ANTIGEN-SPECIFIC T-HELPER CELLS STIMULATE
H-2-COMPATIBLE AND H-2-INCOMPATIBLE B-CELL
BLASTS POLyclONALLY

BY MAX H. SCHREIER,* JAN ANDERSSON,† WALDEMAR LERNHARDT,*, AND
FRITZ MELCHERS*†

From the Basel Institute for Immunology, Basel, Switzerland; and Biomedicum, University of Uppsala,
Uppsala, Sweden

B lymphocytes can be induced to divide and form Ig-secreting clones either by T-
cell help or by mitogens (1, 2). About one in three splenic lymphocytes respond to B-
cell mitogens, and such activation is polyclonal: 1 clone in 1,000 produces antibodies
against sheep erythrocytes (SRC),1 1 in 500 against horse erythrocytes (HRC), and 1
in 50 against trinitrophenyl (TNP) (3–5). The presence of mitogen is required for
each consecutive cell cycle (6; W. Lernhardt and F. Melchers. Manuscript in prepa-
ratation.), but the various B-cell mitogens are largely interchangeable (6). Both the
frequencies of mitogen-reactive cells and their multireactivity suggests that these cells
are also the targets of T-cell help.

T-cell help is generated by the interaction of antigen-specific helper T-cells and
adherent cells (2, 7, 8). By the use of recently developed methods it could be shown
that such antigen-activated T-cell help was as effective in inducing anti-SRC and
anti-HRC clones as was lipopolysaccharide (LPS) or lipoprotein (LP) (9).

By combining the improved method of generating antigen-activated T-cell help (9,
10) and with the techniques developed for testing responses of B blasts at the single cell
level (3), we could investigate three questions: (a) Is T-cell help directed toward the
same set of B cells as are inducible by B-cell mitogens? (b) Is this help limited in any
way, either by the affinity of antigen for cellular receptors or by some other kind of
restriction (11, 12)? (c) Is antigen-activated T-cell help as specific as is its induction?

Materials and Methods

Animals. C57BL/6J/Füll. nu/nu or normal mice (6–8 wk old) and Lewis rats (4 wk old)
were obtained from the Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf,
Switzerland. BALB/c mice (4–6 wk of age) were obtained from Gv. Bomholtgaard, Ry,
Denmark. B10.BR, B10.A, B10(4R), and B10(5R) mice (6 wk–6 mo of age) were obtained from
OLAC Ltd., Blackthorn, Bicester, Oxon, England.

Cell Cultures. Mouse spleen cells, prepared as described previously (3, 4), were cultured in

* Basel Institute for Immunology, Basel, Switzerland.
† Biomedical, University of Uppsala, Uppsala, Sweden. Supported by the Swedish Cancer Society.

Abbreviations used in this paper: IgM-PFC, IgM-secreting plaque-forming cells; HRC, horse erythrocyte(s);
G0, resting state of the cell; LP, lipoprotein; LPS, lipopolysaccharide; PFC, plaque-forming cell(s); SRC, sheep erythrocyte(s); TSRC, TMR, T cells primed to SRC, HRC, TNP, trinitrophenyl; TNP-SRC, trinitrophenylated SRC.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/80/01/0194/10$1.00
Volume 151  January 1980  194-203
Iscove's medium (an enriched modification of Dulbecco's modified Eagle's medium containing additional amino acids and vitamins) (13), containing transferrin, albumin, and soybean lipid as serum replacements and also 2-mercaptoethanol (5 × 10⁻⁵M) and kanamycin (Bio-Cult, Ltd., Irvine, Scotland). Splenic B cells were activated by the smooth form of LPS (LPS-S, a gift from Dr. C. Galanos and Dr. O. Lüderitz, Max-Planck-Institut für Immunobiologie, Freiburg i. Br., West Germany [50 μg/ml]) in culture at 3 × 10⁶ cells per ml for 48 h, as described previously (6). Activated B-cell blasts were separated by velocity sedimentation at unit gravity (14) and collected as described previously (6). The separated B-cell blast fraction was then recultured for limiting-dilution analyses in medium containing 3 × 10⁶ rat thymus cells per ml as filler cells (3).

