Clin. exp. Immunol. (1977) 28, 363–374.

Tolerance, the thymus, and suppressor T cells*

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(*Received* 21 *December* 1976)

It is a privilege to be the first speaker at this year's European Immunology meeting. I shall speak principally of work done in my own laboratory by a group as cosmopolitan as those present at this meeting, among them Gery, Folch, Ha, Bash, Durkin, Namba, Jegasothy, Jayawardena and Wagshal. I wish to introduce the three subjects of my title with a few general remarks, without attempting to cite all the authors who have worked in the designated areas.

TOLERANCE

Tolerance may be broadly defined as 'specific inability to respond to an antigen, induced by prior exposure to the same or a related antigen.' The first well-studied example of which I am aware was described by the immunochemist, H. G. Wells (1911). Guinea-pigs fed crystalline plant proteins or ovalbumin lost the ability to develop anaphylactic sensitization to the same protein upon appropriate challenge. Other early examples involved contact sensitization (Sulzberger, 1929; Chase, 1946), pneumococcal polysaccharides (Felton, 1949), lymphocytic choriomeningitis virus (Traub, 1936), tissue allografts (Owen, 1945; Billingham, Brent & Medawar, 1953; Hasek, Lengerová & Hraba, 1961), and finally tumours (Habel, 1962; Allison, 1964). It should be noted that Wells's example has been restudied with modern techniques (Thomas & Parrott, 1974), but its mechanism remains obscure.

TABLE 1. Techniques for inducing tolerance

Perinatal exposure to antigen
(Lymphocytes not peripheralized)
(Immature 'processing apparatus')
(Low blood-thymus barrier)
(High level of nonspecific suppression in thymus and spleen)
(High sensitivity of B cells to tolerization)
Two zones of antigen dosage
Below immunizing threshhold (T cells)
Massive (B cells)
Use of 'deaggregated' antigen or antigen fragments
(Failure to interact with macrophages)
Use of oral route
(Low dose zone, biofiltration)
Immunosuppression, X-ray
(Mimics perinatal situation, in part)
Blockade
(Prevents interaction of antigen with macrophages)

We now recognize multiple mechanisms of tolerance (Howard & Mitchison, 1975; Dresser, Ed., 1976), but retain the original term for convenience. Table 1 shows techniques which are commonly used for inducing tolerance, with suggestions as to the reason for their effectiveness. Table 2 lists names

* Modified from an address presented at the 3rd European Immunology Meeting, Copenhagen, August 25–27, 1976.

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used to describe related phenomena in which the immune response is suppressed by administration of specific antigen, of other antigens, or indeed of antibody. Table 3 analyses these phenomena in terms of what we know about their mechanism.

Rapidly-reversible receptor-blockade in B cells has been well recognized since the studies of Howard and his colleagues on pneumococcal polysaccharide (reviewed in Howard & Mitchison, 1975) and Borel's work on hapten conjugates with isologous γ-globulin (Aldo-Benson & Borel, 1974). A more recent example is Schrader's 'effector cell blockade' with DNP-gelatin (1975). Irreversible clonal deletion, again of B cells is observed with such antigens as DGL (Katz *et al.*, 1971), dextran, and levan (see Feldman, Howard & Desaymard, 1975; Howard & Mitchison, 1975). Nossal's recent work has established that a similar clonal deletion is much more readily induced in B cells present in the perinatal period than in adult cells (clonal abortion) (Nossal & Pike, 1975; Metcalf & Klinman, 1976).

In vitro models of clonal deletion have made use of such antigens as POL and LPS and of antigen–antibody complexes (Diener & Armstrong, 1967; Britton, 1969; Feldmann & Diener, 1970; see Diener & Feldmann, 1972). The significance of the dose and molecular size of the antigen employed and the density of surface determinants has been well brought out in Feldmann, Howard & Desaymard's recent review (1975). (See also Desaymard, Pearce & Feldmann, 1976). Another class of *in vitro* models involves the use of antibody against B-cell receptors, *e.g.* anti-μ, to clear the cell surface (Pierce, Solliday & Asofsky, 1972). Again reversible clearing and adult and perinatal forms of irreversible 'deletion' are observed (Raff *et al.*, 1975). These mechanisms have all been studied in relation to B cells. Their significance for

TABLE 2. Types of immune modulation

Tolerance and partial tolerance in unprimed individuals
Split tolerance (different antigenic determinants)
Immune deviation (different types of immune response)
Densensitization of primed cells
Competition of antigens
Feedback suppression by antibody
Class, allotype, and idiotype suppression

TABLE 3. Mechanisms of tolerance

I. Direct: Removal of B-cell clone from reactivity
Receptor blockade, reversible
Clonal deletion, adult, irreversible
Clonal abortion, perinatal, irreversible
Reversible and irreversible clearing of B-cell surface with anti-μ, etc.
(Significance of antigen dose, molecular size, density of determinants)
II. Indirect: Action of specific suppressor T cells on T or B cells
Induction by antigen: classical tolerance
Induction by antibody: acting as antigen–antibody complex
Induction by antibody against immunoglobulin receptor
(class, allotype, idiotype): acting as antigen–antibody complex
(Significance of dose and molecular class of antibody)

tolerance at the T-cell level remains conjectural, although specific T-cell deletion has been observed in experiments with highly radioactive antigens such as POL (Cooper & Ada, 1972) or with nonradioactive affinity labels for the T-cell combining site (Godfrey, 1976).

The role in tolerance of suppressor cells, usually suppressor T cells (STC), was established first with heterologous red cells as antigens (McCullagh, 1970; Gershon & Kondo, 1970; 1971), and has since been demonstrated in tolerance to pneumococcal polysaccharide (Baker *et al.*, 1971), in contact allergy (Asherson & Zembala, 1974), tolerance to proteins and conjugates (Basten *et al.*, 1974), and a wide

variety of other systems (Gershon, 1973; Möller, ed., 1975a; Pierce & Kapp, 1976). In general, antigen-induced, STC-mediated tolerance is specific and long lasting, and appears to affect both T and B cells as targets.

