Clin. exp. Immunol. (1980) 39, 257–262.

REVIEW

Current perspectives on the cellular mechanisms of immunologic tolerance

D. E. PARKS & W. O. WEIGLE *Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California, USA*

(*Accepted for publication* 13 *August* 1979)

The ultimate outcome of the exposure of an antigenic substance to the immune system is determined by a variety of factors including the dose of antigen, the form of the antigen within the host, the route of exposure to the antigen, and the immunocompetence of the host. Although the rapid triggering of B lymphocytes to differentiate and synthesize antibody is most commonly associated with exposure to a foreign antigen, this type of response represents only one of the several cellular events initiated by encounter with antigen. Potent immunoregulatory processes are established as a matter of course which regulate the class of antigen, the type of cellular response and the magnitude and duration of the immune response to the antigen. Additionally, a state of intrinsic unresponsiveness in which antigen responsive cells are irreversibly inactivated or deleted can result from antigen exposure. Such immunologic unresponsiveness appears to be the mechanism by which tolerance to self constituents is established very early and maintained intact for the life of the host. The maintenance of tolerance to self is essential to the continued health of the host because any circumvention of this unresponsiveness will result in an autoimmune state possibly leading to disease.

Models of tolerance envisioning the establishment of an intrinsic unresponsive state by the permanent inactivation of responsive cells dominated the theory of tolerance for many years following Burnet's presentation of his clonal deletion theory (Burnet, 1959). However, our understanding of the immune system and the sophisticated regulatory mechanisms embodied in that system have expanded. Attention has been focused on the possibility that suppressive mechanisms may be sufficiently effective or potent to mediate immunologic unresponsiveness and that responsive cells theoretically may not be inactivated, but rather may coexist with such suppressive mechanisms in the tolerant host. Although antigen-specific suppressor cells have been demonstrated in many systems of immunologic unresponsiveness, it has been difficult to establish that these suppressor cells are responsible for the unresponsive state. Alternatively, these cells may be a by-product of the induction of antigen-specific unresponsiveness and serve as the second line of defence, a 'fail-safe' mechanism (Benjamin, 1977a; Doyle *et al.*, 1979) available to maintain unresponsiveness.

EVIDENCE FOR IRREVERSIBLE INACTIVATION OF B LYMPHOCYTES

A less ambiguous approach to determine whether unresponsiveness is established and maintained in the presence or absence of responsive cells is to probe for such cells in states of unresponsiveness. The experimentally induced unresponsive state established to human gammaglobulin (HGG) by a single injection of deaggregated HGG (DHGG) into adult mice is well suited for such investigations. This well characterized state of complete, long-lived specific unresponsiveness is easily established in both helper T and B lymphocytes and can be readily manipulated (reviewed in Doyle *et al.*, 1979). Furthermore, although suppressor cells can be demonstrated, (Basten, 1974; Benjamin, 1975; Doyle, Parks & Weigle, 1976a, b)

Correspondence: D. Elliot Parks, Ph.D., Department of Immunopathology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037, USA.

0099-9104/80/0200-0257$02.00

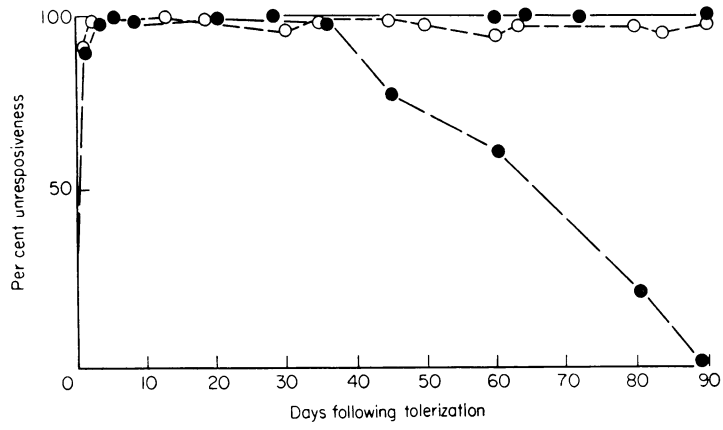

FIG. 1. The kinetics of spontaneous reacquisition of responsive B cells in tolerant A/J mice. 400 μg of antigenic aggregated human gammaglobulin (○ – – – ○, AHGG) and/or 50 μg of lipopolysaccharide (●——●, LPS) were injected at various times after tolerization with 2·5 mg of deaggregated HGG. The indirect plaque-forming cell (PFC) response to HGG in the spleen was determined 6 days after challenge. The per cent unresponsiveness equals 100−(PFC/10^6 for tolerized mice÷PFC/10^6 for control mice)×100. Control mice received AHGG alone. (● – – – ●) AHGG + LPS.

