Proc. Natl. Acad. Sci. USA
Vol. 74, No. 3, pp. 1200–1203, March 1977
Immunology

Endotoxin-induced serum factor controlling differentiation of bone-marrow-derived lymphocytes

(induction/helper factor/antibody production)

MICHAEL K. HOFFMANN, HERBERT F. OETTGEN, LLOYD J. OLD, ANN F. CHIN, AND ULRICH HAMMERLING

Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, N.Y. 10021

Communicated by Lewis Thomas, December 2, 1976

ABSTRACT **Serum from bacillus Calmette-Guerin-infected mice injected with endotoxin induces the appearance of surface immunoglobulin, Ia antigen, and complement receptor on the surface of precursor bone-marrow-derived (B) cells. While endotoxin itself causes phenotypic conversion of both thymus-derived (T) cells and B cells *in vitro*, the endotoxin-induced serum factor was found to be a selective inducer of B cell differentiation. Spleen cells rendered immunodeficient by removal of B cells bearing the complement receptor regained the capacity to cooperate with helper T cells and to produce antibody against red cell antigens *in vitro* upon addition of the serum factor to the culture medium. Thus, a factor that controls selective phenotypic and functional differentiation of B cells has been identified and can now be characterized.**

Lymphocyte differentiation proceeds through a sequence of steps which can be dissected by analyzing the expression of distinctive cell surface components. In the mouse, mature bone-marrow-derived (B) cells have been shown to carry Ig (immunoglobulin) (1, 2), Ia (I-region-controlled antigen) (3), complement receptors (CR) (4), Fc receptors (5) and PC antigens (6), whereas thymus-derived (T) cells characteristically exhibit Thy-1 (thymocyte alloantigen) (7) and Ly antigens (8–10) and also, where genetically appropriate, TL antigens (11). Expression of these markers on the surface of defined populations of precursor cells can be induced *in vitro* in an assay originally described by Komuro and Boyse (12). A number of agents induce the appearance of both B and T cell markers (13). Thymopoietin (14) acts more selectively; it induces differentiation of T cells but not of B cells (15). An agent which induces differentiation of B cells only has yet to be described for the mouse. In chickens, however, a factor isolated from the bursa by Brand *et al.* behaves like a selective B cell inducer (16).

We have reported recently that an endotoxin-induced serum factor in the mouse can replace helper T cells in the immune response to sheep erythrocytes (SRBC) *in vitro* (17). The factor is found in the serum of bacillus Calmette-Guerin (BCG)-infected mice injected with endotoxin (18); serum from normal mice, BCG-infected mice, or from normal mice injected with endotoxin is inactive. Interest in the properties of this serum was stimulated by our finding that it causes acute necrosis of a variety of transplanted tumors in the mouse (18). For this reason the serum has been designated tumor necrosis serum (TNS), and will be referred to as such throughout this report. The tumor necrotizing factor (TNF) of TNS migrates with α_2-macroglobulins, and partial purification indicates that it is a glycoprotein with a molecular weight of approximately 150,000 (19).

We have now found that TNS induces structural and functional changes of B cells *in vitro* but does not induce differentiation of prothymocytes under conditions where thymopoietin is active.

Abbreviations: B cell, bone-marrow-derived cell; T cell, thymus-derived cell; CR, complement receptor; SRBC, sheep erythrocytes; BCG, bacillus Calmette Guerin; TNS, tumor necrosis serum; TNF, tumor necrosis factor; Ia, immune response associated antigen; LPS, bacterial lipopolysaccharide; BSS, balanced salt solution (22); Seph. column, column consisting of Sephadex G-10; Ig, immunoglobulin; Thy-1, thymocyte alloantigen; Ia, I-region-controlled antigen.

MATERIALS AND METHODS

Mice. (C57BL/6 × A)F_1 mice from our colony and (C57BL/6 × DBA/2)F_1 (BDF_1) female mice from Jackson Laboratory, Bar Harbor, Maine, were used. All mice were 3–4 weeks of age at the time of study.

Antisera—Anti-Ia^k. A.TH mice were immunized with lymphoid tissues of A.TL mice. Hyperimmune serum was absorbed with (C57BL/6 × A)F_1 thymus cells (13).