Antibody against exogenous antigen produces so-called enhancement (or 'facilitation'), and it has long been recognized that such antibody may act as an afferent block to immunization (Möller & Möller, 1962) or an efferent blocking factor in, e.g., tumour destruction by specific cytotoxic T lymphocytes (Möller, 1965; Hellström & Hellström, 1974). What is important for the purpose of this discussion is that antibody of the correct molecular class, on forming a complex with antigen, can induce a long-lasting central type of tolerance, apparently mediated by STC specific for the antigen (Voisin & Kinsky, 1962; Voisin, 1971). The effective antibody is described as IgG_1 (mouse antibody acting in the mouse).

It has also recently been recognized that class-specific immunoglobulin suppression (see Lawton & Cooper, 1974), chronic allotype suppression (Jacobson *et al.*, 1972; Herzenberg & Herzenberg, 1974; Möller, Ed., 1975b) and long-term idiotype suppression (Eichmann, 1975; Fitch, 1975; Möller, Ed., 1975; Binz & Wigzell, 1976), all produced by injection of a suitable dose of antibody of the correct molecular class, may also be mediated by STC activation. Here the STC are specific for the immunoglobulin receptor on the B-cell surface or the corresponding T-cell receptor and a long-lasting, specific tolerance-like state is induced, affecting either T or B cells. Since anti-idiotype antibody shows steric complementarity to the idiotype, which forms part of the combining site for antigen, its action is formally comparable to that of antigen acting to induce classical tolerance. The effective antibody in idiotype suppression in mice is described as IgG_2 (guinea-pig) (Eichmann, 1975). *In vitro* models for the induction of an STC response which will mediate tolerance are still lacking; in consequence we cannot yet explain the molecular requirements for effective antigens or for the immunoglobulin components of effective antigen-antibody complexes. Scott, in our laboratory, showed some time ago that antigen (bovine γ-globulin) injected into the intact spleen *in vitro* induces tolerance whereas incubation of the same cells in suspension with antigen fails to do so (Scott & Waksman, 1968; 1969). This might well serve as an *in vitro* STC model.

THYMUS AND TOLERANCE

The view which prevailed until recently was that stem cells from the bone marrow, after entering the thymus, matured in the thymus cortex and passed through the medulla to enter the peripheral T-lymphocyte pool. Recent emigrants (T_1) differed in a number of properties from older cells (T_2) (Raff & Cantor, 1971). The difference between T_1 and T_2 cells in the periphery has been well documented in individual studies e.g. (Kappler *et al.*, 1974; Stutman, 1975), T_1 being large, adherent, dividing cells of low density, largely sessile in the spleen, and T_2 recirculating, small, non-adherent, nondividing cells. With the recognition that there are multiple functional subsets of both T_1 and T_2 cells, and that many if not all these functions can be identified both within the thymus and in the periphery, the original simple scheme has been questioned (Shortman *et al.*, 1975). It has been suggested that there may be a direct outflow of T_1 cells from the thymus cortex to the spleen and of T_2 cells from the medulla to the recirculating T-cell pool, in addition to $T_1 \rightarrow T_2$ maturation, which can apparently take place within both the thymus and the spleen under the influence of thymus hormone (Bach *et al.*, 1974; Stutman, 1975). This suggestion is supported by a variety of evidence from direct studies of cell migration (Iorio *et al.*, 1970; Weissman, 1976; Durkin & Waksman, 1977), and cell-marker studies (Shortman & Jackson, 1974; Ruuskanen, 1975; Barton, Goldschneider & Bollum, 1976; Goldschneider, 1976) to studies of regeneration in the irradiated or cortisol-treated thymus (Jacobsson & Blomgren, 1972; Schlesinger & Israel, 1975).

The use of antigenic markers such as Thy-1 and the Ly system in the mouse (Cantor & Boyse, 1975; Huber *et al.*, 1976; Feldmann *et al.*, 1976) and of RMTA and RBMLA, the homologue of Thy-1 in the rat (Goldschneider, 1976) has permitted us to differentiate a wide variety of T-cell subsets which differ in their recognition function, target cells, and immunological role (Table 4). Those subsets designated T_1 are found in thymus cortex and/or spleen and appear to migrate between them. Those

TABLE 4. Heterogeneity of T cells

T cell	Suggested symbol	T_1/T_2	Markers			Cell recognized	Antigen recognized	Target
			Ly 1	Ly 23	Ia			
Amplifier	T_A	T_1	+	+	−	T_H, T_S	?	T_H, T_S
Nonspecific suppressor	T_{NS}	T_1	+	+	?	Various	?	Various
Helper	T_H	T_2	+	−	−	Mϕ	Ia*	B
MLR	T_{MLR}	T_2	+	−	−	Mϕ	Ia	T_K
CMI	T_{CMI}	T_2	+	−	−	Mϕ	Ia*	Mϕ
Killer	T_K	T_2	−	+	−	Target	K/D*	Various
Specific suppressor	T_S	T_2	−	+	+	Mϕ ?	K/D*	T(Ly 1), B

* Modified Ia or H-2K/D

designated T_2 are present in thymus medulla and the recirculating T cell pool and migration in this system is generally accepted.

