Immunology, 1975, **28**, 243.

Lymphoid Cell Dependence of Eosinophil Response to Antigen

VI. THE EFFECT OF SELECTIVE REMOVAL OF T OR B LYMPHOCYTES ON THE CAPACITY OF PRIMED SPLEEN CELLS TO ADOPTIVELY TRANSFERRED IMMUNITY TO TETANUS TOXOID

N. M. PONZIO* AND R. S. SPEIRS

Department of Anatomy, State University of New York, Downstate Medical Center, Brooklyn, New York, U.S.A.

(*Received* 11*th March* 1974; *accepted for publication* 26*th June* 1974)

Summary. Spleens from mice primed with tetanus toxoid 30 days earlier contain memory cells capable of adoptively transferring secondary type cell-mediated (eosinophil) and humoral (antitoxin) responses to irradiated, reconstituted recipients. Spleen cells derived from 10-day-primed donors, on the other hand, possess the capacity after transfer to elicit secondary type eosinophil responses, but not anamnestic antitoxin responses. Treatment of 30-day-primed cells with anti-θ serum and C′ prevented transfer of memory for both responses, whereas similar treatment with rabbit anti-mouse IgG (RAM–IgG) serum and C′ only inhibited transfer of memory for the antitoxin response. Addition of non-primed spleen cells to antisera-treated primed cells failed to restore secondary type responses. Recombination of 30-day-primed anti-θ and RAM-IgG-treated cells re-established the capacity to transfer these responses. To determine whether the same T cells which mediate the eosinophil response also act as helper cells in antitoxin production, antisera treated 10- and 30-day-primed cells were combined prior to transfer. Ten-day-primed T cells induced eosinophil responses and also co-operated with 30-day-primed B cells to produce antitoxin. In contrast, 30-day-primed T cells elicited eosinophil responses, but were unable to induce antitoxin production when combined with anti-θ-treated 10-day-primed cells. These results indicate that B memory cells are not present in the spleens of the donor mice 10 days after priming, but T memory cells are present.

It is concluded that primed T cells mediated both eosinophil and antitoxin responses, while B memory cells are involved only with antitoxin production. Following subcutaneous priming T memory cells are present in the spleen prior to B memory cells, and T memory cells which mediate the eosinophil response at 10 days after priming also augment the production of antitoxin by B memory cells.

* Present address: Department of Pathology, New York University Medical Center, New York, New York 10016, U.S.A.

Correspondence: Dr R. S. Speirs, Department of Anatomy, State University of New York, Downstate Medical Center, Brooklyn, New York 11203, U.S.A.

INTRODUCTION

The selective removal of T or B lymphocytes from a mixed population of spleen cells can be effected by treatment with anti-θ or anti-immunoglobulin sera plus complement (Raff, 1971). Utilization of these procedures provides a useful tool for investigation of the participation of T and B lymphocytes in immune reactions. It has been demonstrated that inactivation of T lymphocytes in mixed populations of T and B cells by treatment with anti-θ serum and C′ inhibits cell-mediated type reactions (Cerottini, Nordin and Brunner, 1970; Cantor, 1972; Visher, 1972), as well as those humoral responses which are thymic-dependent (Schimpl and Wecker, 1970; Takahashi, Carswell and Thorbecke, 1970). The participation of B lymphocytes in immune reactions can be studied by treating mixed populations of cells with anti-immunoglobulin serum and C′. Such treatment has been shown to inhibit humoral responses to both thymic-dependent and thymic-independent antigens (Takahashi, Mond, Carswell and Thorbecke, 1971; Visher, 1972; Visher and Jaquet, 1972; Herscowitz, 1973).

