rabbit Resources Colony maintained by Dr C. Cohen, at the University of Illinois Medical Center, Chicago, Ill.

*Cells and media*

Spleen and mesenteric lymph node (MLN) cell suspensions were prepared as described previously (Metzger *et al.*, 1977). For *in vitro* cell cultures, cells were incubated in RPMI-1640 medium which had been supplemented as described by Metzger *et al.* (1977) except that foetal calf serum (FCS) was used in some experiments rather than normal rabbit serum (NRS).

For cell separations, spleen and MLN cells ($10^8$) were placed on petri dishes (100 mm, Falcon Plastics, Oxnard, Calif.), which had been coated with specifically purified goat anti-rabbit Ig antibody (1 mg/ml); (Mage, McHugh & Rothstein, 1977). The cells which did not attach to the petri plates, the $Ig^-$ cells, were removed and washed. Immunofluorescent analyses revealed that 90% of the $Ig^-$ cells from spleen and $\geq 95\%$ of the $Ig^-$ cells from MLN reacted with the 9AE10 MAb and $\leq 3\%$ of either cell population reacted with anti-Ig antibody.

*Clone 9AE10.* Clone 9AE10, obtained from the fusion of P3/X 63-Ag8 myeloma cells with spleen cells from mice immunized with rabbit spleen and mesenteric lymph node (MLN) cells was maintained in RPMI-1640 supplemented with 15% FCS (M. A. Bioproducts, Bethesda, Md). Clone 9AE10 was subcloned two times by limiting dilution and supernatant from the subclones was used in all the experiments. These subclones reacted by immunofluorescence with essentially all thymocytes and with T cells in the spleen, MLN, and peripheral blood lymphocytes. By double membrane immunofluorescence, the 9AE10 MAb and $Ig^+$ cells of spleen, MLN and peripheral blood lymphocytes were distinct and non-overlapping populatons. The 9AE10 MAb is of the IgM class and is cytotoxic.

*Antibody-mediated cytotoxicity*

Rabbit spleen or MLN cells were mixed with the 9AE10 MAb (2 ml of five-fold concentrated supernatant fluid/$10^8$ cells) and incubated at 4° for 1 hr. The cells were centrifuged at 900 *g* and the cell pellet was suspended in rabbit complement (4 ml of a 1:4 dilution; (Pel Freeze, Rodgers, Ark.) and were incubated at 37° for 1 hr. Dead cells were removed by centrifugation (900 *g* for 10 min) of the cells through FCS (Mishell & Shiigi, 1980). The cell pellet was treated a second time with 9AE10 MAb and complement in the same manner. From 35% to 55% of the spleen cells and from 45% to 65% of the MLN cells were killed by 9AE10 MAb and complement treatment, as measured by trypan blue dye exclusion. By membrane immunofluorescence, less than 3% of the viable cells remaining after lysis reacted with 9AE10 MAb and from 70% to 80% reacted with anti-Ig.

*Mitogen response assays*

The mitogens used in these experiments included: Con-A, 10–20 μg/ml (Calbiochem, San Diego, Calif.); PHA, 0·06–1·00 μl/ml (Difco, Detroit, Mich.); pokeweed mitogen, 1–2 μl/ml (PWM, Gibco, Grand Island, N.Y.) and goat anti-rabbit L-chain antibody (anti-Ig), 50–200 μg/ml. The concentration of mitogen which resulted in maximum stimulation was determined in every experiment. Within individual experiments, maximum stimulation of each cell population was obtained with the same concentration of mitogen.

Cells were cultured with 0·1 ml mitogen in microtitre plates (Falcon Micro Test II, Cockeysville, Md) in medium containing 5% FCS (for anti-Ig stimulation) or 10% NRS (for Con-A, PHA and PWM stimulation). Each well contained $2 \times 10^5$ cells in a total of 0·2 ml. Cultures were incubated at 37° in 5% $CO_2$ for

72 hr. Eighteen hours before harvest, 1–2 $\mu$Ci [$^3$H]-thymidine were added to each well. Cells were harvested (Brandel Cell Harvester, Rockville, Md) and the radioactivity incorporated was measured in a Packard TriCarb-scintillation counter. The data are presented as the mean counts per minute (c.p.m.) of quadruplicate cultures ± the standard error of the mean.