they do not appear to be responsible for the induction or maintenance of this unresponsive state (reviewed below and in Parks & Weigle, 1979a).

The polyclonal B cell activator, bacterial lipopolysaccharide (LPS) has proven to be a useful tool in investigating responsiveness in B lymphocytes. When injected with antigen, LPS can circumvent the requirement for specific T cell help in the response to T dependent antigen and can stimulate directly antigen-specific B cells in the absence of antigen-specific T cells. Therefore, the presence of antigen-responsive B cells in tolerant animals lacking responsive T cells can be demonstrated by the injection of antigen and LPS (Chiller & Weigle, 1973a). Use of this protocol (Parks & Weigle, 1979b, 1980) has allowed determination of the kinetics of reacquisition of responsiveness in antigen-specific B cells following tolerization with DHGG (Fig. 1). Most T and B cells are rendered unresponsive very rapidly and remain unresponsive for at least 6 weeks after tolerization. After that time HGG-responsive B cells gradually repopulate the spleen of tolerant animals as determined by challenge with antigen (aggregated HGG) and LPS, while helper T cells remain unresponsive for a considerably longer period of time as assessed by challenge with antigen alone.

Exposure of B lymphocytes to LPS has been reported to stimulate polyclonally antigen-responsive B cells possessing LPS reactivity. This polyclonal reactivity to LPS has fostered attempts to demonstrate responsive B cells in states of unresponsiveness established by receptor blockade (Gronowicz & Coutinho, 1975). Receptor blockade can be established by antigen masking or blockading antigen receptors on B cells thereby inhibiting otherwise responsive cells (Aldo-Benson & Borel, 1974; Schrader, 1974b). This inhibition of responsive cells has been demonstrated unambiguously to be temporary and reversible by incubating the cells in the absence of antigen (Gronowicz & Coutinho, 1975) or by enzymatic removal of blockading antigen (Fernandez & Möller, 1978).

However, unresponsiveness to HGG does not appear to be maintained by receptor blockade. Responsive B cells can not be demonstrated in either the bone marrow or spleen of tolerant animals until at least 7 weeks after tolerization by adoptive transfer (reviewed in Doyle *et al.*, 1979). Furthermore, the injection of the polyclonal B cell activator LPS with antigen is unable to demonstrate antigen-responsive cells (Fig. 1) before they can be detected by cell transfer. To exclude further the proposal that tolerogen is blockading or masking the antigen receptors on putatively responsive B cells, spleen cells have been removed from tolerant mice and washed extensively before transfer into lethally irradiated recipients. The pattern of responsiveness to challenge with antigen alone or antigen and LPS after these procedures

is unchanged from that of untransferred spleen cells in the tolerant donors (Parks & Weigle, 1979b, 1980). These data suggest that receptor blockade cannot be implicated in this tolerant state.

Receptor blockade may be responsible for the establishment for unresponsiveness by certain antigens but its role as a universal mechanism of tolerance cannot be supported by evidence from other antigen systems. In addition to the data discussed above from HGG tolerant mice, evidence supporting irreversible inactivation of B lymphocytes has been presented in several unresponsive states by others (Nossal & Pike, 1978; Venkataraman & Scott, 1977; Elson & Taylor, 1977). Furthermore, unresponsive states in which receptor blockade is established initially but supplanted rapidly by irreversible inactivation of B cells have been described (Klaus, 1976; Fidler, 1979).