Previous experiments (Speirs and Wenck, 1955; Speirs, Speirs and Jansen, 1961) have shown that specific challenge of tetanus toxoid-primed mice elicits both a secondary type cell-mediated response, characterized by an accumulation of eosinophils at the injection site, and a secondary humoral response, as indicated by high antitoxin levels. It was also demonstrated that spleen cells derived from primed mice retained the capacity to elicit these responses after adoptive transfer to lethally irradiated recipients reconstituted with bone marrow or foetal liver (McGarry, Speirs, Jenkins and Trentin, 1971). The appearance in the spleen of memory cells with the capacity to adoptively transfer these responses to irradiated mice was correlated with the period of time after initial immunization (Ponzio and Speirs, 1973). Spleen cells taken 10 days after priming transferred only the capacity to mediate a secondary eosinophil response on challenge, whereas spleen cells taken 30 days after priming contained memory cells capable of eliciting both eosinophil and antitoxin responses.

The following experiments were designed to further characterize these memory cells on the basis of cell surface markers, to examine their participation in adoptive secondary responses through selective removal with anti-θ or anti-immunoglobulin sera, and to further investigate in 10-day-primed spleen, the apparent deficiency of memory cells mediating antitoxin production.

MATERIALS AND METHODS

The experimental design, which is similar to that utilized in previous experiments (McGarry, Speirs, Jenkins and Trentin, 1971; Ponzio and Speirs, 1973), involves adoptive transfer of spleen cells into isogeneic, irradiated, reconstituted recipients.

Donors

Adult BAF (C57Bl × A/J) female donor mice were primed with a single subcutaneous injection of 0·2 ml of aluminium phosphate-adsorbed tetanus toxoid (10 Lf/ml) (National Drug Company, Philadelphia, Pennsylvania) in an adjuvant of 0·2 ml of *Pertussis* vaccine (10^9 organisms/ml) (Eli Lilly Drug Company, Indianapolis, Indiana), diluted 1 : 10 with saline (PVTT). At either 10 or 30 days after priming, donors were killed and spleen cell suspensions prepared. The tissues were placed on stainless steel grids, gently teased with

a needle and the cells were washed from the stroma with a jet of cold Hanks's balanced salt solution (HBSS). The cell suspensions were counted with a Coulter counter model B (Coulter Electronics, Hialeah, Florida) and adjusted to the desired concentration.

Recipients

Recipient BA F_1 mice were exposed to 900 rads whole body X-irradiation with a Maxitron 250 KVP unit (General Electric, Waterford, New York). The exposure rate was 32 rads/minute with a 0·25 mm copper and 1·0 mm aluminium filter at a distance of 77 cm. The lymphoid cells were reconstituted by an intravenous (i.v.) injection of 10^7 foetal liver cells suspended in 0·5 ml of HBSS.

Antisera treatment of spleen cells

Anti-θ serum was prepared according to the procedure described by Reif and Allen (1966). AKR mice received weekly intraperitoneal (i.p.) injections of 10^7 CBA thymus cells. After the 5th week, animals were bled from the retro-orbital plexus, the antiserum pooled and heat-inactivated at 56° for 45 minutes. Trypan blue dye exclusion cytotoxicity tests were carried out utilizing anti-θ serum and agarose-absorbed guinea-pig serum (GPS) (Cohen and Schlesinger, 1970) as a source of C′ against BA F_1 thymus cells. Greater than 90 per cent cell lysis was demonstrated in antibody dilutions up to 1:8. Rabbit anti-mouse IgG (RAM–IgG) was purchased from Miles Laboratories (Kankakee, Illinois). Similar cytotoxicity testing revealed its capacity to lyse greater than 90 per cent of spleen cells derived from thymectomized, lethally irradiated, foetal liver reconstituted mice (presumably B lymphocytes) in dilutions up to 1:4.