Anti-Ig. Antisera to myeloma MOPC 104 E or to $F(ab)_2$ fragments of myeloma MOPC 21 were raised in rabbits. Antibodies were isolated by absorption on IgM or IgG/$F(ab)_2$ immunadsorbants and subsequent acid elution (13).

Anti-Thy-1.2. (A/Thy-1.1 × AKR/H-2b)F_1 mice were immunized with the A strain leukemia ASL1 (13).

Anti-SRBC. The 19S fraction of rabbit antiserum to SRBC was obtained from Cordis Laboratories (Miami, Fla.).

Induction Assays. These were performed, as described, for the following surface markers: Thy-1.2 (12), Ig (20), immune response associated antigen (Ia) (13), and CR (13).

Inducing Agents. *Escherichia coli* lipopolysaccharide (LPS) was provided by C. Galanos (Freiburg, Germany); calf-thymopoietin and synthetic thymopoietin by G. Goldstein of this Institute. TNS was prepared in CD-1 mice infected with BCG (Tice strain, Institute for Tuberculosis Research, University of Illinois Medical Center, Chicago, Ill.) by injecting 2×10^7 viable organisms intravenously. Fourteen days after BCG infection, mice were injected with 25 μg of endotoxin (lipopolysaccharide W from *E. coli*, Difco, Detroit, Mich.) intravenously and exsanguinated 2 hr later (18).

Enumeration of Cells Carrying Induced Surface Markers. Cells carrying Thy-1.2, Ig, or Ia were identified by assays of complement-dependent cytotoxicity (12, 21); cells carrying CR were assayed by the rosette assay (4).

Assays of Functional Activity. The culture system described by Mishell and Dutton was used (22).

Preparation of Columns. Sephadex G-10 columns were prepared as described by Ly and Mishell (23) for the removal of macrophages from mouse spleen cells. One volume of Sephadex G-10 was washed five times in five volumes of distilled water and twice in saline. Aliquots (30 ml) of Sephadex G-10 in saline were autoclaved at 110° for 30 min and stored at room temperature. Columns were prepared by loading sterile 10 ml plastic syringe barrels with 8 ml of Sephadex G-10, warmed up to 42°, and the Sephadex was washed with 20 ml of balanced

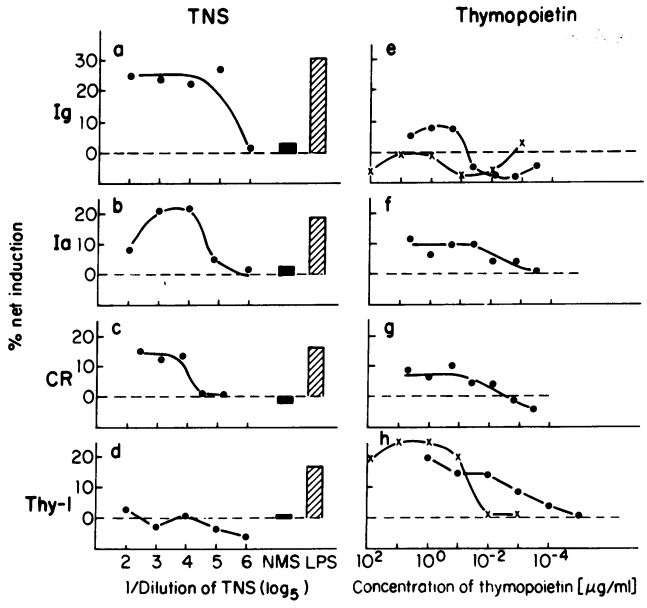

FIG. 1. Comparative activity of TNS and thymopoietin in inducing B cell and T cell markers. Bone marrow cells or spleen cells of (C57BL/6 × A)F_1 mice, 3–4 weeks of age, were fractionated on a discontinuous bovine serum albumin gradient. Cells from the 23 to 26% interface were cultured at a density of 5×10^6 cells per ml in the presence of different dilutions of TNS (*left panels*), 1:25 diluted normal mouse serum (NMS) (solid bar), LPS at 30 μg/ml (shaded bar), thymopoietin (*right panels*, ●—●) or synthetic thymopoietin (*right panels*, x—x), or with no addition (control cultures, broken line). The percentage of cells expressing Ig (*panels a and e*), Ia (*panels b and f*), CR (*panels c and g*), and Thy-1 (*panels d and h*) was determined by standard cytotoxicity or rosette-formation techniques (3, 15). The percent net induction represents the difference of cytotoxicity indices or rosette-forming cells between cultures with inducing agent and control cultures without agent. Cytotoxicity indices were computed according to the formula $(a - b)/c \times 100$ where a = percent of nonviable cells in antiserum and complement, b = percent of nonviable cells in complement, and c = percent of viable cells in complement.