It is quite clear by now that at least two types of T cells are involved in tolerance and related phenomena: nonspecific suppressor cells, which appear to be T_1, and specific suppressors, which are clearly T_2 and related in their observable characteristics to killer T cells (Cantor, Shen & Boyse, 1976; Herzenberg *et al.*, 1976; Feldmann *et al.*, 1976). I shall say more about each in a moment. In addition to these, however, we must also take account of more recently described types of cells (not listed in the table) which might, under appropriate circumstances, be involved in suppressor functions: the so-called prothymocyte, a low Thy-1 cell in the bone marrow and spleen (Roelants *et al.*, 1976; Loor *et al.*, 1976; Smith & Eaton, 1976), which appears to be the same as the NK ('natural' killer) cell (Lamon *et al.*, 1972; Herberman *et al.*, 1973; Greenberg & Playfair, 1974; Kiessling *et al.*, 1975); the M cell (Bennett *et al.*, 1976), the cell responsible for hybrid resistance and the cell which rejects bone marrow grafts (Shearer, Garbarino & Cudkowicz, 1976; Lotzová, Gallagher & Trentin, 1976); another Thy-1+, Ly1+23+ cell, which kills syngeneic tumors (Shiku *et al.*, 1976); and the K cell, which kills certain targets in the presence of antibody and may be a 'pre-B cell' or an immature monocyte (Greenberg *et al.*, 1975; Penfold, Greenberg & Roitt, 1976; Chess *et al.*, 1976).

We must further be prepared for the possibility that suppression will prove in many cases to involve two cell systems (Gershon, 1975). Such a relationship has been recognized, for example, between nonspecific amplifier T cells and specific suppressor T cells (Feldmann *et al.*, 1976); between SIRS (soluble immune response suppressor), a T lymphocyte product (see Pierce & Kapp, 1976), and macrophages; and between the nonspecific suppressor T cell we have studied (details below) and macrophages (Bash, Singer & Waksman, 1976).

The relationship of tolerance to the lymphocyte pool was first demonstrated in cell transfer experiments by Gowans & McGregor (1963) and by Dietrich & Weigle (1964). The thymus was directly implicated in the generation and maintenance of tolerance about a year later by experiments with transplantation antigens (Vojtíšková & Lengerová, 1965) and soluble protein antigens (Isaković, Smith & Waksman, 1965) and the use of thymus grafting. (That tolerance for some antigens can be induced in the absence of a thymus was shown at almost the same time (Follett, Battisto & Bloom, 1966)). Our laboratory carried out a series of further experiments relating tolerance to the thymus (Table 5). Tolerance lasted, in all experiments in which antigen made its way into the intact thymus, approximately 10 weeks. This period was presumed to be related to persistence of antigen and the mechanism was considered to be clonal deletion, since at that time the suppressor T-cell concept still lay in the future. However, Horiuchi & Waksman's finding (1968b) that injection of aggregated or 'soluble' protein antigen into the thymus of normal rats produced specific tolerance lasting several weeks at a much lower dose than

TABLE 5. Induction of tolerance in the thymus

Experiment	Effective antigen dose (mg)	Duration of unresponsiveness (weeks)	Reference
BGG iv: normal rats	20	~10	(Gery & Waksman, 1967)
BGG in the thymus: normal rats	0·02	5–10	(Horiuchi & Waksman, 1968b)
BGG in the thymus: irradiated, thymus-shielded rats	0·02	~10	(Staples, Gery & Waksman 1966; Horiuchi & Waksman, 1968a)
BGG i.p. at 0 and 4 weeks: thymus graft at 10 weeks to Tx X BM rats	70	9–12	(Isaković, Smith & Waksman, 1965)
BSA i.p. three daily doses: thymus graft next day to Tx X BM rats	150	3–6	(Smith, Isaković & Waksman, 1966)
BGG i.p. thymocyte transfer to normal rats	100	0–1	(Ha & Waksman, 1973; Ha, Waksman & Treffers, 1974a, b)
Allogeneic spleen cells at birth: Thymocyte transfer at 3 weeks to syngeneic NTx recipients	—	>10	(Toullet & Waksman, 1966)

* All experiments in Lewis rats except last, which was done in A/J mice tolerant of CBA donor grafts.

antigen given by any other route implied a suppressor cell-mediated mechanism, and this implication was confirmed in subsequent experiments (Ha & Waksman, 1973).

It should be noted here that macromolecular antigen present in the systemic circulation enters the thymus quite readily. An early study which emphasized the presence of a blood-thymus barrier (Marshall & White, 1961) unfortunately persuaded many investigators of the contrary. In the perinatal period, even cellular and particulate materials get into the thymus, and in the adult soluble antigens enter only slightly slower than into other organs (reviewed in Horiuchi, Gery & Waksman (1968)). Two days after injection of 100 mg of OA i.p., a two- or three-fold increase is observed in large- and medium-sized blasts within the thymus (Durkin & Waksman, 1977).

SUPPRESSOR T CELLS

The work in our laboratory on specific suppressor T cells in the thymus (Ha & Waksman, 1973; Ha, Waksman & Treffers, 1974a, b) was parallelled and greatly extended by the elegant studies of Tada and his collaborators, beginning with feedback suppression of the specific IgE response in rats and proceeding to a specific suppression of IgM and IgG responses in rats or mice given large doses of protein antigen (reviewed in Tada, 1975; Tada, Taniguchi & Takemori, 1975; Tada, Taniguchi & David, 1976). More recent studies of specific suppressor cells in the thymus have also been reported by Kapp and her collaborators, using polymers in low-responder strains of mice (Kapp *et al.*, 1974, 1976), and by Fujimoto, Greene & Sehon with tumour antigens (1976a, b). The suppressor cells are present in both the thymus medulla and spleen, are T_2 in character, and bear the Thy-1, Ly 23 (but not Ly 1) and Ia markers. They act by way of a mediator, which can be isolated from the cell membrane and has been shown to consist of an I-region gene product (specific membrane recognition unit?) complexed with a fragment of antigen. The biochemical target of this mediator is unknown.

