Long-term culture of normal mouse B lymphocytes

(B-cell activation/growth factors/proliferation/B-cell lines)

MAUREEN HOWARD*, STEVEN KESSLER†, THOMAS CHUSED‡, AND WILLIAM E. PAUL*

*Laboratory of Immunology and †Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205; and ‡Departments of Medicine and Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

Communicated by Richard M. Krause, May 21, 1981

ABSTRACT A procedure is described for the preparation of long-term lines of normal mouse B lymphocytes. Surface immunoglobulin-bearing splenic B lymphocytes were purified with the fluorescence-activated cell sorter and then cultured with lipopolysaccharide for 1–4 wk. The cells were then transferred into medium supplemented with a T-hybridoma-derived supernatant containing interleukin 2 (IL 2). Continuous feeding with this supernatant led to the establishment of cell lines that also could be propagated in IL 2-free medium containing interleukin 1 but not in culture medium alone. Cell lines have been propagated in this manner for as long as 10 mo. The cells in these lines have the appearance of small, dense lymphocytes, which all bear surface IgM detectable by immunofluorescence, rosetting, and surface radiolabeling and immunoprecipitation. The cells express Ia and lack Thy 1. These cultured B lymphocytes are unresponsive to lipopolysaccharide but can be activated to become more rapidly dividing, immunoglobulin-secreting cells by exposure to culture supernatants containing both T-cell-replacing factor and IL 2.

B-cell lymphomas, myelomas, and hybridomas have provided invaluable resources for the study of antibody diversity, idiotype, and immunoglobulin gene rearrangement and expression (1–3). The limitation of such tumor lines is that they have relatively little immunocompetence and so do not allow the analysis of many of the cellular and biochemical mechanisms related to B-cell activation, isotype switching, idiotype selection, and immunoregulation. The recent development of long-term cultures of antigen-specific immunocompetent T-cell clones (4–8) has prompted speculation that the same technology might be possible for B lymphocytes. Indeed, the technologies for marked enrichment of antigen-specific B lymphocytes (9), for isolation of individual short-lived B-cell clones (10–13), and for isolation of individual immunocompetent antigen-specific B-cell clones (14) are already well established. The one remaining difficulty in producing long-term cloned lines of antigen-specific immunocompetent B lymphocytes lies in the continuous propagation of these cells. Although it has been possible to propagate individual B-cell clones *in vivo* for periods of up to 10 mo (15, 16), previous attempts to culture B lymphocytes for periods longer than 4 wk have not been successful (17). In this report we describe a procedure for the long-term culture of a population of mouse B lymphocytes that is normal in terms of phenotype and growth factor dependence and immunocompetent in terms of activation to immunoglobulin secretion by supernatants containing T-cell-replacing factor (TRF).

MATERIALS AND METHODS

Mice. Five-week-old BALB/cJ mice were purchased from The Jackson Laboratory and used at age 8–12 wk.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Cell Sorting. Cells were washed, resuspended at 50×10^6 cells per ml, and stained on ice with fluorescein isothiocyanate-conjugated Fab fragments of affinity-purified goat anti-mouse Fab. The cells were sorted sterily with a fluorescence-activated cell sorter (FACS-II, Becton Dickinson, Sunnyvale, CA), and the brightest 80% of stained cells were collected.

Mitogens and Growth Factors. *Escherichia coli* 0111:B4 lipopolysaccharide (LPS) was obtained from Difco and used at a final concentration of 50 $\mu\text{g/ml}$. Concanavalin A (Con A) was purchased from Pharmacia and used at 1 $\mu\text{g/ml}$. Purified phytohemagglutinin (PHA) was purchased from Wellcome Laboratories, Beckenham, England, and used at 2 $\mu\text{g/ml}$.

Interleukin 1 (IL 1)-containing supernatants were obtained from cultures of the macrophage cell line P388D₁. Details of this cell line and its constitutive release of IL 1 are given elsewhere (18). Cell-free supernatants were concentrated ≈ 3 -fold by vacuum dialysis. The presence of IL 1 in each batch of supernatant was verified by its ability to induce proliferation of PHA-stimulated thymocytes as described (18).