*One-way mixed lymphocyte reaction*

Stimulating cells were inactivated with mitomycin C (40 $\mu$g/$10^7$ cells/ml). Equal numbers of stimulator and responder cells ($10^6$ cells/well), in medium containing $5 \times 10^{-5}$ M 2-mercaptoethanol and 5% FCS, were co-cultured in microtitre plates for 96 hr at 37° in 5% $CO_2$. The cultures were pulsed with [$^3$H]-thymidine and harvested as described for the mitogen assays. The data are presented as the mean c.p.m. of quadruplicate cultures ± the standard error of the mean.

*Primary* in vivo *and secondary* in vitro *antibody response to SRBC*

Rabbits were injected intravenously with 1 ml of 10% (v/v) sheep red blood cells (SRBC) in saline and in some experiments also with colchicine (1 mg/kg; Shek & Coons, 1978). Four days later, rabbits were killed and the spleen cells were assayed for the number of antibody secreting cells in both direct and indirect plaque forming cell (PFC) assays.

For the secondary *in vitro* response, $5 \times 10^6$ spleen cells from the immunized rabbits were cultured in 1 ml of medium containing 5% NRS and 0·1 ml of 1% (v/v) SRBC in 24-well tissue culture plates (Costar, Cambridge, Mass.) at 37° in 5% $CO_2$. After 5 days, the cells were harvested and washed, and the number of antibody secreting cells was determined in both direct and indirect PFC assays.

The PFC assay was as described by Fauci & Pratt (1976); goat anti-rabbit $Fc_\gamma$ antiserum (1:800) was used to develop the indirect plaques. The data are reported as the mean number of PFC/$10^6$ cells (primary response) or PFC/$10^6$ cultured cells (secondary response) of triplicate determinations. The standard deviation of the mean was always less than 10%.

## RESULTS

### Mitogen responses

The effect of 9AE10 MAb and complement treatment of rabbit spleen and MLN cells on their response to various mitogens was investigated. Lysis of spleen and MLN cells with 9AE10 MAb and complement removed the responsiveness to Con-A, PHA and PWM (Table 1). Whereas the unseparated spleen cells and the Ig$^-$ cells responded to Con-A (156,169 and 187,390 c.p.m. respectively), the response of 9AE10 MAb and complement-treated spleen cells was nearly 100-fold less (2142 c.p.m.). Similarly, the response to PHA was reduced 100-fold after lysis of 9AE10$^+$ cells (56,175 c.p.m. in untreated cells and 602 c.p.m. in treated cells). The mitogenic response to PWM was also eliminated after treatment of spleen cells with 9AE10 MAb and complement (Table 1; 44,744 c.p.m. in the unseparated cells to 405 c.p.m. in the treated cells). Responsiveness to the B-cell mitogen, anti-Ig, was not diminished after lysis of 9AE10$^+$ cells (12,150 c.p.m. in untreated cells and 21,852 c.p.m. in treated cells). As a positive control, the responses of Ig$^-$ cells (≥90% 9AE10$^+$) obtained from anti-Ig coated petri plates were determined. These cells responded to the T-cell mitogens Con-A and PHA as well as did unseparated spleen cells, but, they did not respond to anti-Ig. In similar experiments performed with MLN cells treatment with 9AE10 MAb and complement again abolished responsiveness to Con-A, PHA and PWM but did not significantly decrease the response

**Table 1.** Mitogen responses of unseparated spleen and MLN cells as compared with 9AE10 MAb + C′ treated and Ig$^-$ cell populations