salt solution (BSS), also at 42°. This column will be referred to as Seph column. Seph-Ag columns were prepared by incubating Seph columns with 5 ml of a solution of concanavalin A in BSS (1 mg/ml) for 10 min at room temperature. Con A that was not bound to Sephadex was eluted with 20 ml of BSS. Seph-Ag-Ab columns were prepared by incubating Seph-Ag columns with rabbit antiserum against Con A (diluted 1:20 in BSS) at room temperature, and washing them with 20 ml of BSS. Seph-Ag-Ab-C columns were prepared by incubating Seph-Ag-Ab columns with serum of BDF_1 mice (diluted 1:10 in BSS) for 45 min at 37°. Before addition of spleen cells, each column was washed with 20 ml of BSS (42°), and 5 ml of BSS containing 5% fetal calf serum at 42°. Spleen cells in complete culture medium were kept on ice until used. One milliliter of the cell suspension (10^8 cells) was passed through the column, followed by 5 ml of BSS containing 5% fetal calf serum. The cells recovered in the eluate were washed once in medium before culturing (24).

RESULTS

Induction of lymphocyte surface markers by TNS and thymopoietin

Bone marrow cells [for induction of BAF, Ig, Ia, and Thy-1 (T cell marker)] or spleen cells (for induction of CR) of BDF_1 mice were separated on a discontinuous bovine serum albumin gradient (13, 25) and the cells of the C layer (the 23–26% interface), containing most of the inducible cells, were cultured for 2.5 hr with or without the inducing agent. Cultures were assayed for the appearance of cells carrying the surface markers Ig, Ia, CR, and Thy-1. The combined results of several experiments are shown in Fig. 1. Endotoxin at a standard concentration of 30 μg/ml induced all three B cell markers as well as the T cell marker Thy-1, which is in agreement with the earlier reports (12, 13, 26). TNS induced Ig, Ia, and CR, but not Thy-1. Induction of Ig occurred at a serum dilution of 1:10,000, induction of Ia and CR required a roughly 10-fold higher concentration of TNS (endpoint of induction, approximately 1:625). By contrast, calf thymopoietin induced Thy-1 at concentrations as low as 0.1 ng/ml (Fig. 1), but not Ig, Ia, or CR. Synthetic thymopoietin, a hexadecapeptide (provided by G. Goldstein) did not induce B cell surface markers in the wide dose range tested. Serum from mice that had been treated with either BCG or LPS alone (as well as serum from untreated mice) was inactive.

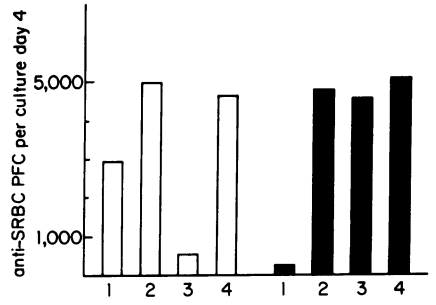

FIG. 2. Restoration of antibody production by spleen cells depleted of macrophages (open bars) or macrophages and CR^+ spleen cells (black bars) by TNS, 1% or LPS, 10 μg/ml (mean of two cultures). (*1*) no addition, (*2*) TNS added, (*3*) LPS added, (*4*) LPS + 1% NMS added. PFC, plaque-forming cells.

Induction of antibody production *in vitro* by TNS

We have shown previously (24) that mouse spleen cells, depleted of CR^+ cells, are deficient in antibody production to SRBC *in vitro*. (Responsiveness of CR^- spleen cells can be restored by addition of macrophages and primed helper T cells.) We proposed that CR^+ cells are the immediate precursors of antibody-secreting cells, and that stimulation by antigen induces differentiation of CR^+ B cells to antibody-secreting cells. If this were true, induction of CR should confer the ability to produce antibody in response to stimulation by antigen. To see whether this occurs, the following experiments were performed.