Interleukin 2 (IL 2)-containing supernatants were harvested from the T-cell hybridoma FS6-14.13 after Con A stimulation as described (19). The cell-free supernatants were concentrated 2- or 3-fold by vacuum dialysis. The presence of IL 2 in each batch of supernatant was verified by its ability to support growth of an IL 2-dependent, long-term cloned T-cell line provided by D. Cohen, (National Institute of Allergy and Infectious Diseases). As demonstrated (19), these IL 2-containing supernatants were found to lack the plaque-forming cell helper activity (designated TRF) described by Schimpl and Wecker (20).

TRF-containing mouse spleen cell supernatants were prepared by incubating spleen cells in serum-free culture medium containing PHA or Con A for 16–20 hr, as described (21). Other growth factor-containing supernatants, prepared from human peripheral blood lymphocytes, rat spleen cell cultures, and long-term T-cell lines, were supplied by B. Sredni (National Institute of Allergy and Infectious Disease).

Culture Conditions. The culture medium used for both growth factor preparation and long-term growth of B lymphocytes was RPMI-1640 (GIBCO) supplemented with 10% fetal calf serum, (Reheis, Kankakee, IL), penicillin (50 $\mu\text{g/ml}$), streptomycin (50 $\mu\text{g/ml}$), gentamicin (100 $\mu\text{g/ml}$), L-glutamine (200 mM), and 2-mercaptoethanol (0.5 μM). Long-term B-cell lines were grown in a 1:1 mixture of fresh medium and the supernatant from growth factor-producing cells. The culture vessels were flat-bottomed 24-well plates (No. 3524, Costar, Cam-

Abbreviations: Con A, concanavalin A; FACS, fluorescence-activated cell sorter; FI, fluorescein conjugate; IL 1, interleukin 1 (lymphocyte-activating factor); IL 2, interleukin 2 (T-cell growth factor); KLH, key-hole limpet hemocyanin; LPS, lipopolysaccharide; PHA, phytohemagglutinin; SRBC, sheep erythrocytes; TRF, T-cell-replacing factor; FS6 medium, IL-2-containing medium from Con A-stimulated FS6-14.13 cells.

bridge, MA) or flat-bottomed 96-well microtiter plates (No. 3596, Costar). Cell cultures were incubated at 37°C in humidified 7.5% CO₂/92.5% air and fed every 3–4 days by removing 50% of the culture-well contents and refilling with an equal volume of fresh factor-supplemented medium.

Immunofluorescence Analysis. Cultured cells were stained and analyzed on a FACS II as described (22). All anti-Ig reagents were affinity-purified rabbit antibodies adsorbed against bovine gamma globulins, pepsin-digested to F(ab')₂ fragments, and directly conjugated with fluorescein isothiocyanate at a fluorescein-to-protein molar ratio of 4 or less. Ia was detected with monoclonal anti-Ia^d reagent (25-9-17; see ref. 23) followed by a fluorescein-conjugated (Fl) F(ab')₂ anti-IgG fragment. A monoclonal anti-Ia^k reagent (17-3-3; see ref. 24) was used as control. Both anti-Ia reagents were provided by K. Ozato and D. Sachs (National Cancer Institute). Biotin-conjugated anti-Thy 1 followed by Fl-avidin was used to test for Thy 1 expression.

Rosetting Procedures. Expression of surface Ig or Thy 1 on cultured cells was assessed by using both the direct rosetting procedure of Parish *et al.* (25) and the two-step rosetting procedure of McKenzie (26) involving protein A-coupled sheep erythrocytes (SRBC). In both cases, surface Ig was detected with a goat or rabbit polyvalent anti-mouse Ig reagent prepared and affinity-purified as described (27). Surface Thy 1 expression was investigated with a monoclonal anti-Thy 1.2 (New England Nuclear).