| Cells | Con-A | PHA | PWM | NRS control | Anti-Ig | FCS control |
|---|---|---|---|---|---|---|
| Spleen | 156,169 ± 1822 | 56,175 ± 942 | 44,744 ± 1269 | 337 ± 30 | 12,150 ± 432 | 1102 ± 152 |
| Spleen-9AE10 + C′treated | 2142 ± 253 | 602 ± 188 | 405 ± 25 | 164 ± 7 | 21,852 ± 629 | 1049 ± 44 |
| Spleen-Ig$^-$ | 187,390 ± 2892 | 60,609 ± 1615 | 103,285 ± 3310 | 1299 ± 266 | 694 ± 193 | 924 ± 93 |
| MLN | 185,325 ± 7097 | 109,481 ± 2069 | 7388 ± 129 | 112 ± 15 | 11,991 ± 271 | 288 ± 19 |
| MLN-9AE10 + C′ treated | 200 ± 23 | 75 ± 12 | 42 ± 9 | 61 ± 16 | 10,851 ± 536 | 214 ± 24 |
| MLN-Ig$^-$ | 202,332 ± 4928 | 104,405 ± 1436 | 16,414 ± 2145 | 54 ± 20 | 657 ± 1 | 110 ± 24 |

**Table 2.** One-way mixed lymphocyte reactions

| Experiment 1 | | |
|---|---|---|
| Responding cells (T118-1) | Stimulating cells | |
| | MLN 0884 cells | Autologous control cells |
| MLN | 27,832±1698 | 603±94 |
| MLN-9AE10+C′ treated | 733± 109 | 114±16 |
| MLN-Ig$^-$ | 11,574±3105 | 328±68 |
| Spleen | 46,630±2455 | 236±28 |
| Spleen-9AE10+C′ treated | 6219± 28 | 730±49 |

| Experiment 2 | | |
|---|---|---|
| Responding cells (T116-2) | Stimulating cells | |
| | MLN 2192 cells | Autologous control cells |
| MLN | 261,593±15,187 | 7379±1635 |
| MLN-9AE10+C′ treated | 19,775± 2414 | 328± 83 |
| MLN-Ig$^-$ | 386,251±44,011 | 1628± 377 |
| Spleen | 177,945±18,692 | 7271±2623 |
| Spleen-9AE10+C′ treated | 24,881± 1418 | 1038± 220 |
| Spleen-Ig$^-$ | 117,964± 7677 | 1690± 110 |

to anti-Ig (Table 1). Similar data were obtained for both MLN and spleen from each of five other rabbits (data not shown).

### Mixed lymphocyte reaction (MLR)

Experiments were performed to determine whether the responder cell in the one-way MLR was sensitive to lysis by 9AE10 MAb and complement. The results of two such experiments are given in Table 2. In the first experiment, mitomycin C-treated MLN cells from rabbit 0884 were used as stimulator cells and the responses of either untreated or 9AE10 MAb and complement-treated spleen and MLN cells from rabbit T118-1 were determined. The response of MLN cells to mitomycin C-treated allogeneic stimulator cells (27,832 c.p.m.) was abrogated by treatment with 9AE10 MAb and complement (733 c.p.m.). In similar experiments, lysis of the 9AE10$^+$ spleen cells resulted in a marked reduction of the response to allogeneic stimulator cells. The data from three additional experiments confirmed these results (data from one of these is shown in Table 2). Thus, lysis of 9AE10$^+$ cells effectively resulted in elimination of the responsiveness of lymphocytes to allogeneic stimulation.

### Antibody response to SRBC

The effect of treatment of spleen cells with 9AE10 MAb and complement on antibody production, as measured with the haemolytic plaque assay, was determined. The number of antibody secreting cells in the

**Table 3.** Primary *in vivo* anti-SRBC response (PFC/$10^6$ cells)

| | Rabbit 1 | | Rabbit 2 | | Rabbit 3 | | Rabbit 4 | | Rabbit 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cells | Direct | Indirect | Direct | Indirect | Direct | Indirect | Direct | Indirect | Direct | Indirect |
| Spleen | 1740 | 4040 | 2100 | 2960 | 1720 | 2400 | 100 | 1680 | 570 | 2600 |
| Sp *B** | 3840 | 4600 | 3520 | 3560 | 6480 | 6800 | 415 | 3440 | 2300 | 8480 |

*9AE10+C′ treated spleen cells.