Primed spleen cells (8×10^7) were incubated in polyethylene test tubes (Falcon Plastics, Oxnard, California) with 1·0 ml of either anti-θ or RAM–IgG sera at 37° for 30 minutes with intermittent shaking. At the end of the incubation period, tubes were centrifuged at 750 rev/minute for 10 minutes, the supernatant fluid discarded, and the cells resuspended in 1·0 ml of agarose-absorbed GPS. The tubes were incubated for an additional 30 minutes at 37° with intermittent shaking. Control tubes, treated in a manner similar to experimental tubes included: spleen cells plus normal GPS (complement present); spleen cells plus complement-inactivated GPS (56° for 45 minutes); and spleen cells plus normal rabbit and guinea-pig sera. Since these controls showed no significant differences, they were combined. At the end of the second incubation period, the content of each tube was diluted to 8·0 ml with HBSS and 1·0 ml was injected into each of eight irradiated recipients. Two hours later, these mice were challenged intraperitoneally with 0·4 ml of aluminium phosphate-adsorbed tetanus toxoid. Some groups of mice received combinations of anti-θ-treated cells and RAM–IgG-treated cells and were similarly challenged. Other groups of recipients received 10^7 normal spleen cells (in 1·0 ml) intraperitoneally in addition to the antiserum-treated cells, as an exogenous source of unprimed T and B lymphocytes.

Killing

The mice were bled from the retro-orbital plexus and antitoxin levels determined by a modification of the bioassay method of Ipsen (1953). This assay method measures the capacity of immune serum to neutralize tetanus toxin, which is known to be a function of the IgG class of antibody (Piazzi, 1970). One unit of antitoxin is defined as the amount of antibody capable of neutralizing a minimal lethal dose of tetanus toxin. Earlier work

(Ponzio and Speirs, 1973) had shown that optimal eosinophil and antitoxin responses were obtained 18 days after transfer and challenge. The mice were then killed, smears made of the peritoneal exudate for May–Grünwald–Giemsa staining and differential counting, and the remaining cells in the peritoneal cavity were washed into collecting vials with 25 ml of cold saline diluent. Total cell counts were performed and absolute numbers of eosinophils estimated by multiplying the percentage obtained from the differential counts by the total number of cells present.

RESULTS

In the first experiments, spleen cell suspensions prepared from mice primed 10 and 30 days earlier were treated *in vitro* with anti-θ serum and C′ and transferred to irradiated recipients. These mice were challenged intraperitoneally with tetanus toxoid and 18 days later, eosinophil and antitoxin levels determined. The results are presented in Table 1.

TABLE 1

CAPACITY OF ANTI-θ-TREATED 10- AND 30-DAY-PRIMED SPLEEN CELLS TO ADOPTIVELY TRANSFER SECONDARY TYPE EOSINOPHIL AND ANTITOXIN RESPONSES

Group	Cell donor treatment	Number of recipients	Eosinophils (per cent)	Eosinophils ($\times 10^6$) ($\pm$s.e.)	Antitoxin units ($\times 10^3$)
1	Non-primed cells*	11	3·7	0·28 ± 0·17	< 0·3
2	10-day-primed cells †	7	28·3	4·32 ± 1·06	< 0·3
3	10-day-primed cells + anti-θ‡ + non-primed cells*	7	22·9	1·89 ± 0·63	< 0·3
4	30-day-primed cells*	22	21·8	2·45 ± 0·36	18·4
5	30-day-primed cells + anti-θ‡	16	6·2	0·28 ± 0·07	1·8
6	30-day-primed cells + anti-θ‡ + non-primed cells*	16	12·3	0·88 ± 0·22	6·8

* Non-primed cells were derived from the spleens of normal mice of the same age as primed donors.
† Controls include primed cells treated either with normal rabbit serum, normal guinea-pig serum, or heat-inactivated guinea-pig serum.
‡ Complement was added following antiserum treatment.

Recipients of non-primed cells (group 1) produced neither an eosinophil nor an antitoxin response. Ten-day-primed spleen cells not treated with anti-θ serum (group 2) contained memory cells capable of eliciting a secondary eosinophil response, but not anamnestic antitoxin production. Anti-θ serum treatment of 10-day-primed spleen cells (group 3) reduced the total number of eosinophils. This was a result of a great reduction in the total number of inflammatory cells present, but the percentage of eosinophils was only slightly reduced. No measurable antitoxin was produced in irradiated recipients of 10-day-primed cells (groups 2 and 3).

In contrast to 10-day-primed cells (group 2), 30-day-primed cells (group 4) were capable of eliciting both secondary type eosinophil and antitoxin responses after adoptive transfer and challenge. It was shown that treatment of 30-day-primed cells with anti-θ serum and C′ (group 5) inhibits both the secondary eosinophil and antitoxin responses. The addition of non-primed spleen cells to anti-θ-treated 30-day-primed cells (group 6) resulted in somewhat higher responses than group 5, but did not approach levels of untreated primed cells (group 4).