Our own further studies have concentrated on nonspecific T_1 suppressor cells in thymus and spleen. Table 6 suggests a logical sequence of investigations, applicable not only to the system which we have studied but also to other systems, and our data are summarized here in a corresponding sequence. Folch was able to demonstrate that the spleen contains an unusual cell population adherent to glass wool and capable of suppressing proliferative responses to mitogen or mixed lymphocyte reactions (Folch, Yoshinaga & Waksman, 1973; Folch & Waksman, 1973a; Folch & Waksman, 1974a, b). Suppression was elicited by mitogen stimulation (PHA, concanavalin A) or by allogeneic stimuli, and was greatly

TABLE 6. Levels of investigation of suppressor phenomena

Suppressor phenomena: specific, nonspecific, feedback
Suppressor cells: T, B, Mϕ; subsets, effectors
Contact mechanisms of suppression *vs* mediator release
Mediators: specific and nonspecific lymphokines and monokines
Mediator release: shedding, early protein, late protein synthesis
Target cells: T, B, Mϕ, other (tumour, parasite)
Target function: membrane function, DNA synthesis and mitosis, differentiation
Second messengers: prostaglandins, cyclic nucleotides
Biologic significance of the system

intensified in animals given a large dose of antigen (Gershon, Gery & Waksman, 1974; Bash & Waksman, 1975) or antigen in complete adjuvant (Bash & Waksman, 1975; Durkin, Bash & Waksman, 1975). In antigen-stimulated rats, suppressor activity was also demonstrated in the otherwise unstimulated thymus and in lymph-node cells. The system is thymus-dependent, and the active cell is presumably a T cell. It was clearly shown not be a macrophage although present evidence suggests that the suppressor cell may interact with a macrophage in producing suppression (Bash, Singer & Waksman, 1976). The responsible cell is adherent to glass wool and nylon wool and sensitive to cyclophosphamide, and its action is inhibited by cycloheximide. To our surprise, we found that nonspecific adherent-cell suppressor activity in the spleen is substantially increased shortly after adult thymectomy, returning to normal some 5 weeks later (Folch & Waksman, 1972, 1973b). This increase was tentatively attributed to removal of the hormonal stimulus to T_1–T_2 transformation within the spleen and a temporary local piling up of T_1 suppressor cells. Daily treatment of thymectomized rats with thymus hormone was partially effective in preventing this change (Bash *et al.*, 1976).

It is probable that the use of mitogen or of very large amounts of antigen (or complete adjuvant mixtures) results in stimulation of a large number of peripheral cells. In the case of antigen, these must be cells with a wide range of combining activities for the antigen. At the same time we have been able to demonstrate, by a number of techniques, that a large dose of systemic antigen in rats is followed by a wave of emigration of thymocytes from the thymus and, in particular, from the thymus cortex to the splenic red pulp and mesenteric lymph nodes (Durkin & Waksman, 1977). The peripheral suppressor population appears to include cells bearing the RBMLA surface marker characteristic of rat thymus cortical cells (Durkin & Goldschneider, unpublished studies). Thus they appear to be T_1, to respond to antigen *in situ*, and to migrate to the periphery in increased numbers after antigen administration. The nonspecific suppressor cells studied by Mosier in neonatal and young mice (Mosier & Johnson, 1975) are also localized in the thymus cortex and spleen and bear both Ly1 and Ly23 (Mosier & Mathison, 1977), and these must be regarded similarly as T_1. Other systems of peripheral nonspecific suppressor cells are under investigation in many laboratories (see review by Pierce & Kapp, 1976).

We have strong evidence that the nonspecific suppressor cell in the thymus cortex and spleen acts by release of a nonspecific glycoprotein mediator, IDS (inhibitor of DNA synthesis). IDS is secreted either after exposure of normal cells to mitogen or of sensitized cells to antigen *in vitro* (Namba & Waksman, 1975a, b; 1976; 1977; Waksman & Namba, 1976), and its production appears linked to cell division. The mol. wt of IDS is 75–80,000, its pI is 3·0, and it is resistant to heat up to 80°C. It is produced within a few hours after stimulation by adherent spleen cells and by adherent, cortisol-sensitive (cortical) thymocytes (Namba, Jegasothy & Waksman, 1977). After a large dose of systemic antigen, IDS production is greatly increased in both thymus and spleen populations (Fig. 1). IDS differs from the specific factors described by Tada (Tada *et al.*, 1975; Tada *et al.*, 1977) and Kapp *et al.* (1976) in mol. wt, in being easily released from the cell, in showing no antigen-specificity and having no relationship to the genes of the major histocompatibility complex, and in acting across strain and even species barrier. It also appears to differ from SIRS, the 'soluble immune response suppressor' of Pierce and his colleagues

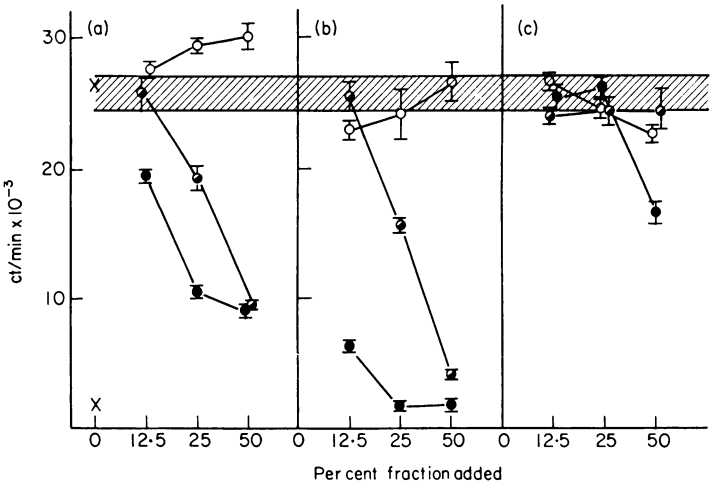

FIG. 1. Production of IDS by adherent and nonadherent subpopulations of thymus (a), spleen (b), and lymph node cells (c) collected 24 hr after i.v. injection of 100 mg ovalbumin. Cells were separated on glass wool columns, cultured 24 hr without stimulant, and IDS-containing supernatant fractions (from Sephadex G-100) were added to PHA-stimulated LN cell cultures at different final concentrations. (◕) Whole cells, (○) non-adherent cells, (●) adherent cells. Shaded area shows range of values obtained with supernatant fractions from lymphoid cells of saline-injected animals. X shows control ct/min values in LN cell cultures with and without PHA, without added fractions. (From Namba, Jegasothy & Waksman, 1977).