Radiolabeling, Immunoprecipitation, and Analysis of Cell Surface Ig. Lactoperoxidase-catalyzed radioiodination of surface membrane proteins was performed essentially as described (28). After lysis of labeled cells with Nonidet P-40, Ig was immunoprecipitated with a polyspecific affinity-purified rabbit anti-mouse Ig antibody plus protein A-bearing staphylococci as described (28, 29). In order to minimize nonspecific precipitation of radiolabeled fetal calf serum components and lactoperoxidase that had become adsorbed to the membranes of cultured cells, it was found advantageous to precoat the staphylococci with specific antibody and then to block all remaining protein A sites by incubation with an indifferent antibody [affinity-purified rabbit anti-keyhole limpet hemocyanin (KLH)]. Immunoprecipitates were washed, and the antigens were eluted, reduced, and identified by fluorography after electrophoresis on 10% (wt/vol) polyacrylamide gel slabs in NaDodSO₄ (29).

Measurement of Ig. Solid-phase competitive radioimmunoassays were performed in disposable flexible polyvinyl chloride microtiter plates (Dynatech Laboratories, Alexandria, VA). Each well was precoated with an IgM myeloma protein (TEPC-183) (Litton Bionetics) by incubation with 10 µg of protein in 100 µl of phosphate-buffered saline for 2 hr at room temperature. Ig release by long-term lines was quantitated by measuring the ability of culture supernatants to inhibit the binding of an affinity-purified ³H-labeled goat anti-mouse Ig to the TEPC-183-coated plates. Standard curves were constructed by diluting known amounts of TEPC-183 in the same culture media as that which had been used to grow the cells and measuring their ability to inhibit binding. Plates were incubated for 2 hr at 37°C with preformed mixtures of inhibitor and ³H-labeled goat anti-mouse Ig (approximately 50,000 cpm per well), washed extensively, and cut into individual wells. Wells were placed in vials containing Ultrafluor scintillant (New England Nuclear), and isotope binding was determined in a liquid scintillation spectrometer.

RESULTS

Long-Term Culture of T Cell-Depleted Splenocytes. Lymphocytes bearing relatively large amounts of membrane Ig were

prepared from BALB/c spleen by treatment with a monoclonal anti-Thy 1.2 and complement, staining with Fl-goat anti-mouse Fab fragment, and sorting on a FACS II. This B-lymphocyte-enriched cell population was immediately cultured in 24-well or 96-well Costar dishes at 10⁵ or 10⁶ cells per ml in culture medium containing LPS (50 µg/ml). Culture wells were fed every 3–4 days with fresh medium containing LPS and split and subcultured according to observed cell proliferation for periods of 1–4 wk. After that time, cells were subcultured (without washing) into a 1:1 mixture of fresh medium and an IL 2-containing medium from Con A-stimulated FS6-14.13 cells (FS6 medium). All cultures were fed with fresh preparations of the same mixture every 3–4 days. After transfer into FS6 medium, a proportion of cells recommenced proliferation at a slow rate, doubling in numbers approximately every 3–4 days. The maximum cell concentration reached in 24-well culture dishes was approximately 10⁵ cells per ml, with some cell death evident in a number of the culture wells. Cell lines have been propagated and maintained in this manner for at least 10 mo. FACS-purified B lymphocytes immediately cultured in IL 2- or IL 1-containing media (i.e., bypassing the initial LPS culture step) failed to produce such long-term cell lines. Instead, these cultures became overgrown with large granular cells that died after 10–14 wk.

Growth Factor Dependence of Long-Term Cell Lines. Growth factor dependence of these long-term cell lines was established by transferring the cells, after an 8- to 10-wk culture in FS6 medium, into a number of other growth factor-supplemented media. Cells continued to proliferate for periods of at least 10 wk in medium containing either IL 1 or various supernatants containing IL 2 and TRF, including Con A- and PHA-activated mouse splenocyte supernatants, Con A-activated rat splenocyte supernatants, PHA-activated human peripheral blood lymphocyte supernatants, and supernatants from some IL 2-dependent, long-term T-cell clones. Proliferation in TRF-containing supernatants was generally more rapid, with a cell-doubling time of approximately 2 days. Cell line propagation was not supported by culture medium that totally lacked growth factor supplements or by factor-free culture medium containing LPS. Under both of these conditions, the cells soon stopped dividing and died after 7–10 days.

Phenotype of Long-Term Cell Lines. Cultures maintained in FS6 medium were composed of a homogeneous population of small dense cells. The cells were analyzed for surface markers by immunofluorescence, rosetting, and membrane iodination, followed by immunoprecipitation and NaDodSO₄/polyacrylamide gel electrophoresis.