In the next experiment, RAM–IgG treated 10- and 30-day-primed spleen cells were

adoptively transferred to irradiated mice. These recipients were challenged and eosinophil and antitoxin levels determined 18 days later (Table 2).

Recipients of RAM–IgG-treated 10-day-primed cells plus non-primed cells (group 7) had fewer eosinophils, but the percentage was unchanged when compared with untreated primed cells (group 2). No measurable antitoxin was produced in these mice. In contrast, the cells remaining after removal of B lymphocytes from a 30-day-primed spleen cell population with RAM–IgG and C′ (group 8) were capable of transferring the capacity for a secondary eosinophil response. The addition of non-primed spleen cells to these RAM–IgG treated cells (group 9) resulted in somewhat lower eosinophil levels, but no detectable antitoxin production.

TABLE 2

CAPACITY OF RAM-IgG-TREATED 10- AND 30-DAY-PRIMED SPLEEN CELLS TO ADOPTIVELY TRANSFER SECONDARY TYPE EOSINOPHIL AND ANTITOXIN RESPONSES

Group	Cell donor treatment	Number of recipients	Eosinophils (per cent)	Eosinophils ($\times 10^6$) ($\pm$s.e.)	Antitoxin units ($\times 10^3$)
7	10-day-primed cells + RAM-IgG† + non-primed cells*	7	24·2	1·30 ± 0·31	< 0·3
8	30-day-primed cells + RAM-IgG†	11	17·7	2·52 ± 0·85	< 0·3
9	30-day-primed cells + RAM-IgG† + non-primed cells*	12	15·1	1·18 ± 0·23	< 0·3

* Non-primed cells were derived from the spleens of normal mice of the same age as primed donors.
† Complement was added following antiserum treatment.

TABLE 3

CAPACITY OF COMBINATIONS OF ANTISERA-TREATED 10- AND 30-DAY-PRIMED SPLEEN CELLS TO ADOPTIVELY TRANSFER SECONDARY TYPE EOSINOPHIL AND ANTITOXIN RESPONSES

Group	Cell donor treatment*	Number of recipients	Eosinophils (per cent)	Eosinophils ($\times 10^6$) ($\pm$s.e.)	Antitoxin units ($\times 10^3$)
10	30-day-primed cells anti-θ-treated (B) + 30-day-primed cells RAM-IgG-treated (T)	5	21·7	2·08 ± 0·40	10·8
11	30-day-primed cells, anti-θ-treated (B) + 10-day-primed cells, RAM-IgG-treated (T)	8	23·2	1·90 ± 0·30	9·2
12	10-day-primed cells, anti-θ-treated (B) + 30-day-primed cells, RAM-IgG-treated (T)	7	22·5	2·59 ± 0·61	< 0·3

* 10^7 non-primed spleen cells were added to the primed cells following antisera treatment. Parentheses show the cell type remaining after antisera treatment.

Since the data in Table 1 indicate that transfer of both the secondary eosinophil and antitoxin responses is dependent on memory cells possessing θ antigen (T cells), experiments were designed to determine whether T memory cells that mediate the eosinophil response also co-operate with B memory cells to produce antitoxin. Spleen cells from donors primed 10 and 30 days earlier were treated *in vitro* with antisera and C′. Prior to transfer, various combinations of 10- and 30-day-primed anti-θ and RAM–IgG-treated cells were prepared. The irradiated recipients were challenged, killed 18 days later, and eosinophil and antitoxin levels were measured. The results of these experiments are presented in Table 3.

In group 10, separate aliquots of 30-day-primed spleen cells were treated with antisera

and C′, and prior to transfer anti-θ-treated cells were recombined with RAM–IgG treated cells. It was noted that such recombination restored the capacity for transfer of both eosinophil and antitoxin responses.