The data indicating that receptor blockade is not responsible of unresponsiveness to HGG are strengthened by the fact that this approach employs an antigen-directed system which focuses on antigen-responsive B cells. On the other hand, the use of polyclonal B cell activators alone to overcome receptor blockade possesses inherent shortcomings. First, data from polyclonal activation are more ambiguous than direct means of demonstrating responsive B cells such as enzymatic digestion and antigen-free incubation which remove the interfering antigen from the cell membrane. Second, polyclonal activators stimulate B cells independent of their antigenic specificity thereby releasing antibodies of lower average affinities than those released by antigenic stimulation which is affinity dependent (Andersson, Sjoberg & Möller, 1972; Nilsson, Sultzer & Bullock, 1973; Coutinho *et al.*, 1974). It has been reported that polyclonal B cell activation stimulates fewer cells which release high affinity antibody than does activation by specific antigen (Coutinho *et al.*, 1974). Therefore, the majority of the cells stimulated by polyclonal B cell activation may possess Ig receptors bearing antigen affinities below the threshold required to bind sufficient antigen to mediate either antigenic or tolerogenic stimulation. Even in unresponsive states where antigen-responsive B cells have been inactivated irreversibly, low affinity antibodies may be detected by polyclonal B cell activation (Scott, Venkataraman & Jandinski, 1979; Venkataraman & Scott, 1979).

The detection of low affinity antibody-producing cells which escape affinity dependent tolerization may explain the low levels of polyclonally induced antibody to self antigen reported following polyclonal activation (Primi *et al.*, 1977; Izui *et al.*, 1977; Ortiz-Ortiz *et al.*, 1980). Self antigen alone may be unable to induce these B cells which may have little relevance to the aetiology of autoimmune disease. The production of antibody-mediated autoimmune disease would normally be everted because helper T cells are tolerant to nearly all T dependent self antigens and because large concentrations of polyclonal B cell activator would be required to release the low affinity antibody from untolerized B cells. The cells detected by polyclonal B cell activation may represent a threat to self-tolerance only in those cases in which cells and/or antibody of low affinity can promote autoimmune pathology. The formation of large aggregates of complexes of rheumatoid factor and self antigen may represent one such example of autoimmune disease mediated by polyclonal B cell activation of low affinity antibody (Slaughter *et al.*, 1978).

Direct evidence that polyclonal activation stimulates B cells independent of their affinity for antigen has been provided by the demonstration that tolerization inactivates only the high affinity cells, leaving lower affinity cells intact and susceptible to polyclonal activation (Scott *et al.*, 1979; Venkataraman & Scott, 1979). The affinity dependence of tolerization was established sometime ago with the demonstration that antibody producing cells of higher affinities are tolerized before cells possessing lower affinities whether assessed by challenge with antigen (Theis & Siskind, 1968; Celada, Schmidt & Strom, 1969) or LPS (Gronowicz & Coutinho, 1975). Furthermore, the physical removal of high affinity cells from a lymphoid cell population renders the recipient of that depleted population unresponsive to antigen challenge (Fournier, Muller & Bach, 1978).

Considerable evidence has been amassed over the years supporting the concept of irreversible inactivation as a mechanism of immunologic tolerance as discussed above and reviewed elsewhere (Nossal *et al.*, 1979; Metcalf, Schrater & Klinman, 1979). However, an interesting restriction has been postulated recently (Fernandez & Möller, 1977, 1978; Möller & Fernandez, 1978). The latter authors have proposed, from experimental data with the T independent antigen dextran, that irreversible inactivation of B cells is accomplished through interaction between both the antigen and the receptor for polyclonal B cell

activation on B cells. Extrapolating this model to T dependent antigens, we have argued that all T dependent B cells could be inactivated irreversibly by this mechanism but that this inactivation would require the presence of helper T cells to provide a signal analogous to the polyclonal signal provided by the T independent antigen (Parks & Weigle, 1979a). All T dependent B cells are, by definition, responsive to the theoretical polyclonal signal provided by helper T cells. This mechanism has been supported by recent evidence indicating that an intrinsic polyclonal B cell activator (Con A stimulated soluble factor) is unable to activate B cells responding to self antigens whereas an extrinsic activator (LPS) can stimulate such cells (Primi *et al.*, 1978). Although the implications of this model have not been fully explored experimentally, these data are compatible with the suggestion that self antigen alone may inactivate irreversibly high affinity cells whereas antigen in concert with an intrinsic polyclonal B cell activator, possibly helper T cells, can extend this irreversible unresponsiveness to cells of lower affinities.