**Table 4.** Secondary *in vivo* anti-SRBC response (PFC/$10^6$ cells)

| | Rabbit 1 | | Rabbit 2 | | Rabbit 3 | | Rabbit 4 | | Rabbit 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cells | Direct | Indirect | Direct | Indirect | Direct | Indirect | Direct | Indirect | Direct | Indirect |
| Spleen | 1680 | — | 65,280 | 89,600 | — | — | 2920 | 5160 | — | — |
| Sp *B** | 0 | — | 70 | 130 | 1920 | 1600 | 110 | 110 | 340 | 480 |
| *B*+*T*† | — | — | — | — | 5040 | 5680 | 1520 | 2920 | 8040 | 9370 |

*9AE10+C′ treated spleen cells.
†Co-cultures of equal numbers of 9AE10+C′ treated spleen cells and $Ig^-$ spleen cells.

spleens of rabbits immunized *in vivo* with SRBC was determined on untreated and on 9AE10 MAb and complement-treated spleen cells (Table 3). In five experiments, the number of PFC's was not decreased, and in fact was somewhat increased, by treatment with 9AE10 MAb and complement even though from 35% to 55% of the spleen cells were killed by this treatment. The apparent increase in PFC's after treatment presumably reflects enrichment of PFC's following the removal of the $9AE10^+$ cells.

In the secondary *in vitro* antibody response to the T cell-dependent antigen, SRBC, treatment of the spleen cells with 9AE10 MAb and complement before culture with antigen resulted in a marked decrease in PFC's (Table 4). Although the absolute number of PFC's varied considerably between rabbits, lysis of $9AE10^+$ cells consistently abrogated antigen responsiveness. Thus, treatment of splenic lymphocytes with 9AE10 MAb and complement removed cells required for the secondary *in vitro* antibody response to SRBC but did not directly effect the antibody secreting cells as measured by PFC's.

## DISCUSSION

A monoclonal antibody 9AE10, which reacts with essentially all rabbit thymocytes and $Ig^-$ peripheral lymphocytes has been described (McNicholas *et al.*, 1981) and in the present paper we have functionally characterized the cells remaining after removal of $9AE10^+$ cells with antibody and complement treatment. Lysis of the $9AE10^+$ cells abrogated the responses of spleen and/or MLN cells to T-cell mitogens, to allogeneic cells and to a T-dependent antigen in a secondary *in vitro* antibody assay. The 9AE10 MAb and complement lysed 35% to 55% and 45% to 65% of spleen and MLN cells, respectively; 70% to 80% of the cells remaining after this treatment were identified as $Ig^+$ cells by membrane immunofluorescence. The identity of the remaining 20% to 30% of the cells is not known; they could be cells with a low density of surface Ig or null cells which bear neither surface Ig nor the 9AE10 T cell antigen.

The spleen and MLN cells which survived treatment with 9AE10 MAb and complement responded poorly to the mitogens Con-A and PHA, but did respond well to anti-Ig stimulation. The response of the treated cells to Con-A and PHA was 1% of the response of either untreated cells or the $Ig^-$ cell population. Several investigators have reported that rabbit spleen cells which survived cytotoxic treatment with heterologous anti-rabbit thymocyte antiserum and complement (B cells) responded to anti-Ig stimulation but not to stimulation by Con-A or PHA; in contrast, nylon wool passed spleen cells, or the $Ig^-$ spleen cells, did not respond to anti-Ig but did respond to Con-A and PHA stimulation (Sheppard *et al.*, 1976; Teodorescu *et al.*, 1976; Ozer & Waksman, 1974; Shek *et al.*, 1974). These results indicate that Con-A and PHA are specific T cell mitogens in the rabbit as they are in other species (mouse, rat, human; Stobo & Paul, 1973; Folch & Waksman, 1972; Nowell, 1960). Since treatment of rabbit spleen and/or MLN cells with 9AE10 MAb and complement-abrogated responsiveness to Con-A and PHA, the 9AE10 MAb must identify a T-cell population responsive to Con-A and PHA.