Cell concentrations covered the range of 3 × 10⁵ to 1 B-cell blast per culture in 3.3-fold steps. 10 cultures for each dilution were set up in Falcon Microtest II plates (catalog No. 3040, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). Helper T-cells specific for SRC or HRC (T₅SRC, T₅HRC) were obtained from irradiated, thymocyte-reconstituted, antigen-primed C57BL/6J/Fül mice (9). Spleen cells from these mice were irradiated (3,300 rad with a Philips RT 305 x-ray machine, Philips Electronic Instruments, Inc., Mahwah, N. J.) and 2 × 10⁵ cells were added to each 0.2 ml culture.

Assays. Cultures were assayed for IgM-secreting cells by the protein A-SRC plaque assay (15). B-cell clones secreting antibodies against SRC, HRC, or trinitrophenylated SRC (TNP₆ SRC) were detected by the appropriate antigen-specific plaque assays (16).

The anti-IgM serum dilution used to develop protein A plaques completely inhibited the appearance of direct SRC- or HRC-plaque-forming cells (PFC) in the antigen-specific PFC assay. The sensitivity of the two kinds of plaque assay is comparable as they score indistinguishable clone sizes (i.e., numbers of PFC) when single clones are studied (4, 5).

Experimental Protocol. Splenic B cells were stimulated for 2 d with LPS and the activated B-cell blasts separated by velocity sedimentation. These B-cell blasts were then cultured at different concentrations in the presence or absence of homologous mitogen (LPS), with or without antigen (HRC or SRC, 2.5 × 10⁵/ml), or in the presence of activated T₅SRC or T₅HRC cells (2 × 10⁵ cells/culture) and antigen. The T-helper cells used in these experiments were activated in vivo either to SRC or to HRC.

The frequency estimates of reactive B cells (summarized in Tables II, III, and IV) rest on 3.3-fold dilutions of B-cell blasts with 10 cultures for one type of stimulus at a given dilution. IgM-secreting PFC IgM-PFC were assayed at day 3 of restimulation (i.e., at day 5 after original stimulation with LPS). Cultures with more than five IgM-PFC per culture were scored as positive. The average number of IgM-PFC per culture represents the mean of all 10 cultures, irrespective of the number of positive cultures within the group.

The general controls for experiments reported in this paper have been summarized in Table I. As can be seen, the preparation of irradiated helper T cells contained no B cells reactive to LPS either in the presence or absence of antigen.

The LPS-stimulated B-cell blasts cultured under our conditions for an additional 3 d remain viable and are detected as PFC (6). Such B-cell blasts cultured in the presence of thymus filler cells or T-helper cells, but in the absence of stimulation by mitogen or antigen, do not multiply and yield, on day 3 of reculture, ~1,000 IgM-PFC per 3 × 10⁶ blasts or 5–18 IgM-PFC per 3 × 10⁵ B-cell blasts. Similar cultures supplemented with LPS give 320–450 IgM-PFC per 3 × 10⁶ cells. However, the growth and maturation of LPS-activated B-cell blasts in cultures containing helper T cells (and adherent cells) is strictly dependent on the presence of specific antigen: no IgM-PFC arise in the presence of an antigen unrelated to that used for in vivo priming of the T-helper cells.

Results

Frequencies of LPS-activated B-Cell Blasts Reactive to LPS- or to Antigen-activated T-Cell help. The experiment tested the growth of B-cell blasts in the presence of the homologous stimulus used to generate the blasts, i.e., LPS, or in the presence of the heterologous stimulus, i.e., antigen-activated T-cell help. The frequency of B-cell blasts so stimulated was determined by limiting-dilution analyses (3). Assuming a
Random distribution, one reactive B-cell blast (yielding a clone of IgM-PFC) is present at concentrations of B blasts where 37% of all cultures are negative.