A population of cells established 18 wk earlier and maintained in IL 2-containing medium was tested for membrane Ig, Ia, and Thy 1 by staining with appropriate fluorescent reagents, followed by analysis on the FACS. These cells were clearly positive with Fl-anti-µ and Fl-anti-κ chains; neither Fl-anti-δ nor Fl-anti-γ chains gave any greater staining than the control reagent Fl-anti-KLH (Fig. 1). Staining patterns on normal BALB/c spleen cells are shown for comparison. Although the fluorescence intensity of the cultured cells stained with Fl-anti-µ chain overlapped that of cultured cells stained with Fl-anti-KLH, the fact that a single peak was observed suggests that virtually all of the cells bore IgM. Failure of the lines to express membrane IgD is consistent with previous reports that many splenic B cells lose membrane IgD after activation by LPS (30, 31). The cultured cells were also stained by anti-Ia^d followed by Fl-anti-IgG. The control reagent (anti-Ia^k) caused no staining above background. No staining with a biotin-conjugated anti-Thy 1 followed by Fl-avidin was observed (data not shown).

More than 95% of cells from a long-term line grown in FS6

FIG. 1. FACS analysis of membrane isotypes expressed by long-term (18 week) B-cell lines (*Left*) or normal BALB/c spleen (*Right*). Cells were stained with affinity-purified F1 rabbit anti-mouse μ chain ($\alpha\mu$), rabbit anti-mouse κ chain ($\alpha\kappa$), rabbit anti-mouse γ chain ($\alpha\gamma$), rabbit anti-mouse δ chain ($\alpha\delta$), or rabbit anti-KLH (α KLH).

medium for 16–20 wk formed rosettes with anti-Ig-conjugated SRBC (Table 1). These rosettes were inhibited by the presence of soluble anti-Ig. No rosettes were formed with trinitrophenylated SRBC. Similarly, more than 90% of cells from a long-term line formed rosettes with a mixture of goat anti-mouse Ig and protein A–SRBC. In contrast, the cells did not form rosettes with anti-Thy 1.2 and protein A–erythrocytes, although 39% of normal spleen cells formed rosettes under these conditions.

Cells from a line which had been maintained in FS6 medium for 22 wk were surface-labeled by lactoperoxidase-catalyzed iodination. Fig. 2 shows NaDodSO₄/polyacrylamide gel electrophoresis analysis of immunoprecipitates with anti-fetal calf serum and anti-lactoperoxidase from spleen cell (lane a) and

FIG. 2. NaDodSO₄/polyacrylamide gel electrophoresis of immunoprecipitates from lysates of radiolabeled mouse spleen cells (lanes a, b, e, f, and g) or a B-cell line established 22 wk earlier (lanes c, d, h, i, and j). Lanes: a and c, precipitates prepared with staphylococci coated with rabbit anti-fetal calf serum and anti-lactoperoxidase antibodies and then by an excess of rabbit anti-KLH antibodies; b and d, subsequent precipitates from these lysates using staphylococci coated with rabbit anti-Ig and an excess of rabbit anti-KLH; e and h, material obtained by incubating similar lysates with uncoated staphylococci. These pretreated lysates were then split into two portions. One portion was precipitated with staphylococci coated with rabbit anti-Ig and anti-KLH (lanes f and i), and the second portion was precipitated with staphylococci coated only with rabbit anti-KLH (lanes g and j). Arrows, positions of marker proteins: BSA (bovine serum albumin), B γ (bovine γ chains), M γ (mouse γ chains), and ML (mouse light chains).

Table 1. Surface Ig and Thy 1 status of long-term B-cell lines

Rosetting procedure	Rosette-forming cells, %	
	B-cell lines*	BALB/c spleen
RaMIg–SRBC	>95	ND
RaMIg [†] + RaMIg–SRBC	12	ND
Trinitrophenylated SRBC	3	ND
GaMIg + protein A–SRBC	>90	43
α Thy 1.2 + protein A–SRBC	0	39
Protein A–SRBC	2	1

ND, not determined; RaMIg, rabbit anti-mouse Ig; GaMIg, goat anti-mouse Ig; α Thy 1.2, anti-Thy 1.2.