In similar recombination experiments, 10-day-primed T cells could co-operate with 30-day-primed B cells to induce both high eosinophil levels and high antitoxin titres upon challenge (group 11). On the other hand, in group 12, 30-day-primed T cells were unable to induce antitoxin production in combination with 10-day-primed spleen cells treated with anti-θ serum. Thus, it appears that at 10 days after priming, T cells capable of inducing an eosinophil response are present, but there is an absence of primed B lymphocytes necessary to induce secondary type antitoxin titres.

DISCUSSION

These experiments give further information on the characteristics of memory cells involved in the secondary type eosinophil and antitoxin responses to tetanus toxoid. Spleen cells derived from mice primed 10 days earlier (group 2) induced a secondary eosinophil response after adoptive transfer, but lacked the capacity to elicit a secondary antitoxin response. In contrast, 30-day-primed spleen cells (group 4) contained memory cells capable of eliciting both eosinophil and antitoxin responses after adoptive transfer.

Selective removal of T lymphocytes from 10-day-primed cells by the use of anti-θ serum and C′ (group 3) was found to remove the capacity to elicit a secondary type eosinophil response. T cell-depleted 30-day-primed spleen cells (group 5) likewise were unable to transfer an eosinophil response and, in addition, lacked the capacity to induce secondary antitoxin production. Previous reports (Basten, Boyer and Beeson, 1970; Cohn, Athanassiades and Speirs, 1971; Walls, Basten, Leuchars and Davies, 1971; Speirs, Gallagher, Rauchwerger, Heim and Trentin, 1973) have suggested that thymus-derived cells are involved in induction of memory cells capable of eliciting eosinophil responses, since neonatal thymectomy, anti-lymphocyte serum and thoracic duct drainage reduce the capacity of animals to have an eosinophil response on challenge. Furthermore, adoptive transfer of primed spleen cells derived from donors previously thymectomized, lethally irradiated and reconstituted, failed to induce secondary eosinophil or antitoxin responses in irradiated mice (McGarry, Speirs, Jenkins and Trentin, 1971). Pretreatment of thymectomized and irradiated mice with thymus cells prior to priming restored the capacity of their spleen cells to transfer these responses (Ponzio and Speirs, 1973).

The data concerning treatment of 30-day-primed spleen cells with RAM–IgG (group 8) shows that selective removal of B lymphocytes abolishes the capacity for transfer of secondary humoral antitoxin production, but does not impair the capacity of surviving T lymphocytes to transfer a secondary eosinophil response. This is in agreement with reports (Chapuis and Brunner, 1971; Mond, Takahashi and Thorbecke, 1972) indicating that anti-Ig does not inhibit T-cell function in the secondary response.

The bioassay method utilized in these studies measures primarily toxin-neutralizing antibodies (IgG). It is possible that other classes of antibodies, not capable of neutralizing toxin, may be present. In this regard, the IgE class of immunoglobulins has been implicated in generating factors chemotactic for eosinophils (Parish, 1970, 1972; Kay and Austen, 1974). In addition, co-operative interactions between adoptively transferred T and B lymphocytes have been reported in the development of an anamnestic IgE response to DNP (Hamaoka, Katz and Benacerraf, 1973).

Treatment of 30-day-primed cells with RAM–IgG and C′ would remove B memory cells bearing IgG on their surface. However, since the anti-IgG utilized in these experiments is known to react with both heavy and light chains, it is expected that cells bearing light chains of other immunoglobulin classes would be similarly affected. This makes it unlikely that the eosinophil responses reported here are due solely to IgE antibodies. This is also indicated in other experiments, reported elsewhere (Ponzio and Speirs, 1974a), in which separation of eosinophil and antibody responses was obtained on the basis of the differential sensitivity of T and B memory cell to *in vitro* X-irradiation, the B cells being more susceptible than T cells.

Priming with tetanus toxoid induces formation of both T and B memory cells. B memory cells appear to be involved only in transfer of the secondary humoral response, while T memory cells are involved in both the secondary type eosinophil and anti-toxin responses.