The average clone size after 3 d of restimulation, assayed either as total IgM-PFC or as specific (SRC, HRC, or TNP-SRC) PFC was found to be between 6 and 15, either in LPS-, or in LPS-plus-antigen-, or in antigen-activated T-help-stimulated B-cell clones. This clone size tallies with the previously determined doubling time of 18 h for mitogen-induced B-cell clones growing under similar culture conditions (3).

The two culture systems used to grow B-cell blasts at limiting dilutions support the induction of SRC- or HRC-specific B-cell clones equally well (5, 9) and may be expected to allow the clonal expansion of any inducible B cell.

Table II lists the frequencies of LPS-activated B-cell blasts that continue clonal growth and maturation to IgM-PFC. These frequencies are about the same in the presence of SRC-specific (experiment I) or HRC-specific (experiment II) T-helper cells, activated by the specific antigen, and after restimulation with the homologous mitogen LPS. Thus, one in approximately three B-cell blasts continues to grow after either kind of stimulus. Without LPS or antigen-activated T-cell help or in the presence of T-helper cells cocultured with an unrelated antigen, <1 in 1,000 blasts form clones. From these results we conclude: (a) The stimulation of B-cell blasts to growth and maturation is mitogen, or antigen-activated T-cell-help dependent. (b) The stimulation of B-cell blasts by antigen-activated T-cell help is nonspecific and polyclonal. Specific, activated T-helper cells, therefore, provide mitogenic stimuli for growth and maturation of B-cell blasts. (c) About one-third of B-cell blasts is reactive to antigen-activated T-cell help or to LPS.

**Frequencies of Antigen-specific Clones among LPS-activated B-Cell Blasts Restimulated with either LPS or Antigen-activated T-Cell Help.** The frequencies of specific B-cell clones were enumerated in an extension of the experiments presented in Table II. Table III

### Table I

| Contents of culture* | Number of PFC at day 3 of stimulation‡ |
|---------------------|----------------------------------------|
|                     | IgM-PFC  | Anti-HRC-PFC | Anti-SRC-PFC |
| Thac                | 0        | 0            | 0            |
| Thac + LPS          | 0        | ND           | 0            |
| Thac                | 0        | 0            | 0            |
| Thac + HRC + LPS    | 0        | ND           | 0            |
| Thac + SRC + LPS    | 0        | 0            | 0            |
| Thac + B blasts (3 × 10⁴) | 1,000   | 1           | 0            |
| Thac + B blasts (3 × 10⁵) | 5     | 0           | 0            |
| Thac + B blasts (3 × 10⁶) + LPS | 3,250  | 4          | 2            |
| Thac + SRC + B blasts (3 × 10⁴) | 1,100  | 2          | 1            |
| Thac + SRC + B blasts (3 × 10⁵) | 18  | 0           | 0            |
| Thac + SRC + B blasts (3 × 10⁶) + LPS | 3,700  | 5          | 2            |

ND, not done.

* For details see Materials and Methods.

‡ Average of 10 cultures.


M. SCHREIER, J. ANDERSSON, W. LERNHARDT, AND F. MELCHERS

TABL~ II

Limiting-Dilution Analysis of the Frequencies of LPS-activated B-Cell Blasts Yielding IgM-secreting Clones under Stimulation by LPS or by Antigen-activated T-Cell Help