* Sixteen- to 20-wk cells.

[†] Cells were preincubated with soluble rabbit anti-mouse Ig (RaMIg) for 45 min at 4°C before addition of RaMIg–SRBC.

long-term cell line lysates (lane c) and subsequent precipitations from the same lysates with anti-Ig (lanes b and d, respectively). The spleen cell immunoprecipitate contains bands with the mobility of membrane μ , membrane δ , and L chains (lane b); the pattern of the cell line lysate is generally similar (lane d). However, more detailed analysis of cell line lysates revealed that the situation is somewhat more complex. Thus, lane h represents a precipitation prepared with staphylococci alone, and lanes i and j represent subsequent precipitations from the same lysate with anti-Ig or anti-KLH, respectively. A band with the mobility of L chain is found only in lane i. Bands with the mobility of μ chain are found in lanes h, i, and j, although the intensity in lane i is substantially greater than in lane j, suggesting that membrane μ chain has been specifically precipitated. In summary, these results indicate that L chains are present on long-term B-cell lines and suggest the presence of membrane IgM. Similar results have been obtained by iodination of lines grown in either IL 1- or TRF-containing medium. Table 2 summarizes the phenotypic analysis of long-term B-cell lines maintained in FS6 medium.

Ig Synthesis by B-Cell Lines. The secretion of Ig by B-cell lines propagated in various growth factors was assessed by an inhibition radioimmunoassay with the IgM myeloma protein TEPC-183 as a standard. B cells (10^5) propagated for 3-day periods in IL 2-containing media, IL 1-containing media, or IL 1- plus IL 2-containing media released 1–10 ng of Ig per ml. Whether this level of Ig release represents active synthesis by a small number of antibody-producing cells, low-level secretion by all of the cultured B lymphocytes, or “leakage” of membrane Ig has not been established.

B cells propagated in TRF-containing mouse spleen supernatants (e.g., PHA-induced spleen supernatants) appeared to release substantially more Ig when culture supernatants were analyzed in parallel experiments. The amount of Ig secreted by the cell lines was difficult to quantitate due to the high background levels of Ig in these factor-supplemented media alone (e.g., 100–500 ng of Ig per ml). In an attempt to overcome this problem, B cells which had been propagated for at least 14 wk in either IL 1- or IL 2-containing media were transferred into media supplemented with supernatant produced by PHA-stimulated human peripheral blood lymphocytes. This human lymphocyte supernatant, although considerably less potent than the PHA-induced mouse spleen supernatant, was functionally active in both IL 2 and TRF mouse bioassays. In contrast to the mouse supernatant, however, the human supernatant did not contain Ig detectable in the above radioimmunoassay. Over the course of 7 days, the two types of B-cell lines showed 40-fold and 60-fold increases in Ig release in this TRF-containing human lymphocyte supernatant (Fig. 3). Although most cells in cultures propagated in TRF-containing supernatants still resembled small dense lymphocytes, the population contained some lymphoblasts and a small proportion of immature plasma cells.

Table 2. Phenotype of long-term B-cell lines

Characteristic	FACS analysis	Rosetting	Membrane radioiodination and immunoprecipitation
nIg	+	+	+
mIgM	+	ND	+
mIgD	—	ND	—
mIgG	—	ND	—
Ia ^d	+	ND	ND
Thy 1	—	—	ND

ND, not determined; m, membrane.

FIG. 3. Release of Ig by B-cell lines in media containing human PHA-activated lymphocyte supernatant (●, ■) or LPS (○, □). B cells (10^5) previously propagated in IL 1-containing media (■, □) or IL 2-containing media (●, ○) were transferred into test media on day 0, and culture supernatants were collected at 3.5-day intervals thereafter. Controls of media alone showed no Ig (Δ).

The increase in Ig synthesis and change in morphological characteristics indicate that TRF-containing supernatants activate a proportion of both IL 1-propagated and FS6-propagated B cells to active secretion of Ig.

To assess the responsiveness of long-term B-cell cultures to LPS, cells cultured for 20 wk in either IL 1- or IL 2-containing media were transferred to LPS-containing, growth factor-free media. Supernatants collected at 3- to 5-day intervals from a starting number of 10^5 cells per well contained less than 1 ng of Ig per ml (Fig. 3), as did supernatants collected from 10^5 cells grown in media alone.