The recombinations of antisera-treated primed cells substantiate and extend these findings. Neither the 30-day-primed cells treated with anti-θ (group 5) nor the 30-day-primed cells treated with RAM–IgG (group 8) transfer the capacity to have secondary antitoxin responses to challenge. The addition of non-primed spleen cells as an exogenous source of T and B cells (groups 6 and 9) does not restore this capacity. Restoration of the capacity for the secondary antitoxin response in irradiated mice can, however, be effected by a combination of primed T and primed B cells (group 10). Thus, secondary type antitoxin production must involve both primed T and primed B lymphocytes. A similar carriage of immunological memory for humoral responses has been demonstrated by other investigators (Miller and Sprent, 1971) in both T- and B-cell populations.

Recent reports (Jimenez, Bloom, Blume and Oettgen, 1971; Kettman, 1972) have suggested that the same T lymphocytes involved in cell-mediated reactions also act as helper cells in humoral responses. Spleen cells taken 10 days after priming transfer memory for an eosinophil response, but are not capable of transferring secondary humoral responsiveness to irradiated recipients. It appears that the deficit does not involve insufficient numbers or undifferentiated B memory cells, since increasing the number of cells transferred or delaying challenge after transfer did not elicit measurable antitoxin (Ponzio and Speirs, 1974b).

These experiments confirm previous studies indicating at least two separate populations of memory cells (Ponzio and Speirs, 1973). In addition, differences in populations of memory cells were noted in the spleens of mice at 10 and 30 days after priming. Memory cells derived from 10-day-primed spleens (group 2) possess a greater eosinophil-inducing capacity than 30-day-primed memory cells (group 4). This reflects the absence of the B memory cells associated with antitoxin production in the spleen at 10 days. We have observed (Ponzio and Speirs, 1973) that with increasing time after immunization, as the capacity to adoptively transfer antitoxin responses increases, the ability to transfer eosinophil responses diminishes somewhat. Furthermore, it has also been demonstrated (Speirs and Turner, 1969) that passively administered antitoxin will suppress the eosinophil response to tetanus toxoid. The decreased eosinophil response may therefore reflect in large measure the higher antitoxin levels induced by B memory cells.

In addition, 10-day-primed cells appear to be less susceptible to the cytotoxic effects of anti-θ serum than 30-day-primed cells. This may be due to a greater proportion of T memory cells in the spleen soon after priming, or alternatively, a greater resistance of

these memory cells to anti-θ serum. Such a resistance of 'early memory' cells to sheep red cells has been demonstrated by Byfield and Salerno (1973).

Addition of non-primed spleen cells to anti-θ-treated primed cells partially restored secondary type eosinophil and antitoxin responses. The increase observed in levels of eosinophils and antitoxin (group 6) may be due to several factors. Some T memory cells may have been better able to survive anti-θ treatment by the addition of non-primed cells. Alternatively, the B memory cells remaining after anti-θ treatment may carry some component of memory, which could conceivably be activated by the presence of normal T-cells.

Addition of non-primed spleen cells to T memory cells resulted in a slightly reduced eosinophil response (group 3), but did not contribute to the capacity of the cells to transfer secondary antitoxin production. The decrease in the eosinophil response when increased numbers of cells were transferred has been noted before (Ponzio and Speirs, 1974b). An increase in the number of cells transferred would increase the number of macrophages and neutrophils capable of ingesting and catabolizing the antigen. It therefore seems likely that this would decrease the amount of antigen available to stimulate T memory cells.

We interpret these data to indicate that following a single s.c. injection of tetanus toxoid: (1) T and B memory cells are produced; (2) T memory cells are formed and distributed earlier than B-memory cells, since B-memory cells are not present in the spleen at 10 days after priming, but are present at 30 days after priming; (3) T memory cells present at 10 days which are capable of mediating the eosinophil response can also co-operate with B memory cells present at 30 days, resulting in secondary antitoxin production. The cell transfer system described provides a convenient model for demonstrating and manipulating anamnestic cell-mediated and humoral responses to a single antigen in the same animal.

ACKNOWLEDGMENTS

The authors wish to thank Mr Raman Patel for technical assistance and Mrs E. E. Speirs for aid in the preparation of the manuscript.