Experiment I. With T~RC Stimulation

| Number of LPS-activated B-cell blasts per culture* | Stimulation          |          |
|--------------------------------------------------|----------------------|----------|
|                                                  | LPS + SRC            | LPS      | T~RC + SRC          |
|                                                  | Percent positive cultures ‡ | Average number of IgM-PFC per culture § | Percent positive cultures ‡ | Average number of IgM-PFC per culture § | Percent positive cultures ‡ | Average number of IgM-PFC per culture § |
|                                                  | %                    | %        | %                   | %        |
| 300                                              | ND                   | ND       | ND                  | 100      | 320 |
| 100                                              | ND                   | ND       | ND                  | 100      | 195 |
| 30                                               | 100                  | 120.2    | 100                 | 160.1    | 100 |
| 10                                               | 100                  | 59.2     | 90                  | 45.7     | 90  |
| 3                                                 | 80                   | 18.4     | 70                  | 18.2     | 70  |
| 1                                                 | 40                   | 6.1      | 50                  | 8.4      | 30  |

Experiment II. With T~ac Stimulation

| Number of LPS-activated B-cell blasts per culture* | Stimulation          |          |
|--------------------------------------------------|----------------------|----------|
|                                                  | LPS + HRC            | LPS      | T~ac + HRC          |
|                                                  | Percent positive cultures ‡ | Average number of IgM-PFC per culture § | Percent positive cultures ‡ | Average number of IgM-PFC per culture § | Percent positive cultures ‡ | Average number of IgM-PFC per culture § |
|                                                  | %                    | %        | %                   | %        |
| 30                                               | 100                  | 450      | 100                 | 320      | 100 |
| 10                                               | 100                  | 124.3    | 100                 | 75.8     | 100 |
| 3                                                 | 90                   | 39.8     | 80                  | 29.4     | 100 |
| 1                                                 | 60                   | 14.2     | 50                  | 19.3     | 60  |

ND, not done. Controls, included in each experiment, are collected in Table I.

* C57BL/6J nu/nu spleen cells activated for 48 h with LPS (50 µg/ml) enriched for blast cells by 1-g sedimentation (Materials and Methods).

‡ Boldface figures indicate the point nearest to one precursor per culture (= 63% positive assuming Poisson's distribution of reactive precursors).

§ The average number of PFC is based on 10 cultures.

summarizes the results. (a) B-cell clones producing SRC-, HRC- or TNP-SRC-binding IgM molecules occur with approximately the same frequency among B-cell blasts restimulated either by the homologous mitogen LPS or by antigen-activated T-cell help. This restates that LPS-activated B-cell blasts producing a particular antibody will continue to grow under the stimulating influence of T-helper cells induced by specific antigen. (b) The frequencies of clones specific for a given antigen are like the frequencies obtained by mitogenic activation of resting B cells by LPS or LP (5): 1 in 1–3 × 10^3 specific for SRC, 1 in 1–3 × 10^3 specific for HRC, and 1 in 1–10 × 10^2 specific for TNP_30-SRC. Antigen-activated T-helper cells are seen to act as polyclonal activators of LPS blasts irrespective of antigen specificity. The overall frequencies, compiled from the data of Tables II and III, are given in Table IV.
### Table III

**Limiting-Dilution Analysis of the Frequencies of LPS-activated B-Cell Blasts Yielding Antigen-Specific PFC under Homologous (LPS) Stimulation or Antigen-activated T-Cell Help**

**Experiment I. With T-cell Stimulation**

| Number of LPS-activated B-cell blasts per culture* | Stimulation | LPS + SRC |  | LPS | T-cell + SRC |
|---------------------------------------------------|-------------|-----------|---|----------|------------|
|                                                   |             | Percent positive cultures | Average number of PFC per culture | Percent positive cultures | Average number of PFC per culture |
|                                                   |             | plaque assay on SRC | plaque assay on SRC | plaque assay on SRC | plaque assay on SRC |
| 3 × 10⁴                                           |             | 90 | 28.3 | 100 | 26.4 |
| 1 × 10⁴                                           |             | 60 | 10.4 | 70 | 11.2 |
| 3 × 10⁴                                           |             | 30 | 3.5  | 30 | 4    |
| 1 × 10⁴                                           |             | 0  | 0    | 0  | 0    |