DISCUSSION

We describe here a procedure for the extended cell culture of a population of mouse spleen cells that are largely if not entirely B cell in nature. Two distinct types of B-cell lines have been produced: (i) slowly dividing, small, dense B cells that release a small amount of Ig into the culture medium and are propagated by media containing either IL 1 or IL 2; and (ii) more rapidly dividing B cells that include a range of differentiation states from small lymphocytes to plasma cells, that secrete larger amounts of Ig into the culture medium and that are propagated by supernatants produced by mitogen-activated spleen cells. The latter supernatants were shown to contain IL 2 and TRF and probably include a number of other lymphokines and monokines (e.g., IL 1, immune interferon, colony-stimulating factor, etc.). As the activation of proliferating B cells into antibody-forming cells is a property previously ascribed to TRF (32, 33), we propose that exposure of B-cell lines grown in IL 1- or IL 2-containing media to a source of TRF drives some of these cells to differentiate into antibody-secreting cells. Whether TRF alone is sufficient to sustain proliferation of these lines is not yet clear.

The procedure for long-term B-cell culture outlined in this report requires further analysis to clarify several important

points. The type and differentiation state of B cells capable of propagation in our technique must be assessed. Similarly, we wish to emphasize that the nature of the B-cell growth factor(s) within the IL 1- and IL 2-containing media has not yet been established. Whether these will prove identical to IL 1 and IL 2 or represent separate molecular entities will be important to determine. It is of interest that B cells can be propagated by both IL 1-containing media and by IL 2-containing media, a feature which distinguishes the procedure from T-cell cloning (34).

Several aspects of the procedure already contribute to our current understanding of B-cell activation and development. In particular, the need for an LPS activation step provides some insight concerning the relative roles of LPS and the various growth factors. This initial LPS step appears to fulfill two requirements: (i) the elimination of a numerically small contaminating population of growth factor-responsive non-B-cells and (ii) the transformation of resting B cells into cells responsive to growth factors. The latter situation is analogous to procedures for long-term T-cell growth (4–8) in which T lymphocytes must be activated by antigen or lectin before they become responsive to IL 2 (21, 35, 36). Based on the findings outlined in this report and separate experiments investigating the effect of IL 1-, IL 2-, and TRF-containing media on resting B cells (unpublished data), we propose the following model: that resting or nonstimulated B cells are unresponsive to IL 1- or IL 2-containing media, or both, in terms of both proliferation and Ig synthesis; that LPS can render a population of B cells responsive to IL 1- or IL 2-containing media, or both; that IL 1- or IL 2-containing media, or both, deliver proliferative signals to such activated or responsive B lymphocytes; and that the differentiation signal required for Ig synthesis is provided by TRF.

Finally, we wish to acknowledge that, in parallel with the work reported here, B. Sredni *et al.* (personal communication) have independently developed a similar procedure for the long-term culture of human B lymphocytes.

We thank R. Asofsky for the gift of goat anti-mouse Ig, K. Ozato and D. Sachs for monoclonal anti-Ia reagents, J. Titus for providing P388D₁ culture supernatants, J. Kappler for providing FS6-14.13 cells, and B. Johnson for excellent technical assistance. M.H. is supported by a C. J. Martin Fellowship from the National Health and Medical Research Council of Australia. This work was supported in part by the Uniformed Services University of the Health Sciences Grant No. CO8310.