ROLE OF SUPPRESSOR CELLS IN IMMUNOLOGIC TOLERANCE AND AUTOIMMUNITY

Antigen-specific and antigen-non-specific suppressor cells have been implicated in the regulation of immune responsiveness not only in experimental animal models but also in human disease states. Although antigen-specific suppressor cells are apparently generated by the same immunologic processes that lead to responsiveness and are only transiently associated with immune responses, considerable speculation as to their role in the establishment of immunologic tolerance has appeared. Again, experimentally-induced unresponsiveness to human gammaglobulin has provided considerable data pertaining to suppressor cells in tolerance to this protein antigen (reviewed by Parks & Weigle, 1979a).

The basic approach to the question of the necessity for and responsibility of suppressor cells in tolerance has employed experimental attempts to establish unresponsiveness in the absence of detectable suppressor cells. Antigen-specific unresponsiveness to HGG has been established both in nude mice congenitally devoid of mature thymus-dependent cells (Parks, Doyle & Weigle, 1977; Etlinger & Chiller, 1977) and in mice surgically depleted of T cells (Chiller *et al.*, 1974). Of relevance to the induction of tolerance to self which is established early in life are the reports that the unresponsiveness established in recipients of foetal liver cells (Elson, 1977), *in utero* (Waters *et al.*, 1979), and in neonates (Benjamin, 1977b) all lack detectable suppressor cells. These findings are supported by the observation that tolerance to gammaglobulins can be established in the absence of suppressor cells by administering pharmacologic agents like cyclophosphamide (Basten *et al.*, 1974) or colchicine (Parks, Shaller & Weigle, 1979) which interfere with the induction of suppressor cells at the time of tolerization. In the latter case, the unresponsive state established in the presence of colchicine was investigated extensively and found to be indistinguishable in either the helper T cell or B cell populations from the unresponsiveness established in the presence of suppressor cells (Parks *et al.*, 1979). Several other reports of the establishment of unresponsiveness lacking suppressor cells have been reported with HGG (Chiller & Weigle, 1973b; Chiller *et al.*, 1974; Zolla & Naor, 1974; Parks, Doyle & Weigle, 1978), sheep gammaglobulin (Scott, 1973) and fowl gammaglobulin (Schrader, 1974a).

The failure to re-induce suppressor cells in tolerant animals after the initial, transient suppressor cells had disappeared also argues against the role for suppressor cells in the prevention of self-reactivity (Benjamin, 1977a). Because memory suppressor cells can be generated by exposure to HGG (Loblay, Pritchard-Briscoe & Basten, 1978) this inability to re-establish suppression indicates that suppressor cells or their precursors may be rendered unresponsive as are helper T cells and B cells. These findings imply that antigens present at birth and remaining in contact with the immune system in tolerogenic concentrations throughout life may establish a persistent unresponsiveness in suppressor T cell populations. Although the susceptibility of suppressor cells to tolerization has not been established experimentally and the relative amounts of antigen required to tolerize helper *vs* suppressor T cells have not been determined, tolerization of suppressor cells could preclude the activity of these T cells in the maintenance of tolerance to most self antigens. The presence of suppressor T cells specific for self antigens in the absence of responsive helper T cells may therefore identify an incomplete unresponsive state in which

suppressor cells have avoided the induction of tolerance or escaped its maintenance.

In the light of numerous observations that suppressor cells are neither necessary for the establishment of immunologic unresponsiveness nor the mandatory result of such an unresponsive state, they cannot be invoked as a likely mechanism for the induction or maintenance of immunologic unresponsiveness to self. The detection of suppressor cells in an unresponsive state is not sufficient evidence to implicate these cells as the mechanism for this unresponsiveness. Suppressor cells should only be considered responsible for unresponsiveness when they can be shown to parallel the unresponsive state both in magnitude and in duration. Even then, care must be taken to establish that suppressor cells are responsible for the unresponsive state and not merely an ancillary result of the establishment of unresponsiveness. Such ancillary cells may provide a secondary mechanism reinforcing the unresponsive state but not responsible for it.