**Experiment II. With H-cell Stimulation**

| Number of LPS-activated B-cell blasts per culture* | Stimulation | LPS + HRC |  | LPS | T-cell + HRC |
|---------------------------------------------------|-------------|-----------|---|----------|------------|
|                                                   |             | Percent positive cultures | Average number of PFC per culture | Percent positive cultures | Average number of PFC per culture |
|                                                   |             | plaque assay on SRC | plaque assay on SRC | plaque assay on SRC | plaque assay on SRC |
| 1 × 10⁵                                           |             | 100 | 100 | ND | 81 |
| 3 × 10⁴                                           |             | 90  | 100 | ND | 310 |
|                                                   |             | 100 | 100 | ND | 320 |
|                                                   |             | 100 | 100 | ND | 320 |
|                                                   |             | 100 | 100 | ND | 320 |

**Discussion**

H-2-unrestricted Stimulation of B-Cell Blasts by Antigen-activated T-Cell Help. Possible H-2 restriction at the level of activated B-cell blasts reacting to antigen-activated T-cell help were tested in limiting-dilution analyses of the number of B-cell blasts from partially or totally H-2-incompatible mice reactive to HRC-activated T-cell help produced with H-2-compatible (C57BL/6) = H-2b) helper T cells and adherent cells.

Results in Table V show that no H-2 restriction exists for LPS-activated B-cell blasts to react to antigen-activated T-cell help.
TABLE IV
Frequencies* of SRC-, HRC-, or TNP-SRC-specific and Total IgM-secreting B Cells after Stimulating LPS-activated B-Cell Blasts either with LPS or with Antigen-activated T-Cell Help

| Experiment I. With T_{SRC} | Precursor frequency |
|---------------------------|---------------------|
|                           | Total IgM-PFC | Anti-SRC | Anti-HRC | Anti-TNP-SRC |
| LPS                       | 1 in 3        | 1 in 10^4 |          |              |
| LPS + SRC                 | 1 in 3-1      | 1 in 10^4 |          |              |
| T_{SRC} + SRC             | 1 in 3        | 1 in 1-3 X 10^4 |          |              |

| Experiment II. With T_{HRC} | Precursor frequency |
|-----------------------------|---------------------|
|                            | Total IgM-PFC | Anti-SRC | Anti-HRC | Anti-TNP-SRC |
| LPS                         | 1 in 1-3      | 1 in 3-10 X 10^3 | 1 in 3 X 10^2 | 1 in 3 X 1-3 X 10^2 |
| LPS + HRC                   | 1 in 1-3      | 1 in 1-3 X 10^3 | 1 in 1-3 X 10^2 | 1 in 1 X 10^2 |
| T_{HRC} + HRC               | 1 in 1-3      | 1 in 1-3 X 10^3 | 1 in 1-3 X 10^2 | 1 in 3-10 X 10^2 |

* Extracted from data of Tables II and III.

TABLE V
H-2-Unrestricted, Polyclonal Stimulation of LPS-activated B-Cell Blasts Expressing Different H-2 Haplotypes

| LPS-activated B-cell blasts | Frequency of reactive B-cell blasts upon restimulation by |
|-----------------------------|--------------------------------------------------------|
| H-2 haplotype               | HRC-activated T-cell help (C57BL/6J: bbbbb b) | LPS |
|                             | Strain | K | I | A | B | J | E | C | 1 in 3 | 1 in 3-1 |
| C57BL/6J                    | b      | b | b | b | b | b | b | b | 1 in 3 | 1 in 3-1 |
| BALB/c                      | d      | d | d | d | d | d | d | d | 1 in 1-3 | 1 in 1-3 |
| B10.BR                      | k      | k | k | k | k | k | k | k | 1 in 1-3 | 1 in 1-3 |
| B10.A                       | k      | k | k | k | k | k | d | d | 1 in 3-10 | 1 in 3-10 |
| B10.A (4R)                  | k      | k | b | b | b | b | b | b | 1 in 1-3 | 1 in 3 |
| B10.A (5R)                  | b      | b | b | b | b | k | k | d | 1 in 1-3 | 1 in 1-3 |

or adherent cells produce the helper principle (factors) effective on B cells, we expect that specificity resides at the level of the T-helper cells carrying antigen-specific receptors (10, 22-24). It seems reasonable to believe therefore that the induction of T-helper cells to their effector stage should be antigen specific, whereas the effect on B cells may be either specific or nonspecific.