CR^+ cells and macrophages were eliminated from suspensions of spleen cells by passage through Sephadex G-10 columns coated with antigen/antibody/complement complexes (24). Control cells were passed through uncoated Sephadex G-10 columns (which remove only macrophages) (23). The cells (5×10^6 per ml) were then cultured as described by Mishell and Dutton (22) except that the medium was supplemented with 2-mercaptoethanol to compensate for the lack of macrophages (27). Antibody production to SRBC by CR^+ and CR^- spleen cells was assayed under four conditions (Fig. 2): (*i*) no addition, (*ii*) TNS added, (*iii*) endotoxin added, and (*iv*) endotoxin and normal mouse serum added. As expected, CR^+ spleen cells produced antibody but CR^- spleen cells did not. Addition of TNS enabled CR^- spleen cells to produce antibody (and increased antibody production by CR^+ spleen cells). Addition of LPS had the same effect on CR^- spleen cells, but suppressed antibody production by CR^+ spleen cells (28). This inhibitory effect of endotoxin was reversed by adding normal mouse serum to the cultures.

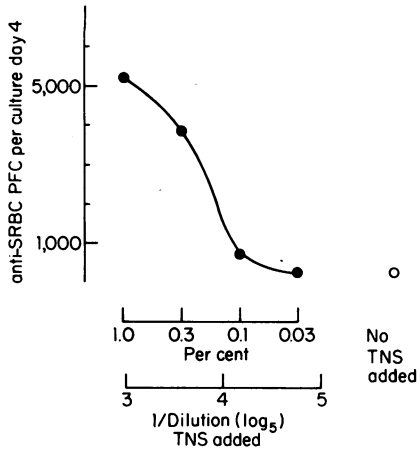

FIG. 3. Relationship between restoration of antibody production and concentration of TNS in cultures of CR^- mouse spleen cells (mean of two cultures). PFC, plaque-forming cells.

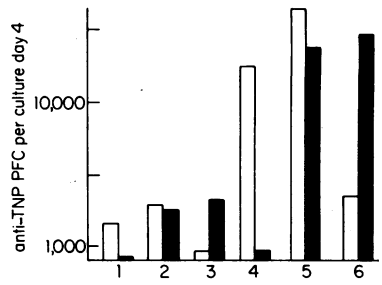

FIG. 4. Interaction of CR^- spleen cells with primed helper T cells in presence of TNS (or LPS), as measured by anti-hapten (TNP) plaque-forming cells response. CR^- spleen cells (black bars) or CR^+ spleen cells (open bars) were cultured (*1*) with no addition, (*2*) with 1% TNS, (*3*) with 10 μg/ml of LPS, (*4*) with 5×10^5 CR^- SRBC-primed spleen cells, (*5*) as (*4*), with 1% TNS, (*6*) as (*4*), with 10 μg/ml of LPS.

Influence of TNS on collaboration of B cells with primed helper T cells

We have shown that TNS (*i*) induces $CR^- \rightarrow CR^+$ conversion in spleen cells and (*ii*) enables CR^- spleen cells to produce antibody under conditions where they do not produce antibody in its absence. While there is no direct evidence that expression of CR is a prerequisite for antibody formation, the similar dose-response relation between TNS and augmented antibody production by CR^- spleen cells (Fig. 3) and between TNS and induced expression of CR, is compatible with this view. Further support comes from experiments, where cooperation between primed helper T cells and CR^- or CR^+ spleen cells was measured. Spleen cells from mice immunized with SRBC, and depleted of CR^+ cells (and macrophages) by passage through a Sephadex G-10 column coated with antibody/antigen/complement (24), were used as a source of primed helper T cells. One part SRBC-primed spleen cells was mixed with 9 parts unprimed CR^+ or CR^- spleen cells, and the cultures were challenged with trinitrophenyl-conjugated SRBC (29). As shown in Fig. 4, addition of primed helper T cells resulted in considerably increased antibody production by CR^+ spleen cells, but not by CR^- spleen cells. Addition of TNS and primed helper T cells increased greatly the response of CR^- spleen cells. In the absence of helper cells, TNS restores the response of CR^- spleen cells only to the level of the primary response observed with cultures of CR^+ spleen cells (see also Fig. 2). The additional increase seen here in the presence of helper T cells suggests that TNS enables CR^- B cells to cooperate with them. [Endotoxin had the same action on cultures of CR^- cells, but suppressed in the absence of normal mouse serum the response of CR^+ spleen cells (17).] As it has been shown that CR^- spleen cells are deficient in their capacity to cooperate with helper T cells (24), the results are consistent with the suggestion that the TNS-induced differentiation of $CR^- \rightarrow CR^+$ cells is the basis for the observed change in functional capacity.