(Tadakuma *et al.*, 1976), but may be identical with the factor studied by Thomas *et al.* (1975a, b). It acts not only on lymphocytes, but also on fibroblasts and various tumour cells.

IDS shows multihit kinetics and thus can act only at short range (Namba & Waksman, 1975a). It acts much more strongly on lymphocytes than on fibroblasts. Thus it is almost ideally suited for immune regulation (Waksman & Namba, 1976). Its target action is limited to G_1, both in stimulated lymphocytes (Namba & Waksman, 1975a) and in synchronized fibroblasts (Wagshal, Jegasothy & Waksman, 1977). This result implies the presence of a receptor for IDS in or on susceptible target cells only during this brief period of the cell cycle. It stimulates cell membrane adenylate cyclase and raises target cell cyclic AMP as much as five-fold, far more than is required to shut off DNA synthesis, within a few seconds (Jegasothy, Pachner & Waksman, 1976). Again this IDS action is seen only in G_1 (Fig. 2). Similar cell cycle specificity has been demonstrated in other systems which involve soluble mediators acting to raise cAMP; an example is melanocyte-stimulating hormone which acts on melanocytes in culture only during G_2 (Varga *et al.*, 1974). The relevance of the IDS-stimulated rise in cAMP to suppressor-cell action has been established by showing that agents which stimulate or inhibit phosphodiesterase (imidazole, isobutyl methylxanthine) inhibit or potentiate IDS action accordingly (Jegasothy & Waksman, in preparation). IDS thus resembles many hormones which act by way of adenylate cyclase and cAMP.

I wish to finish my remarks by suggesting that the nonspecific suppressor-cell system outlined here may play many *in vivo* roles. The most apparent, but perhaps least important biologically, would be its role in competition of antigens, depression of immunological responses during graft-*vs*-host reactions, and feedback suppression after intense stimulation. A second would be its role in the early phase of tolerance induction to exogenous antigens, e.g. (Liacopoulos & Neveu, 1964; Liacopoulos, Couderc & Gille, 1971). More important, as suggested by Mosier's studied (Mosier & Johnson, 1975; Mosier & Mathison, 1977, submitted for publication), might be its role holding back responses to autoantigens in young individuals until the specific tolerance mechanism is fully developed and has acted to remove these from the danger list. Perhaps equally important in a negative way is the role of this system in chronic intracellular and other infections, in which nonspecific suppression results from continuous exposure to antigen and paves the way for specific tolerance to the same or other antigens and for failure of the host's resistance mechanisms. We have demonstrated that such a system is operative in mice infected

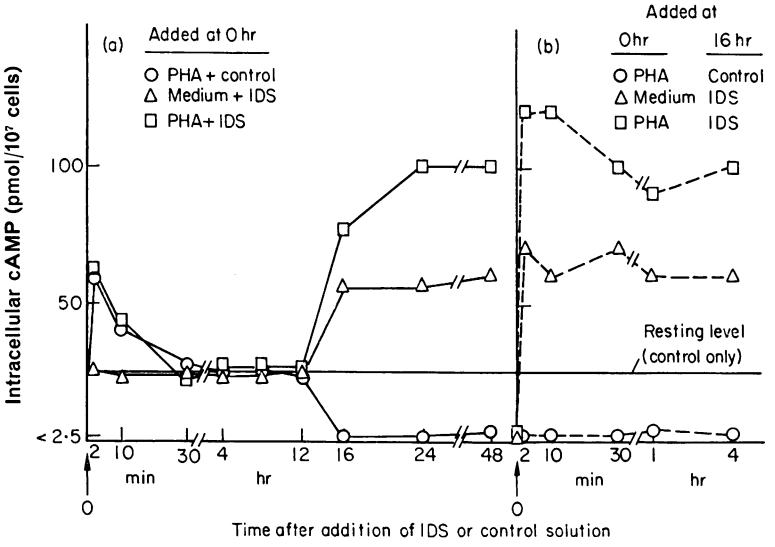

FIG. 2. Changes in cyclic AMP in rat LN cell cultures stimulated with PHA with or without addition of IDS at 0 or 16 hr. Resting levels (cells incubated in medium alone or with added control solution) varied between 24 and 26 pmole/10^7 cells. (From Jegasothy, Pachner & Waksman, 1976). Copyright 1976 by the American Association for the Advancement of Science.

with *T. brucei*, which show a progressive increase in the spleen of weakly adherent, thymus-dependent suppressor cells acting by way of a soluble mediator (Jayawardena & Waksman, 1977). The system may play a similar negative role as an 'introducer' of specific tolerance to tumours (Thomas, Talmage & Roberts, 1975b). Conversely, since both chronic infections and tumors progress from low dose to high dose situations, it may ultimately prove that specific tolerance precedes the state of nonspecific suppression in some or all cases.

The author's research, described here, is supported by NIH grants AI-06112 and AI-065455 and NCI contract CB-43926.

REFERENCES

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

ALLISON, A.C. (1964) Relationship of cellular and humoral immunity in virus carcinogenesis. *Life Sci.* **3**, 1415.

ASHERSON, G.L. & ZEMBALA, M. (1974) T cell suppression of contact sensitivity in the mouse. III. The role of macrophages and the specific triggering of nonspecific suppression. *Europ. J. Immunol.* **4**, 804.

BACH, J.-F., DARDENNE, M., PLEAU, J.M. & BACH, M.-A. (1974) Isolation, biochemical characteristics, and biological activity of a circulating thymic hormone in the mouse and in the human. *Ann. N.Y. Acad. Sci.* **249**, 186.

BAKER, P.J., PRESCOTT, B., BARTH, P.F., STASHAK, P.W. & AMSBAUGH, D.F. (1971) Immunological paralysis to type III pneumococcal polysaccharide as assessed by an immune-plaque procedure. *Ann. N.Y. Acad. Sci.* **181**, 34.