Our results show that this helper effect on B cell blasts is polyclonal and, therefore, nonspecific. Antigen-activated T-cell help provides growth stimuli for B-cell blasts, as do LPS, LP, or other B-cell mitogens (6). Mitogen, and thus help, is needed during each cell cycle and is therefore likely to be used up by the B-cells. Antigen, however, is not needed for the initiation and completion of new rounds of division. Limiting
concentrations of help will lead to submaximal numbers of B cells initiating growth for submaximal numbers of cell division. Although a large fraction of LPS-activated B blasts could be restimulated by T-cell help, it is evident that the clone size of LPS-stimulated B-cell blasts is larger than that of the B-cell blasts stimulated by antigen-activated T-cell help (see Tables II and III). The most likely explanation of this disparity in clone sizes is that the B-cell growth factors provided by antigen-activated T-cell help are limited in amount and are used up by the dividing B-cell blasts (M. H. Schreier, J. Andersson, W. Lernhardt, and F. Melchers. Manuscript in preparation.). In comparison to LPS, therefore, a similar number of B-cell blasts initiate growth with antigen-activated T-cell help, they do, however, not grow as long because the B-cell growth factors of antigen-activated T-cell help become limiting at later divisions. It is also clear, from the data summarized in Table IV, that not even all LPS blasts are restimulated by the homologous mitogen. The experimental conditions for the separation and reculturing of these very fragile blasts may have something to do with this. It is likely, therefore, that the frequencies of LPS-activated blasts, whether induced by T-cell help or by mitogens, may be higher.

It is noteworthy that human T-cells stimulated either by tentanus toxoid (25), or by allogeneic or autologous mixed lymphocyte reactions (26) have been found to lead to the production of nonspecific helper factors that behave like polyclonal B-cell activators. We have also found similar helper factors in the supernatant media of our antigen-activated T-cell help. Their action on B-cell blasts and on resting, small B-cells will be described in a subsequent publication (M. H. Schreier, J. Andersson, W. Lernhardt, and F. Melchers. Manuscript in preparation.). The cellular origin and molecular nature of T-cell help is obscure. T cells and/or adherent cells could be involved. The effect could require cell-to-cell contacts or be mediated by soluble factors. Our preliminary experiments and those of others (10, 17-21, 24) favor the latter. This help shows no H-2 restriction or preference (8, 11, 12) for activated B-cell blasts. We, therefore, conclude that neither antigen nor H-2 (I region) compatibility is needed to stimulate a B-cell blast into the next round of division. This probably means that neither Ig nor Ia have to be occupied by specific ligands.

The polyclonal nature of B-blast activation by antigen-activated T-helper cells is also obvious from the frequencies of SRC-, HRC-, and TNP30-SRC-specific B-cell clones. This distribution is very similar to the one found with LPS-activated B cells (5), a result which is expected if restimulation, concerning the same populations, is random and independent of V-region expression. It remains to be seen whether the approximate two-thirds of resting B cells not activated by LPS (3, 4) will show differences, either in their capacity to be induced by T-cell help or in their repertoire of V-regions. It is also an open question whether B-cell clones switching to other classes of Ig, such as IgG (27, 28) appear with the same frequency after stimulation by T-cell help.