This research was supported by United States Public Health Service Grant number 5 TO1 GM 00379–12.

REFERENCES

Basten, A., Boyer, M. H. and Beeson, P. B. (1970). 'Mechanism of eosinophilia. I. Factors affecting the eosinophil response of rats to *Trichinella spiralis*.' *J. exp. Med.*, **131**, 1271.

Byfield, P. and Salerno, A. (1973). 'Relative resistance of early memory cells in mice primed with sheep erythrocytes to cytotoxic effects of anti-θ serum.' *Cell. Immunol.*, **7**, 328.

Cantor, H. (1972). 'The effects of anti-theta antiserum upon graft-versus-host activity of spleen and lymph node cells.' *Cell. Immunol.*, **3**, 461.

Cerottini, J. C., Nordin, A. A. and Brunner, K. T. (1970). 'Specific *in vitro* cytotoxicity of thymus derived lymphocytes sensitized to alloantigens.' *Nature: New Biology*, **228**, 1308.

Chapuis, B. and Brunner, K. T. (1971). 'Cell-mediated reactions *in vitro*. Reactivity of lymphocytes from animals sensitized to chicken erythrocytes, tuberculin or transplantation antigens.' *Int. Arch. Allergy*, **40**, 321.

Cohen, A. and Schlesinger, M. (1970). 'Absorption of guinea pig serum with agar. A method for elimination of its cytotoxicity for murine thymus cells. *Transplantation*, **10**, 130.

Cohn, D. A., Athanassiades, T. J. and Speirs, R. S. (1971). 'Inhibition of eosinophil and mononuclear responses to antigen following neonatal thymectomy.' *Fed. Proc.*, **30**, 510 (abstract).

Hamaoka, T., Katz, D. H. and Benacerraf, B. (1973). 'Hapten-specific IgE antibody responses in mice. II. Cooperative interactions between adoptively transferred T and B lymphocytes in the

development of IgE responses.' *J. exp. Med.*, **138,** 538.

HERSCOWITZ, H. B. (1973). 'Suppression of the *in vitro* anamnestic response of rabbit lymph node cells by anti-IgG serum.' *J. Immunol.*, **110,** 444.

IPSEN, J. (1953). 'Bioassay for four tetanus toxoids (aluminum precipitated) in mice, guinea-pigs and humans.' *J. Immunol.*, **70,** 426.

JIMENEZ, L., BLOOM, B. R., BLUME, M. R. and OETTGEN, H. F. (1971). 'On the number and nature of antigen sensitive lymphocytes in the blood of delayed-hypersensitive human donors.' *J. exp. Med.*, **133,** 740.

KAY, A. B. and AUSTEN, K. F. (1971). 'The IgE-mediated release of an eosinophil leukocyte chemotactic factor from human lung.' *J. Immunol.*, **107,** 899.

KETTMAN, J. (1972). 'Delayed hypersensitivity: is the same population of thymus derived cells responsible for cellular immunity reactions and the carrier effect?' *Immunol. Commun.*, **1,** 289.

MCGARRY, M. P., SPEIRS, R. S., JENKINS, V. K. and TRENTIN, J. J. (1971). 'Lymphoid cell dependence of eosinophil response to antigen.' *J. exp. Med.*, **134,** 801.

MILLER, J. F. A. P. and SPRENT, L. (1971). 'Cell to cell interactions in the immune response. VI. Contribution of thymus derived cells and antibody-forming cell precursors to immunologic memory.' *J. exp. Med.*, **134,** 66.

MOND, J. J., TAKAHASHI, T. and THORBECKE, G. J. (1972). 'Surface antigens on immunocompetent cells. III. *In vitro* studies of the role of B and T cells in immunological memory.' *J. exp. Med.*, **136,** 663.

PARISH, W. E. (1970). 'Investigations on eosinophilia. The influence of histamine, antigen–antibody complexes containing γ^1 or γ^2 globulins, foreign bodies (phagocytosis) and disrupted mast cells.' *Brit. J. Derm.*, **82,** 42.