Reproducible responses in the mixed lymphocyte reaction have been difficult to obtain with rabbit peripheral blood lymphocytes (PBL). Milthrop & Richter have, however, shown that reproducible results with stimulation indices of 2 to 75 can be obtained with PBL and spleen, if spleen, MLN or Peyer's patch cells are used as stimulators (Milthrop & Richter, 1979). In our studies, we have used spleen and MLN cells as both stimulator and responder cells, and have consis-

tently obtained stimulation indices of 20 to 200. These high stimulation indices may be due to the incorporation of 2-ME in the culture media. With these culture conditions, 86% to 97% of the one-way MLC response was eliminated by treatment of spleen or MLN cells with 9AE10 MAb and complement. These data are compatible with earlier findings that in mouse, human and rabbit, the responder cell in mixed lymphocyte reactions is a T cell (Tynan & Ness, 1972; Plate & McKenzie, 1973; Bach & Segall, 1972).

The effects of 9AE10 MAb and complement treatment on antibody responsiveness of spleen cells to the T-dependent antigen, SRBC, confirmed the T-cell specificity of this monoclonal antibody. The splenic plaque forming cells themselves were not sensitive to lysis by 9AE10 MAb and complement, but some cells necessary for the secondary *in vitro* antibody response were removed by this treatment. In four out of five experiments, treatment with 9AE10 MAb and complement reduced the number of direct and indirect PFC's in the secondary *in vitro* antibody response by at least 90%. Because there were few, if any, PFC's in MLN cells from antigen-primed rabbits, the effect of lysis by 9AE10 MAb and complement on these cells could not be determined. The low number of PFC's in MLN may be due to route of immunization since injection of SRBC directly into appendix or Peyer's patches results in significant numbers of PFC's in MLN (W. Carey Hanly, personal communication).

Thus, the 9AE10 MAb identifies functional T lymphocytes of the rabbit, as characterized by *in vitro* assays of lymphocyte responsiveness to mitogen stimulation, to allogenic stimulation in the MLC and to antigenic stimulation by SRBC. It will be important to isolate specifically, the $9AE10^+$ cell population and to directly examine their funtional capabilities in various *in vitro* assays. It will be of particular interest to separate $9AE10^+$ and $Ig^+$ peripheral blood lymphocytes and to compare their mitogenic responses. Although we have shown that $9AE10^+$ and $Ig^+$ cells are distinct and non-overlapping populations by surface markers, it is possible that these cells have overlapping functional properties as suggested by Sell & Sheppard, 1973. This well defined, monospecific antibody will be useful in such investigations of the rabbit cellular immune system and is available for distribution to other investigators.

## ACKNOWLEDGMENT

This research was supported by PHS grant A1 11234 and A1 16611 from the National Institutes of Health.

## REFERENCES

BACH F.H. & SEGALL M. (1972) The genetics of the mixed lymphocyte culture response–a re-examination. *Transplantn. Proc.* **4,** 205.

FANGER M.W., PELLEY R.P. & REESE A.L. (1972) *In vitro* demonstration of two antigenically-distinct rabbit lymphocyte populations. *J. Immunol.* **109,** 294.

FAUCI A.S. & PRATT K.R. (1976) Activation of human B lymphocytes. I. Direct plaque-forming cell assay for the measurement of polyclonal activation and antigenic stimulation of human B lymphocytes. *J. exp. Med.* **144,** 674.

FRADELIZI D.P., CHOU C.T., CINADER B. & DUBISKI S. (1973) A membrane antigen of rabbit thymus cells. *Cell. Immunol.* **7,** 484.

FOLCH H. & WAKSMAN B.H. (1972) *In vitro* response of rat lymphocytes following adult thymectomy. *J. Immunol.* **109,** 1046.

LANCKI D.W., TISSOT R.G. & COHEN C. (1979) Histocompatibility in the rabbit. Genetic control of rabbit mixed leukocyte culture reactivity. *Transplantation,* **27,** 79.

MCNICHOLAS J.M., RAFFELD M., LOKEN M.R., REITER H. & KNIGHT K.L. (1981) Monoclonal antibodies to rabbit lymphoid cells. *Mol. Immunol.* (In press.)

MAGE M., MCHUGH L.L. & ROTHSTEIN T.L. (1977) Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. immunol. Meth.* **15,** 47.

METZGER D.W., HENDRICKS R.L., TEODORESCU M. & DRAY S. (1977) Primary *in vitro* antibody response of rabbit lymphoid cells and T–B cell collaboration in the absence of detectable mitogens. *Cell. Immunol.* **32,** 23.