The basic finding of this paper, that T-cell help, specifically induced by antigen, acts polyclonally and is H-2 unrestricted on B-cell blasts, poses the question of how such polyclonal, unrestricted B-cell stimulation could lead to the exclusive and H-2-restricted (11, 12) response of antigen-specific B cells wherever the immune system is specifically stimulated. First, the concentration of T-cell help may never be as high in vivo as it is under our in vitro conditions. The effect may be limited to the vicinity of
the activated helper cells, leading to nonspecific activation of neighboring B cells, as frequently seen in immune responses in vivo (29). Second, it appears reasonable to expect that the immune system safeguards itself against nonspecific, polyclonal activation by the resting state of the cell (G0) in which most antigen-reactive lymphocytes are found. We already have some evidence that small, resting B cells are not activated by T-cell help at concentrations which stimulate B-cell blasts polyclonally. Resting B cells, like resting T cells (30), may need additional stimuli to arise from the G0 and become thus amenable to the polyclonally stimulating principle of T-cell help. The two most likely possibilities for the activation of a resting, G0 B cell from this resting state are (a) binding of antigen or anti-Ig antibodies to surface Ig, and (b) occupancy of surface Ia by compatible structures provided in T-cell help.

Summary

Lipopolysaccharide (LPS)-activated B-cell blasts from C57BL/6J nu/nu spleen cells develop into IgM-secreting clones after stimulation by antigen-specific T-helper cells of C57BL/6J origin. Although induction of help is antigen-dependent, help itself acts polyclonally. 1 of 1-3 B-cell blasts is restimulated in a homologous fashion by LPS, or in a heterologous fashion by sheep erythrocyte (SRC)- or horse erythrocyte (HRC)-activated T-helper cells. The repertoire of activated B-cell blasts reflects the polyclonal nature of activation: ~1 in 1,000-3,000 restimulated B-cell blasts is specific for SRC, 1 in 300-1,000 is specific for HRC, and 1 in 100-300 specific for trinitrophenylated SRC (TNP30-SRC).

B-cell blasts that are either H-2 compatible or H-2 incompatible with the antigen-activated T-cell help are stimulated polyclonally in similar high frequencies. Thus, neither antigen nor H-2 compatibility are required to stimulate a B-cell blast into the next cell cycle.

We thank Dr Stephen Fazekas de St. Groth for help with the manuscript; The able technical assistance of Ms. Deborah Norman and Ms. Reet Tees is gratefully acknowledged.

Received for publication 9 July 1979.