BARTON, R., GOLDSCHNEIDER, I. & BOLLUM, F.J. (1976) The distribution of terminal deoxynucleotidyl transferase (TdT) among subsets of thymocytes in the rat. *J. Immunol.* **116**, 462.

BASH, J.A., SINGER, A.M. & WAKSMAN, B.H. (1976) The suppressive effect of immunization on the proliferative response of rat T cells *in vitro*. II. Abrogation of antigen-induced suppression by selective cytotoxic agents. *J. Immunol.* **116**, 1350.

BASH, J.A. & WAKSMAN, B.H. (1975) The suppressive effect of immunization on the proliferative responses of rat T-cells *in vitro*. *J. Immunol.* **114**, 782.

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

BENNETT, M., BAKER, E.E., EASTCOTT, J.W., KUMAR, V. & YONKOSKY, D. (1976) Selective elimination of marrow precursors with the bone-seeking isotope ^{89}Sr: Implications for hemopoiesis, lymphopoiesis, viral leukemogenesis and infection. *J. Reticuloend. Soc.* **20**, 71.

BILLINGHAM, R.E., BRENT, L. & MEDAWAR, P.B. (1953) Actively acquired tolerance of foreign cells. *Nature (Lond.)*, **172**, 603.

BINZ, H. & WIGZELL, H. (1977) Naturally occurring antigen-binding, idiotypic T cell receptors. *Origins of Lymphocyte Diversity, XLI Cold Spring Harbor Sympos. on Quantitative Biology*. p. 275. Cold Spring Harbor Laboratory, New York.

BRITTON, S. (1969) Regulation of antibody synthesis against *Escherichia coli* endotoxin. IV. Induction of paralysis *in vitro* by treating normal lymphoid cells with antigen. *J. exp. Med.* **129**, 469.

CANTOR, H. & BOYSE, E.A. (1975) Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. exp. Med.* **141**, 1376.

CANTOR, H., SHEN, F.W. & BOYSE, E.A. (1976) Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: after immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. *J. exp. Med.* **143**, 1391.

CHASE, M.W. (1946) Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. *Proc. Soc. exp. Biol. (N.Y.)* **61**, 257.

CHESS, L., EVANS, R., HUMPHREYS, R.E., STROMINGER, J.L. & SCHLOSSMAN, S.F. (1976) Inhibition of antibody-dependent cellular cytotoxicity and immuno-globulin synthesis by an antiserum prepared against a human B-cell Ia-like molecule. *J. exp. Med.* **144**, 113.

COOPER, M.G. & ADA, G.L. (1972) Delayed-type hypersensitivity in the mouse. III. Inactivation of thymus-derived effector cells and their precursors. *Scand. J. Immunol.* **1**, 247.

DESAYMARD, C., PEARCE, B. & FELDMANN, M. (1976) Role of epitope density in the induction of tolerance and immunity with thymus-independent antigens. III. Interaction of epitope density and receptor avidity. *Europ. J. Immunol.* **6**, 646.

DIENER, E. & ARMSTRONG, W.D. (1967) Induction of antibody formation and tolerance *in vitro* to a purified protein antigen. *Lancet*, **ii**, 1281.

DIENER, E. & FELDMANN, M. (1972) Relationship between antigen and antibody induced suppression of immunity. *Transplant. Rev.* **8**, 76.

DIETRICH, F.M. & WEIGLE, W.O. (1964) Immunologic unresponsiveness to heterologous serum proteins induced in adult mice and transfer of the unresponsive state. *J. Immunol.* **92**, 167.

DRESSER, D.W., ED. (1976) Immunological tolerance. *Brit. med. Bull.* **32**, 99.

DURKIN, H.G., BASH, J.A. & WAKSMAN, B.H. (1975) Separation of T cell subpopulations capable of DNA synthesis, lymphotoxin release, and regulation of antigen and phytohemagglutinin responses on the basis of density and adherence properties. *Proc. nat. Acad. Sci. (Wash.)*, **72**, 5090.

DURKIN, H.G. & WAKSMAN, B.H. (1977) Antigen induces cortical thymocyte migration to splenic red pulp. *Fed. Proc.* **36**, (Abstract).

EICHMANN, K. (1975) Idiotype suppression, II. Amplification of a suppressor T cell with anti-idiotypic activity. *Europ. J. Immunol.* **5**, 511.

FELDMANN, M., BEVERLY, P.C.L., DUNKLEY, M., ERB, P., HOWIE, S., MAOZ, A., MATHIES, M., MCKENZIE, I. & WOODY, J. (1977) Heterogeneity of T lymphocytes: Relationship to cell interactions in antibody production *in vitro*. *Origins of Lymphocyte Diversity, XLI Cold Spring Harbor Sympos. on Quantitative Biology* p. 113, Cold Spring Harbor Laboratory, New York.

FELDMANN, M. & DIENER, E. (1970) Antibody-mediated suppression of the immune response *in vitro*. II. A new approach to the phenomenon of immunological tolerance. *J. exp. Med.* **132**, 31.

FELDMANN, M., HOWARD, J.G. & DESAYMARD, C. (1975) Role of antigen structure in the discrimination between tolerance and immunity by B cells. *Transplant. Rev.* **23**, 78.

FELTON, L.D. (1949) The significance of antigen in animal tissues. *J. Immunol.* **61**, 107.

FITCH, F.W. (1975) Selective suppression of immune responses. Regulation of antibody formation and cell-mediated immunity. *Progr. Allergy* **19**, 195.

FOLCH, H. & WAKSMAN, B.H. (1972) *In vitro* responses of rat lymphocytes following adult thymectomy. I. Rapid decrease and recovery of responses to mitogens and hemiallogeneic cells. *J. Immunol.* **109**, 1046.