This is Publication No. 1863 from the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California. Supported in part by United States Public Health Service grants AI-07007 and AG-00783, American Cancer Society grant IM-421, and Biomedical Research Support Program grant RRO-5514.

D.E. Parks is a recipient of Junior Faculty Research Award JFRA-8 from the American Cancer Society.

REFERENCES

ALDO-BENSON, M. & BOREL, Y. (1974) Direct evidence for receptor blockade by tolerogen. *J. Immunol.* **112**, 1793.

ANDERSSON, J., SJOBERG, O. & MÖLLER, G. (1972) Mitogens as probes for immunocyte activation and cellular cooperation. *Transplant. Rev.* **11**, 131.

BASTEN, A. (1974) Specific suppression of the immune response by T cells. *Immunological Tolerance: Mechanisms and Potential Therapeutic Applications.* (Ed. by D.H. Katz and B. Benacerraf), p. 107. Academic Press, New York.

BASTEN, A., MILLER, J.F.A.P., SPRENT, J. & CHEERS, C. (1974) Cell-to-cell interaction in the immune response. X. T-cell-dependent suppression in tolerant mice. *J. exp. Med.* **140**, 199.

BENJAMIN, D.C. (1975) Evidence for specific suppression in the maintenance of immunologic tolerance. *J. exp. Med.* **141**, 635.

BENJAMIN, D.C. (1977a) Suppressor cells in tolerance to HGG: kinetics and cross-suppression in high dose tolerance—absence in low dose tolerance. *J. Immunol.* **118**, 2125.

BENJAMIN, D.C. (1977b) Neonatally induced tolerance to HGG: duration in B cells and absence of specific suppressor cells. *J. Immunol.* **119**, 311.

BURNET, F.M. (1959) *The Clonal Selection Theory of Acquired Immunity.* Cambridge University Press, London.

CELADA, F., SCHMIDT, D. & STROM, R. (1969) Determination of avidity of anti-albumin antibodies in the mouse. Influence of the number of cells transferred on the quality of the secondary adoptive response. *Immunology*, **17**, 189.

CHILLER, J.M., LOUIS, J.A., SKIDMORE, B.J. & WEIGLE, W.O. (1974) Cellular parameters of the tolerant state induced to human γ globulin in mice and of its modulation by bacterial lipopolysaccharides. *Immunological Tolerance: Mechanisms and Potential Therapeutic Applications* (ed. by D.H. Katz and B. Benacerraf), p. 373. Academic Press, New York.

CHILLER, J.M. & WEIGLE, W.O. (1973a) Termination of tolerance to human gamma globulin in mice by antigen and bacterial lipopolysaccharide (endotoxin). *J. exp. Med.* **137**, 740.

CHILLER, J.M. & WEIGLE, W.O. (1973b) Restoration of immunocompetency in tolerant lymphoid cell populations by cellular supplementation. *J. Immunol.* **110**, 1051.

COUTINHO, A., GRONOWICZ, E., BULLOCK, W.W. & MÖLLER, G. (1974) Mechanism of thymus-independent immunocyte triggering. Mitogenic activation of B cells results in specific immune responses. *J. exp. Med.* **139**, 74.

DOYLE, M.V., PARKS, D.E. & WEIGLE, W.O. (1976a) Specific suppression of the immune response by HGG tolerant spleen cells. I. Parameters affecting the level of suppression. *J. Immunol.* **116**, 1640.

DOYLE, M.V., PARKS, D.E. & WEIGLE, W.O. (1976b) Specific, transient suppression of the immune response by HGG tolerant spleen cells. II. Effector cells and target cells. *J. Immunol.* **117**, 1152.

DOYLE, M.V., PARKS, D.E., ROMBALL, C.G. & WEIGLE W.O. (1979) Immunoregulation in tolerance and autoimmunity. *Mechanisms of Immunopathology* (ed. by P.A. Ward and R.T. McCluskey), p. 107. John Wiley & Sons, New York.