DISCUSSION

Cell surface markers characteristic for B lymphocytes and T lymphocytes can be induced in precursor cells by a variety of agents (13, 15). A feature shared by all such agents is that they raise intracellular levels of cyclic AMP, and an increased ratio of cyclic AMP/cyclic GMP has been considered a signal of induction (30). To the extent that inducing agents act on surface receptors common to T and B lymphocyte precursors such as β-adrenergic receptors, they can be expected to induce phenotypic conversion of precursor cells belonging to both the T and B cell series. This has indeed been demonstrated for epinephrine, isoproterenol, and ubiquitin (15). Other inducing agents are more selective in their action. Thymopoietin, for example, induces differentiation of T cell precursors but not B cell precursors at subnanogram concentration (induction of B cell markers is seen only at much higher thymopoietin concentrations). TNS, on the other hand, appears to induce differentiation of B cell precursors but not T cell precursors. The selective action on B cell precursors argues against the possibility that residual LPS is the active principle (LPS induces phenotypic conversion of T and B cells at similar concentrations). Three B precursor subpopulations, characterized by the surface phenotypes $Ig^-Ia^-CR^-$, $Ig^+Ia^-CR^-$, and $Ig^+Ia^+CR^-$ acquire, on induction with TNS, Ig, Ia, or CR, respectively. They show decreasing sensitivity, in that order, to induction by TNS, and TNS does not induce the plasma cell antigen PC.1 in what are probably $Ig^+Ia^+CR^+$ precursor cells. As these inducible B cell subpopulations are thought to represent successive stages in B cell ontogeny (26) the failure to induce PC.1 may reflect the need for a unique inductive stimulus for this late differentiation step. The fact that TNS also substitutes for helper T cells (17) points to a possible connection between the signals imparted to B cells by helper T cells and the active principle in TNS—both promoting differentiation of B lymphocytes.

Induction of B cell markers by TNS was accompanied by functional maturation, measured in terms of antibody production *in vitro*. We showed previously that spleen cells depleted of CR^+ B cells are deficient in their capacity to produce antibody to SRBC in Mishell-Dutton cultures, and we proposed that CR^- B cells must differentiate to CR^+ B cells for optimal cooperation with helper T cells. This view is now supported by the finding that both TNS and LPS (which induce CR on the surface of CR^- B cells) restore the primary antibody response of CR^- spleen cells to SRBC, and permit the cooperation of CR^- B cells with primed helper T cells.

Whether CR merely marks a crucial stage in B cell differentiation, or represents a cell surface component essential for the cooperation of T and B cells, is at the moment unclear. It should be emphasized that the inability of CR^- spleen cells to produce antibody *in vitro* may well depend on the procedure and conditions used in the experiments described here. While some published studies are in agreement with our results as far as this deficiency of CR^- spleen cells is concerned (31, 32), others are not (33). In part, conflicting results may be due to differences in the methods that have been used for the purifi-

cation of CR^- cells. Comparative studies with CR^- B cells obtained by various techniques are now in progress.