FOLCH, H. & WAKSMAN, B.H. (1973a) Regulation of lymphocyte responses *in vitro*. V. Suppressor activity of adherent and non-adherent rat lymphoid cells. *Cell. Immunol.* **9**, 12.

FOLCH, H. & WAKSMAN, B.H. (1973b) *In vitro* responses of rat lymphocytes following adult thymectomy. II. Increased inhibition by splenic adherent cells of responses to phytohemagglutinin. *Cell. Immunol.* **9**, 25.

FOLCH, H. & WAKSMAN, B.H. (1974a) The splenic suppressor cell. I. Activity of thymus-dependent adherent cells. Changes with age and stress. *J. Immunol.* **113**, 127.

FOLCH, H. & WAKSMAN, B.H. (1974b) The splenic suppressor cell. II. Suppression of the mixed lymphocyte reaction by thymus-dependent adherent cells. *J. Immunol.* **113**, 140.

FOLCH, H., YOSHINAGA, M. & WAKSMAN, B.H. (1973) Regulation of lymphocyte responses *in vitro*. III. Inhibition by adherent cells of the T-lymphocyte response to phytohemagglutinin. *J. Immunol.* **110**, 835.

FOLLETT, D.A., BATTISTO, J.R. & BLOOM, B.R. (1966) Tolerance to a defined chemical hapten produced in adult guinea-pigs after thymectomy. *Immunology*, **11**, 73.

FUJIMOTO, S., GREENE, M.I. & SEHON, A.H. (1976a) Regulation of the immune response to tumor antigens. I. Immunosuppressor cells in tumor-bearing hosts. *J. Immunol.* **116**, 791.

FUJIMOTO, S., GREENE, M.I. & SEHON, A.H. (1976b) Regulation of the immune response to tumor antigens. II. The nature of immunosuppressor cells in tumor-bearing hosts. *J. Immunol.* **116**, 800.

GERSHON, R.K. (1973) T cell control of antibody production. *Contemp. Topics Immunobiol.* **3**, 1.

GERSHON, R.K. (1975) A disquisition on suppressor T cells. *Transplant. Rev.* **26**, 170.

GERSHON, R.K., GERY, I. & WAKSMAN, B.H. (1974) Suppressive effects of *in vivo* immunization on PHA responses *in vitro*. *J. Immunol.* **112**, 215.

GERSHON, R.K. & KONDO, K. (1970) Cell interactions in the induction of tolerance. The role of thymic lymphocytes *Immunology*, **18**, 723.

GERSHON, R.K. & KONDO, K. (1971) Infectious immunological tolerance. *Immunology*, **21**, 903.

GERY, I. & WAKSMAN, B.H. (1967) Role of the thymus in tolerance. V. Suppressive effect of treatment with non-aggregated and aggregated bovine γ-globulin on specific immune responses in normal adult rats. *J. Immunol.* **98**, 446.

GODFREY, H.P. (1976) The use of 1-fluoro-2,4-dinitrobenzene as an affinity label for the antigen receptor of delayed hypersensitivity. *Immunology* **31**, 665.

GOLDSCHNEIDER, I. (1976) Identification of two antigenically distinct lines of T cells in the rat. *Fed. Proc.* **35**, 277 (abstract).

GOWANS, J.L. & MCGREGOR, D.D. (1963) The origin of antibody forming cells. *III International Sympos. on Immunopathology* (ed. by P. Grabar and P.A. Miescher), p. 89, Schwabe & Co., Stuttgart.

GREENBERG, A.H. & PLAYFAIR, J.V.L. (1974) Spontaneously

arising cytotoxicity to the P-815-X mastocytoma in NZB mice. *Clin. exp. Immunol.* **16**, 99.

GREENBERG, A.H., SHEN, L., WALKER, L., ARNAIZ-VILLENA, A. & ROITT, I.M. (1975) Characteristics of the effector cells mediating cytotoxicity against antibody-coated target cells. II. The mouse nonadherent K cell. *Europ. J. Immunol.* **5**, 474.

HA, T.-Y. & WAKSMAN, B.H. (1973) Role of the thymus in tolerance. X. 'Suppressor' activity of antigen-stimulated rat thymocytes transferred to normal recipients. *J. Immunol.* **110**, 1290.

HA, T.-Y., WAKSMAN, B.H. & TREFFERS, H.P. (1974a) The thymic suppressor cell. I. Separation of subpopulations with suppressor activity. *J. exp. Med.* **139**, 13.

HA, T.-Y., WAKSMAN, B.H. & TREFFERS, H.P. (1974b) The thymic suppressor cell. II. Metabolic requirements of suppressor activity. *Immunol. Communic.* **3**, 351.

HABEL, K. (1962) The relationship between polyoma virus multiplication, immunological competence, and resistance to tumor challenge in the mouse. *Ann. N.Y. Acad. Sci.* **101**, 173.

HAŠEK, M., LENGEROVÁ, A. & HRABA, T. (1961) Transplantation immunity and tolerance. *Advanc. Immunol.* **1**, 1.

HELLSTRÖM, K.E. & HELLSTRÖM, I. (1974) Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. *Advanc. Immunol.* **18**, 209.

HERBERMAN, R. NUNN, M.E., LAVRIN, D.H. & ASOFSKY, R. (1973) Effect of antibody to theta antigen on cell mediated immunity induced in syngeneic mice by murine sarcoma virus. *J. nat. Cancer Inst.* **51**, 1509.

HERZENBERG, L.A. & HERZENBERG, L.A. (1974) Short-term and chronic allotype suppression in mice. *Contemp. Topics Immunobiol.* **3**, 41.

HERZENBERG, L.A. OKUMURA, K., CANTOR, H., SATO, V.L., SHEN, F.-W., BOYSE, E.A. & HERZENBERG, L.A. (1976) T-cell regulation of antibody responses: Demonstration of allotype-specific helper T-cells and their specific removal by suppressor T-cells. *J. exp. Med.* **144**, 330.