ELSON, C.J. (1977) Tolerance in differentiating B lymphocytes. *Eur. J. Immunol.* **7**, 6.

ELSON, C.J. & TAYLOR, R.B. (1977) Cellular basis of persistent tolerance induced by an aggregate free heterologous immunoglobulin. *Immunology*, **33**, 635.

ETLINGER, H.M. & CHILLER, J.M. (1977) Induction of tolerance in athymic mice with an antigen which is highly immunogenic in euthymic mice. *Cell. Immunol.* **33**, 297.

FERNANDEZ, C. & MÖLLER, G. (1977) Induction of immunological tolerance requires that the B cells can respond to the polyclonal B-cell-activating properties of the thymus-independent antigens. *J. exp. Med.* **146**, 308.

FERNANDEZ, C. & MÖLLER, G. (1978) Irreversible immunological tolerance to thymus-independent antigens is restricted to the clone of B cells having both Ig and PBA receptors for the tolerogen. *Scand. J. Immunol.* **7**, 137.

FIDLER, J.M. (1979) The induction of hapten-specific immunological tolerance and immunity in B lymphocytes. VI. Differential tolerance susceptibility in adult spleen as a function of B cell maturation level. *J. exp. Med.* **150**, 491.

FOURNIER, C., MULLER, J.-Y. & BACH, J.-F. (1978) Induction of specific unresponsiveness in normal mouse spleen cells by removing the minority of high avidity antigen-binding cells. *Cell. Immunol.* **39**, 55.

GRONOWICZ, E. & COUTINHO, A. (1975) Hapten-induced B cell paralysis. II. Evidence for trivial mechanisms of tolerance. *Eur. J. Immunol.* **5**, 413.

IZUI, S., LAMBERT, P.-H., FOURNIÉ, G.J., TÜRLER, H. & MIESCHER, P.A. (1977) Features of systemic lupus erythematosus in mice injected with bacterial lipopolysaccharides. *J. exp. Med.* **145**, 1115.

KLAUS, G.G.B. (1976) B cell tolerance induced by polymeric antigens. VI. Kinetics and reversibility of the inhibition of antibody-forming cells by antigen. *Eur. J. Immunol.* **6**, 389.

LOBLAY, R.H., PRITCHARD-BRISCOE, H. & BASTEN, A. (1978) Suppressor T-cell memory. *Nature*, **272**, 620.

METCALF, E.S., SCHRATER, A.F. & KLINMAN, N.R. (1979) Murine models of tolerance induction in developing and mature B cells. *Immunol. Rev.* **43**, 143.

MÖLLER, G. & FERNANDEZ, C. (1978) Immunological tolerance to the thymus-independent antigen dextran can be abrogated by thymus-dependent dextran conjugates: evidence against clonal deletion as the mechanism of tolerance induction. *Scand. J. Immunol.* **8**, 29.

NILSSON, B.S., SULTZER, B.M. & BULLOCK, W.W. (1973) Purified protein derivative of tuberculin induces immunoglobulin production in normal mouse spleen cells. *J. exp. Med.* **137**, 127.

NOSSAL, G.J.V. & PIKE, B.L. (1978) Mechanisms of clonal abortion tolerogenesis. I. Response of immature hapten-specific B lymphocytes. *J. exp. Med.* **148**, 1161.

NOSSAL, G.J.V., PIKE, B.L., TEALE, J.M., LAYTON, J.E., KAY, T.W. & BATTYE, F.L. (1979) Cell fractionation methods and the target cells for clonal abortion of B lymphocytes. *Immunol. Rev.* **43**, 185.

ORTIZ-ORTIZ, L., PARKS, D.E., RODRIGUEZ, M. & WEIGLE, W.O. (1979) Polyclonal B lymphocyte activation during *Trypanosoma cruzi* infection. *J. Immunol.* **124** (in press).

PARKS, D.E., DOYLE, M.V. & WEIGLE, W.O. (1977) Effect of lipopolysaccharide on immunogenicity and tolerogenicity of HGG in C57Bl/6J nude mice. Evidence for a possible B cell deficiency. *J. Immunol.* **119**, 1923.