References

1. Andersson, J., O. Sjöberg, and G. Möller. 1972. Induction of immunoglobulin and antibody synthesis "in vitro" by lipopolysaccharide. Eur. J. Immunol. 2:349.
2. Miller, J. F. A. P., and G. F. Mitchell. 1969. Thymus and antigen-reactive cells. Transplant. Rev. 1:3.
3. Andersson, J., A. Coutinho, W. Lernhardt, and F. Melchers. 1977. Clonal growth and maturation to immunoglobulin secretion in vitro of every growth-inducible B-lymphocyte. Cell. 10:27.
4. Andersson, J., A. Coutinho, and F. Melchers. 1977. Frequencies of mitogen-reactive B-cells in the mouse. I. Distribution in different lymphoid organs from different inbred strains of mice at different ages. J. Exp. Med. 145:1511.
5. Andersson, J., A. Coutinho, and F. Melchers. 1977. Frequencies of mitogen-reactive B-cells in the mouse. II. Frequencies of B-cells producing antibodies which lyse sheep or horse erythrocytes, and trinitrophenylated or nitroiodophenylated sheep erythrocytes. J. Exp. Med. 145:1520.
6. Andersson, J., A. Coutinho, and F. Melchers. 1979. Mitogen activated B-cell blasts reactive to more than one mitogen. J. Exp. Med. 149:553.
7. Mosier, D. E. 1967. A requirement for two cell types for antibody formation in vitro. Science (Wash. D. C.). 158:1575.
8. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirements for histocompatible macrophages and lymphocytes. J. Exp. Med. 138:1194.
9. Schreier, M. H. 1978. B-cell precursors specific to sheep erythrocytes. Estimation of frequency in a specific helper assay. J. Exp. Med. 148:1612.
10. Schreier, M. H. Interaction of specific helper T-cells and antigen: conversion of specific into nonspecific signals. In Proceedings of the 2nd International Lymphokine Workshop. A. de Weck and M. Landy, editors. Academic Press, Inc., New York. In press.
11. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histocompatible T and B lymphocytes. III. Demonstration that the H-2-gene complex determines successful physiologic lymphocyte interactions. Proc. Natl. Acad. Sci. U. S. A. 70:2624.
12. Sprent, J. 1978. Role of H-2 gene products in the function of T-helper cells from normal and chimeric mice in vivo. Immunol. Rev. 42:108.
13. Iscove, N. N., and F. Melchers. 1978. Complete replacement of serum by albumin, transferrin, and soybean lipid in cultures of lipopolysaccharide-reactive B lymphocytes. J. Exp. Med. 147:923.
14. Miller, R. G., and R. A. Phillips. 1969. Separation of cells by velocity sedimentation. J. Cell. Physiol. 73:191.
15. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. Eur. J. Immunol. 6:588.
16. Jerne, N. K., A. H. Nordin, and C. Henry. 1963. The agar plaque technique for recognizing antibody-producing cells. In Cell-bound Antibodies. B. Amos and H. Koprowski, editors. Wistar Institute Press, Philadelphia. 109.
17. Hartmann, K.-U. 1970. Induction of a hemolysin response in vitro. Interaction of cells of bone-marrow origin and thymic origin. J. Exp. Med. 132:1267.
18. Hartmann, K.-U. 1971. Interaction of bone marrow-derived cells and thymus dependent cells in the immune response against erythrocytes in vitro. In Cell Interactions and Receptor Antibodies in Immune Response. O. Mäkelä, A. Cross, and T. U. Kosunen, editors. Academic Press, Inc., New York. 373.
19. Vann, D. C., and J. R. Kettmann. 1972. In vitro cooperation of cells of bone-marrow and thymus origins in the generation of antibody-forming cells. J. Immunol. 108:73.
20. Playfair, J. H. L. 1972. Response of mouse T and B lymphocytes to sheep erythrocytes. Nat. New Biol. 235:115.
21. Waldmann, H., and A. Munro. 1973. T cell-dependent mediator in the immune response. Nature (Lond.). 243:356.
22. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. Eur. J. Immunol. 1:18.
23. Dutton, R. W., M. M. McCarthy, R. I. Mishell, and D. J. Raidt. 1970. Cell components in the immune response. IV. Relationship and possible interactions. Cell. Immunol. 1:196.
24. Waldmann, H. 1975. T cell-dependent mediator in the immune response. III. The role of non-specific factor (NSF) in the in vitro immune response. Immunology 28:497.
25. Geha, R. S., F. Mudawwas, and E. Schneeberger. 1977. The specificity of T-cell helper factor in man. J. Exp. Med. 145:1436.
26. Chiorazzi, N., S. M. Fu, and H. G. Kunkel. 1979. Induction of polyclonal antibody synthesis by human allogeneic and autologous helper factors. J. Exp. Med. 149:1543.
27. Andersson, J., A. Coutinho, and F. Melchers. 1978. The switch from IgM to IgG secretion in single mitogen-stimulated B cell clones. J. Exp. Med. 147:1744.
28. Pernis, B., L. Forni, and L. Amante. 1971. Immunoglobulins as cell receptors. Ann. N. Y. Acad. Sci. 190:820.
29. Pachmann, K., D. Killander, and H. Wigzell. 1974. Increase in intracellular immunoglobulins in the majority of splenic lymphoid cells after primary immunization. *Eur. J. Immunol.* 4:138.

30. Andersson, J., K. O. Grönvik, E. L. Larsson, and A. Coutinho. 1979. Studies on T lymphocyte activation. I. Requirements for the mitogen-dependent production of T cell growth factors. *Eur. J. Immunol.* 9:581.