We have recently found that peritoneal macrophages from normal mice enable CR^- spleen cells to cooperate with primed helper T cells (but not with unprimed T cells), and that the supernatant of cultures of activated peritoneal macrophages (but not of nonactivated macrophages) supports, like TNS, the cooperation of CR^- B cells with both primed and unprimed helper T cells (24). This points to the activated macrophage as a possible source of the active factor in TNS, and proliferation and activation of macrophages is in fact the most striking phenomenon during the priming phase for TNS-production (18). Some effects of TNF, on the other hand, mimic effects of factors assumed to be T cell-derived (M. K. Hoffmann and U. Hammerling, unpublished observations). Current attempts to determine the cellular source of the factors that have now been defined in TNS, including (*i*) the B cell-inducing factor, (*ii*) the T cell helper factor, (*iii*) tumor necrosis factor (TNF), and (*iv*) the cytotoxic factor for tumor cells *in vitro*, and to ascertain whether these functional activities reside in a single molecule by chromatographic separation and other procedures have not succeeded.

This work was supported in part by grants from the National Institutes of Health (CA17673, CA16889, CA17085, and HO-08415), and by the American Cancer Society Grants (IM82 and IM87).

1. Raff, M. C., Sternberg, M. & Taylor, R. B. (1970) "Immunoglobulin determinants on the surface of mouse lymphoid cells," *Nature* **225**, 553–554.
2. Takahashi, T., Old, L. J., McIntire, R. K. & Boyse, E. A. (1971) "Immunoglobulin and other surface antigens of cells of the immune system," *J. Exp. Med.* **134**, 815–832.
3. Hammerling, G., Deak, B. D., Mauve, G., Hammerling, U. & McDevitt, H. O. (1974) "B lymphocyte alloantigens controlled by the I region of the major histocompatibility complex in mice," *Immunogenetics* **1**, 68–81.
4. Bianco, C., Patrick, R. & Nussenzweig, V. (1970) "A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes," *J. Exp. Med.* **132**, 702–720.
5. Dickler, H. & Kunkel, H. G. (1972) "Interaction of aggregated gammaglobulin with B lymphocytes," *J. Exp. Med.* **136**, 191–196.
6. Takahashi, T., Old, L. J. & Boyse, E. A. (1970) "Surface alloantigens of plasma cells," *J. Exp. Med.* **131**, 1325–1341.
7. Reif, A. E. & Allen, J. M. V. (1964) "The AKR thymic antigen and its distribution in leukemias and nervous tissues," *J. Exp. Med.* **120**, 413–434.
8. Boyse, E. A., Miyazawa, M., Aoki, T. & Old, L. J. (1968) "Ly-A and Ly-B: two systems of lymphocyte isoantigens in the mouse," *Proc. Roy. Soc. London, Ser. B.* **170**, 175–193.
9. Shiku, H., Kisielow, P., Bean, M. A., Takahashi, T., Boyse, E. A., Oettgen, H. F. & Old, L. J. (1975) "Expression of T-cell differentiation antigens on effector cells in cell-mediated cytotoxicity *in vitro*. Evidence for functional heterogeneity related to the surface phenotype of T cells," *J. Exp. Med.* **141**, 1376–1389.
10. Kisielow, P. Hirst, J. A., Shiku, H., Beverley, P. C. L., Hoffmann, M. K., Boyse, E. A. & Oettgen, H. F. (1975) "Ly antigens as markers for functionally distinct subpopulations of thymus-derived lymphocytes of the mouse," *Nature* **253**, 219–220.
11. Boyse, E. A., Old, L. J. & Stockert, E. (1965) in *Immunopathology IV International Symposium, Monaco*, eds. Grabar, P. & Miescher, P. A. (Schwabe and Co., Basel, Switzerland), pp. 23–40.
12. Komuro, K. & Boyse, E. A. (1973) "*In vitro* demonstration of thymic hormone in the mouse by conversion of precursor cells into lymphocytes," *Lancet* **i**, 740–743.
13. Hammerling, U., Chin, A. F., Abbott, J. & Scheid, M. P. (1975) "The ontogeny of murine lymphocytes. I. Induction of phenotypic conversion of Ia^- to Ia^+ lymphocytes," *J. Immunol.* **115**, 1425–1431.