PARKS, D.E., DOYLE, M.V. & WEIGLE, W.O. (1978) Induction and mode of action of suppressor cells generated against human gamma globulin. I. An immunologic unresponsive state devoid of demonstrable suppressor cells. *J. exp. Med.* **148**, 625.

PARKS, D.E., SHALLER, D.A. & WEIGLE, W.O. (1979) Induction and mode of action of suppressor cells generated against human gamma globulin. II. Effects of colchicine. *J. exp. Med.* **149**, 1168.

PARKS, D.E. & WEIGLE, W.O. (1979a) Regulation of B cell unresponsiveness by suppressor cells. *Immunol. Rev.* **43**, 217.

PARKS, D.E. & WEIGLE, W.O. (1979b) Evidence for irreversible inactivation of B lymphocytes in tolerance to T dependent antigens. *T and B Lymphocytes: Recognition and Function.* 1979 *ICN-UCLA Symposium on Molecular and Cellular Biology* (ed. by F.L. Bach, B. Bonavida, E.S. Vitetta and C.F. Fox), vol. 16. Academic Press, New York. (In press.)

PARKS, D.E. & WEIGLE, W.O. (1980) Maintenance of immunologic unresponsiveness to human gamma globulin: evidence for irreversible inactivation in B lymphocytes. (Submitted for publication.)

PRIMI, D., SMITH, C.I.E., HAMMARSTRÖM, L. & MÖLLER, G. (1977) Polyclonal B-cell activators induce immunological response to autologous serum proteins. *Cell. Immunol.* **34**, 367.

PRIMI, D., HAMMARSTRÖM, L., SMITH, C.I.E. & MÖLLER, G. (1978) Immunological unresponsiveness of self-recognizing B lymphocytes to the PBA property of a soluble T cell factor. *Cell. Immunol.* **41**, 320.

SCHRADER, J.W. (1974a) Induction of immunological tolerance to a thymus-dependent antigen in the absence of thymus-derived cells. *J. exp. Med.* **139**, 1303.

SCHRADER, J.W. (1974b) Effector cell blockade: a new mechanism of immune hyporeactivity induced by multivalent antigens. *J. exp. Med.* **139**, 1582.

SCOTT, D.W. (1973) Cellular events in tolerance. I. Failure to demonstrate activation of lymphocytes, blocking factors, or suppressor cells during the induction of tolerance to a soluble protein. *J. Immunol.* **111**, 789.

SCOTT, D.W., VENKATARAMAN, M. & JANDINSKI, J.J. (1979) Multiple pathways of B lymphocyte tolerance. *Immunol. Rev.* **43**, 241.

SLAUGHTER, L., CARSON, D.A., JENSEN, F.C. HOLBROOK, T.L. & VAUGHAN, J.H. (1978) *In vitro* effects of Epstein–Barr virus on peripheral blood mononuclear cells from patients with rheumatoid arthritis and normal subjects. *J. exp. Med.* **148**, 1429.

THEIS, G.A. & SISKIND, G.W. (1968) Selection of cell populations in induction of tolerance: affinity of antibody formed in partially tolerant rabbits. *J. Immunol.* **100**, 138.

VENKATARAMAN, M. & SCOTT, D.W. (1977) Cellular events in tolerance. VI. Neonatal *vs* adult B cell tolerance: differences in antigen-binding cell patterns and lipopolysaccharide stimulation. *J. Immunol.* **119**, 1879.

VENKATARAMAN, M. & SCOTT, D.W. (1979) Cellular events in tolerance. VII. Decrease in tolerant spleens of PFC precursors stimulated *in vitro* by specific antigen or mitogen. *Cell. Immunol.* **47**, 323.

WATERS, C.A., PILARSKI, L.M., WEGMANN, T.G. & DIENER, E. (1979) Tolerance induction during ontogeny. I. Presence of active suppression in mice rendered tolerant to human γ-globulin *in utero* correlates with the breakdown of the tolerant state. *J. exp. Med.* **149**, 1134.

ZOLLA, S. & NAOR, D. (1974) Restoration of immune competence in tolerant mice by parabiosis to normal mice. *J. exp. Med.* **140**, 1421.