- Potter, M. (1977) *Adv. Immunol.* **25**, 141–211.
- Melchers, F., Potter, M. & Warner, N., eds. (1978) *Current Topics in Microbiology and Immunology* (Springer, Berlin), Vol. 81.
- Sakano, H., Maki, R., Kurosawa, Y., Reeder, W. & Tonegawa, S. (1980) *Nature (London)* **286**, 676–683.
- Gillis, S. & Smith, K. A. (1977) *Nature (London)* **268**, 154–156.
- Fathman, C. G. & Hengartner, H. (1978) *Nature (London)* **272**, 617–618.
- Nabholz, M., Engers, H., Collavo, D. & North, M. (1978) *Curr. Top. Microbiol. Immunol.* **81**, 176–187.
- Augustin, A. A., Julius, M. H. & Cosenza, H. (1979) *Eur. J. Immunol.* **9**, 665–670.
- Sredni, B., Tse, H. Y. & Schwartz, R. H. (1980) *Nature (London)* **283**, 581–583.
- Haas, W. & Layton, J. E. (1975) *J. Exp. Med.* **141**, 1004–1014.
- Metcalfe, D., Nossal, G. J. V., Warner, N., Miller, J. F. A. P., Mandel, T., Layton, J. & Gutman, G. (1975) *J. Exp. Med.* **142**, 1534–1549.
- Metcalfe, D., Wilson, J., Shortman, K., Miller, J. F. A. P. & Stocker, J. (1976) *J. Cell Physiol.* **88**, 107–116.
- Kincade, P., Ralph, P. & Moore, M. A. S. (1976) *J. Exp. Med.* **143**, 1265–1270.
- Sredni, B., Rozenszajn, L. A., Kalechman, I. & Michlin, H. (1975) *Exp. Hematol.* **3**, 44–56.
- Pillai, S. & Scott, D. (1981) *J. Immunol.* **126**, 1883–1886.
- Askonas, B. A., Williamson, A. R. & Wright, B. E. G. (1970) *Proc. Natl. Acad. Sci. USA* **67**, 1398–1403.
- Williamson, A. & Askonas, B. (1972) *Nature (London)* **238**, 337–339.
- Melchers, F., Coutinho, A., Heinrich, G. & Andersson, J. (1975) *Scand. J. Immunol.* **4**, 853–858.
- Lachman, L. B., Hacker, M. P., Blyden, G. T. & Haudschmader, R. E. (1977) *Cell Immunol.* **34**, 416–419.
- Harwell, L., Skidmore, B., Marrack, P. & Kappler, J. (1980) *J. Exp. Med.* **152**, 893–904.
- Schimpl, A. & Wecker, E. (1972) *Nature (London) New Biol.* **237**, 15–17.
- Lafferty, K., Warren, H., Woolnough, J. & Talmage, D. (1978) *Blood Cells* **4**, 395–404.
- Scher, I., Berning, A. K., Kessler, S. & Finkelman, F. D. (1980) *J. Immunol.* **125**, 1686–1693.
- Ozato, K. & Sachs, D. (1981) *J. Immunol.* **126**, 317–321.
- Ozato, K., Mayer, N. & Sachs, D. (1980) *J. Immunol.* **124**, 533–540.
- Parish, C., Kirov, S., Bown, N. & Blanden, R. V. (1974) *Eur. J. Immunol.* **4**, 808–815.
- Sandrin, M., Potter, T., Morgan, G. & McKenzie, I. (1978) *Transplantation* **26**, 126–130.
- Sieckmann, D., Asofsky, R., Mosier, D., Zitron, I. & Paul, W. (1978) *J. Exp. Med.* **147**, 814–829.
- Kessler, S. W. (1976) *J. Immunol.* **117**, 1482–1490.
- Kessler, S. W., Woods, V. L., Finkelman, F. D. & Scher, I. (1979) *J. Immunol.* **123**, 2772–2778.
- Bourgeois, A., Kitajima, K., Hunter, I. & Askonas, B. (1977) *Eur. J. Immunol.* **7**, 151–153.
- Sitia, R., Abbott, J. & Hammerling, U. (1979) *Eur. J. Immunol.* **9**, 859–864.
- Dutton, R. (1975) *Transplant. Rev.* **23**, 66–77.
- Schimpl, A. & Wecker, E. (1975) *Transplant. Rev.* **23**, 176–188.
- Oppenheim, J., Northoff, H., Greenhill, A., Mathieson, B., Smith, K. & Gillis, S. (1979) in *Biochemistry of Lymphokines*, ed. DeWeck, A. (Academic, New York), pp. 399–404.
- Larsson, E. L. & Coutinho, A. (1979) *Nature (London)* **280**, 239–241.
- Andersson, J., Gronvik, K. O., Larsson, E. L. & Coutinho, A. (1979) *Eur. J. Immunol.* **9**, 581–587.