14. Goldstein, G. (1974) "Isolation of bovine thymin: A polypeptide hormone of the thymus," *Nature* **247**, 11–14.
15. Scheid, M. P., Goldstein, G., Hammerling, U. & Boyse, E. A. (1975) "Lymphocyte differentiation from precursor cells *in vitro*," *Ann. N.Y. Acad. Sci.* **249**, 531–538.
16. Brand, A., Gilmour, D. G. & Goldstein, G. (1976) "Lymphocyte-differentiating hormone of bursa of Fabricius," *Science* **193**, 319–321.
17. Hoffmann, M. K., Green, S., Old, L. J. & Oettgen, H. F. (1976) "Serum containing endotoxin-induced tumor necrosis factor substitutes for helper T cells," *Nature* **263**, 416–417.
18. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) "An endotoxin-induced serum factor that causes necrosis of tumors," *Proc. Natl. Acad. Sci. USA* **72**, 3666–3670.
19. Green, S., Dobransky, A., Carswell, E. A., Kassel, R. L., Old, L. J., Fiore, N. & Schwartz, M. (1976) "Partial purification of a serum factor that causes necrosis of tumors," *Proc. Natl. Acad. Sci. USA* **73**, 381–385.
20. Abbott, J., Hoffmann, M. K., Chin, A. F. & Hammerling, U. (1976) in *Progress in Differentiation Research*, ed. Muller-Berat, N. (North-Holland Publishing Co., Amsterdam, The Netherlands), pp. 545–550.
21. Gorer, P. A. & O'Gorman, P. (1956) "The cytotoxic activity of isoantibodies in mice," *Transplant. Bull.* **3**, 142–144.
22. Mishell, R. I. & Dutton, R. W. (1967) "Immunization of dissociated spleen cell cultures from normal mice," *J. Exp. Med.* **126**, 423–442.
23. Ly, I. A. & Mishell, R. I. (1974) "Separation of mouse spleen cells by passage through columns of Sephadex G-10," *J. Immunol. Method* **5**, 239–247.
24. Hoffmann, M. K., Hammerling, U., Simon, M. & Oettgen, H. F. (1976) "Macrophage requirements of CR^- and CR^+ B-lymphocytes for antibody production *in vitro*," *J. Immunol.* **116**, 1447–1451.
25. Dicke, K. A., Lina, P. H. C. & van Bekkum, D. W. (1970) "Adaptation of albumin density gradient centrifugation to human bone marrow fractionation," *Rev. Eur. Etud. Clin. Biol.* **15**, 305–308.
26. Hammerling, U., Chin, A. F. & Abbott, J. (1976) "Ontogeny of murine B lymphocytes: Sequence of B-cell differentiation from surface-immuno-globulin-negative precursors to plasma cells," *Proc. Natl. Acad. Sci. USA* **73**, 2008–2012.
27. Chang-Chen, M. B. & Hirsch, J. A. (1972) "Restoration of antibody-forming capacity in cultures of nonadherent spleen cells by mercaptoethanol," *Science* **176**, 60–61.
28. Hoffmann, M. K., Weiss, O., Koenig, S., Hirst, J. A. & Oettgen, H. F. (1975) "Suppression and enhancement of the T cell-dependent production of antibody to SRBC *in vitro* by bacterial lipopolysaccharide," *J. Immunol.* **114**, 738–741.
29. Hoffmann, M. K. & Kappler, J. W. (1973) "Regulation of the immune response. II. Qualitative and quantitative differences between thymus- and bone marrow-derived lymphocytes in the recognition of antigen," *J. Exp. Med.* **137**, 721–739.
30. Hammerling, U., Chin, A. F., Abbott, J., Goldstein, G., Sonenberg, M. & Hoffmann, M. K. (1977) "The ontogeny of B lymphocytes. III. Opposite signals transmitted to B lymphocyte precursor cells by inducing agents and hormones," *Eur. J. Immunol.*, in press.
31. Mason, D. W. (1976) "The requirement for C3 receptors on the precursors of 19S and 7S antibody forming cells," *J. Exp. Med.* **143**, 1111–1121.
32. Dukor, P., Dietrich, F. M., Gisler, R. H., Schumann, G. & Bitter-Suermann, D. (1974) in *Progress in Immunology, II*, eds. Brent, L. & Holborow, J. (North-Holland Publishing Co., Amsterdam), Vol. 3, p. 99.
33. Parish, C. R. (1975) "Separation and functional analysis of subpopulations of lymphocytes bearing complement and Fc receptors," *Transplant. Rev.* **25**, 98–101.