MILTHORP P. & RICHTER M. (1979) The cells involved in cell-mediated and transplantation immunity in the normal outbred rabbits. X. The organ sources of the stimulator and responder cells in the mixed leukocyte culture reaction. *Immunology,* **36,** 13.

MISHELL B.B. & SHIIGI S.M. (1980) *Selected Methods in Cellular Immunology,* p. 27. G. H. Freeman and Co. San Francisco.

NOWELL P.C. (1960) Phytohemagglutinin: an initiator of mitosis in cultures of human leukocytes. *Cancer Research,* **20,** 462.

OZER H. & WAKSMAN B.H. (1972) Appendix and γM antibody formation. V. Appendix and thymus cell synergism in the direct and indirect plaque forming cell responses to sheep erythrocytes in the rabbit. *J. Immunol.* **109,** 410.

OZER H. & WAKSMAN B.H. (1974) The response of rabbit lymphocytes to mitogens and alloantigens; evidence for T cell heterogeneity. *J. Immunol.* **113,** 1780.

PLATE J.M.D. & MCKENZIE I.F.C. (1973) B-cell stimulation of allogeneic T-cell proliferation in mixed lymphocyte cultures. *Nature (Lond.),* **245,** 247.

REDELMAN D., SCOTT C.B., SHEPPARD H.W. & SELL S. (1976) *In vitro* studies of the rabbit immune system: II. Functional characterization or rabbit T and B populations separated by adherence to nylon wool or lysis with antithymocyte serum and complement. *Cell. Immunol.* **24,** 11.

SELL S. & SHEPPARD H.W. (1973) Rabbit blood lymphocytes may be T cells with surface immunoglobulin. *Science,* **182,** 586.

SHEK P.N., CHOU C.-T., DUBISKI S. & CINADER B. (1974) Mitogen stimulation of rabbit spleen cells before and after complement-mediated cell kill with an antiserum directed against the thymus antigen RTLA. *Int. Arch. Allergy*, **46**, 753.

SHEK P.N. & COONS A.H. (1978) Effect of Colchicine on the antibody response: enhancement of antibody formation in mice. *J. exp. Med.* **147**, 1213.

SHEPPARD H.W., REDELMAN D. & SELL S. (1976) *In vitro* studies of the rabbit immune system. IV. Differential mitogen responses of isolated T and B cells. *Cell. Immunol.* **24**, 34.

SHEPPARD H.W., SELL S., POLER S.M. & REDELMAN D.D. (1977) Mixed lymphocyte reactions in the rabbits using peripheral blood cells: the effects of cell preparation and skin grafting. *J. immunol. Meth.* **16**, 185.

STAVITSKY A.B. & COOK R.G. (1974) *In vitro* anamnestic response of rabbit lymph node cells: evidence for cell collaboration in induction and regulation. *J. Immunol.* **112**, 583.

STOBO J.D. & PAUL W.E. (1973) Functional homogeneity of murine lymphoid cells. III. Differential responsiveness of "T" cells to phytohemagglutinin and concanavalin A as a probe for T cell subsets. *J. Immunol.* **110**, 362.

TEODORESCU M., MAYER E.P., REITER H. & DRAY S. (1976) Rabbit lymphocyte subpopulations. I. Separation of $Ig^+$ and $Ig^-$ cells and their interaction in cultures stimulated by mitogens. *Cell. Immunol.* **22**, 66.

TYNAN M.L. & NESS D.B. (1972) Modification of the mixed leukocyte reaction with various antigens. *Transplantation* **13**, 198.

WILSON A.B., GURNER B.W. & COOMBS R.R.A. (1975) Observations on rabbit thymocytes and peripheral T cells; *Int. Arch. Allergy* **48**, 383.

WILSON B.S., TEODORESCU M. & DRAY S. (1976) Enumeration and isolation of rabbit T and B lymphocytes by using antibody coated erythrocytes. *J. Immunol.* **116**, 1306.

ZIMMERMAN D.H., OKUMURA K., RABKIN C. & KERN M. (1974) Differentiation of lymphoid cells: the use of specific antisera to characterize the cells required for the induction of immunoglobulin M production *in vitro*. *J. Immunol.* **113**, 1891.