PARISH, W. E. (1972). 'Eosinophilia. I. Eosinophilia in guinea-pigs mediated by passive anaphylaxis and by antigen–antibody complexes containing homologous IgG1a and IgG1b.' *Immunology*, **22,** 1087.

PIAZZI, S. E. (1970). 'Metodo di preparazione ed analisi delle gamma-globuline antitetaniche purificate dal siero di bovini iperimmunizate.' *Progr. Immunobiol. Standardization*, **4,** 114.

PONZIO, N. M. and SPEIRS, R. S. (1973). 'Lymphoid cell dependence of eosinophil response to antigen. III. Comparison of the rate of appearance of two types of memory cells in various lymphoid tissues at different times after priming.' *J. Immunol.*, **110,** 1363.

PONZIO, N. M. and SPEIRS, R. S. (1974a). 'Lymphoid cell dependence of eosinophil response to antigen. IV. Effects of *in vitro* X-irradiation on the adoptive transfer of anamnestic cellular and humoral responses to tetanus toxoid. *Proc. Soc. exp. Biol. (N.Y.)*, **145,** 1178.

PONZIO, N. M. and SPEIRS, R. S. (1974b). 'Lymphoid cell dependence of eosinophil response to antigen. V. Evidence for the induction of separate memory cells mediating cellular and humoral responses to tetanus toxoid.' *Z. Immun.-Forsch.*, **146,** 405.

RAFF, M. C. (1971). 'T and B lymphocytes in mice studied by using antisera against surface antigenic markers.' *Amer. J. Path.*, **65,** 467.

REIF, A. E. and ALLEN, J. N. (1966). 'Mouse thymic iso-antigens.' *Nature (Lond.)*, **209,** 521.

SCHIMPL, A. and WECKER, E. (1970). 'Inhibition of the *in vitro* immune response to SRBC by treatment of spleen cell suspensions with anti-theta serum.' *Nature: New Biology*, **226,** 1258.

SPEIRS, R. S., GALLAGHER, M., RAUCHWERGER, J., HEIM, L. and TRENTIN, J. J. (1973). 'Lymphoid cell dependence of eosinophil response to antigen. II. Location of memory cells and their dependence upon thymic influence.' *Exp. Hematol.*, **1,** 150.

SPEIRS, R. S., SPEIRS, E. E. and JANSEN, V. (1961). 'A quantitative approach to the study of inflammatory cells.' *Proc. Soc. exp. Biol. (N.Y.)*, **106,** 248.

SPEIRS, R. S. and TURNER, M. X. (1969). 'The eosinophil response to toxoids and its inhibition by antitoxin.' *Blood*, **34,** 320.

SPEIRS, R. S. and WENCK, U. (1955). 'Eosinophil response to toxoids in actively and passively immunized mice.' *Proc. Soc. exp. Biol. (N.Y.)*, **90,** 571.

TAKAHASHI, T., CARSWELL, E. A. and THORBECKE, G. J. (1970). 'Surface antigens of immunocompetent cells. I. Effects of theta and PC-1 alloantisera on the ability of spleen cells to transfer immune responses.' *J. exp. Med.*, **132,** 1181.

TAKAHASHI, T., MOND, J. J., CARSWELL, E. A. and THORBECKE, G. J. (1971). 'The importace of theta and Ig bearing cells in the immune response to various antigens.' *J. Immunol.*, **107,** 1520.

VISHER, T. (1972). 'Immunoglobulin-like surface molecules and theta antigen during the specific and non-specific stimulation of mouse spleen cells *in vitro*.' *Clin. exp. Immunol.*, **11,** 523.

VISHER, T. and JAQUET, C. (1972). 'Effect of antibodies against immunoglobulins and theta antigen on the specific and non-specific stimulation of mouse spleen cells *in vitro*.' *Immunology*, **22,** 259.

WALLS, R. S., BASTEN, A., LEUCHARS, E. and DAVIES, A. J. S. (1971). 'Mechanisms for eosinophilic and neutrophilic leucocytes.' *Brit. med. J.*, **3,** 5767.