HORIUCHI, A., GERY, I. & WAKSMAN, B.H. (1968) Role of the thymus in tolerance. VII. Distribution of non-aggregated and heat-aggregated bovine gamma globulin in lymphoid organs of normal newborn and adult rats. *Yale J. Biol. Med.* **41**, 13.

HORIUCHI, A. & WAKSMAN, B.H. (1968a) Role of the thymus in tolerance. VI. Tolerance to bovine-globulin in rats given a low dose of irradiation and injection of non-aggregated or aggregated antigen into the shielded thymus. *J. Immunol.* **100**, 974.

HORIUCHI, A. & WAKSMAN, B.H. (1968b) Role of the thymus in tolerance. VIII. Relative effectiveness of nonaggregated and heat-aggregated bovine γ-globulin, injected directly into lymphoid organs of normal rats, in suppressing immune responsiveness. *J. Immunol.* **101**, 1322.

HOWARD, J.G. & MITCHISON, N.A. (1975) Immunological tolerance. *Progr. Allergy*, **18**, 43.

HUBER, B., CANTOR, H., SHEN, F.W. & BOYSE, E.A. (1976) Independent differentiative pathways of Ly 1 and Ly 23 subclasses of T cells. Experimental production of mice deprived of selected T-cell subclasses. *J. exp. Med.* **144**, 1128.

IORIO, R.J., CHANANA, A.D., CRONKITE, E.P. & JOEL, D.D. (1970) Studies on lymphocytes. XVI. Distribution of bovine thymus lymphocytes in the spleen and lymph nodes. *Cell Tissue Kinet.* **3**, 161.

ISAKOVIĆ, K., SMITH, S.B. & WAKSMAN, B.H. (1975) Role of the thymus in tolerance. I. Tolerance to bovine gamma globulin in thymectomized, irradiated rats grafted with thymus from tolerant donors. *J. exp. Med.* **122**, 1103.

JACOBSON, E.B., HERZENBERG, L.A., RIBLET, R. & HERZENBERG, L.A. (1972) Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. *J. exp. Med.* **135**, 1163.

JACOBSSON, H. & BLOMGREN, H. (1972) Changes of the PHA-responding pool of cells in the thymus after cortisone or x-ray treatment of mice. Evidence for an inverse relation between the production of cortical and medullary thymocytes. *Cell. Immunol.* **4**, 93.

JAYAWARDENA, A.N. & WAKSMAN, B.H. (1977) Suppressor cells in experimental trypanosomiasis. *Nature (Lond.)*, **265**, 539.

JEGASOTHY, B.V., PACHNER, A.R. & WAKSMAN, B.H. (1976) Cytokine inhibition of DNA synthesis: Effect on cyclic adenosine monophosphate in lymphocytes. *Science* **193**, 1260.

KAPP, J.A., PIERCE, C.W., DE LA CROIX, F. & BENACERRAF, B. (1976) Immunosuppressive factor(s) extracted from lymphoid cells of nonresponder mice primed with L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). I. Activity and antigenic specificity. *J. Immunol.* **116**, 305.

KAPP, J.A., PIERCE, C.W., SCHLOSSMAN, S. & BENACERRAF, B. (1974) Genetic control of immune responses *in vitro*. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). *J. exp. Med.* **140**, 648.

KAPPLER, J.W., HUNTER, P.C., JACOBS, D. & LORD, E. (1974) Functional heterogeneity among T-derived lymphocytes of the mouse. Analysis by adult thymectomy. *J. Immunol.* **113**, 27.

KATZ, D.H., DAVIE, J.M., PAUL, W.E. & BENACERRAF, B. (1971) Carrier function in anti-hapten antibody responses. IV. Experimental conditions for the induction of hapten-specific tolerance or for the stimulation of anti-hapten anamnestic responses by 'non-immunogenic' hapten-polypeptide conjugates. *J. exp. Med.* **134**, 201.

KIESSLING, R., KLEIN, E., PROSS, H. & WIGZELL, H. (1975) 'Natural' killer cells in the mouse. *Europ. J. Immunol.* **5**, 112, 117.

LAMON, E.W., SKURZAK, H.M., KLEIN, E. & WIGZELL, H. (1972) *In vitro* cytotoxicity by a nonthymus-processed lymphocyte population with specificity for a virally determined tumor cell surface antigen. *J. exp. Med.* **136**, 1072.

LAWTON, A.R. III & COOPER, M.D. (1974) Modification of B lymphocyte differentiation by anti-immunoglobulins. *Contemp. Topics Immunobiol.* **3**, 193.

LOOR, F., AMSTUTZ, H., HÄGG, L.-B., MAYOR, K.S. & ROELANTS, G.E. (1976) T lineage lymphocytes in nude mice born from homozygous nu/nu parents. *Europ. J. Immunol.* **6**, 663.

LIACOPOULOS, P., COUDERC, J. & GILLE, M.F. (1971) Competition of antigens during induction of low zone tolerance. *Europ. J. Immunol.* **1**, 359.

LIACOPOULOS, P. & NEVEU, T. (1964) Non-specific inhibition of the immediate and delayed types of hypersensitivity during immune paralysis of adult guinea-pigs. *Immunology* **7**, 26.

LOTZOVÁ, E., GALLAGHER, M.T. & TRENTIN, J.J. (1976) Macrophage involvement in genetic resistance to bone marrow transplantation. *Transplant. Proc.* **8**, 477.

MARSHALL, A.H.E. & WHITE, R.G. (1961) The immunological reactivity of the thymus. *Brit. J. exp. Path.* **42**, 379.

MCCULLAGH, P.J. (1970) The transfer of immunological

competence to rats tolerant of sheep erythrocytes with lymphocytes from normal rats. *Aust. J. exp. Biol. med. Sci.* **48**, 351.

METCALF, E.S. & KLINMAN, N.R. (1976) *In vitro* tolerance induction of neonatal murine B cells. *J. exp. Med.* **143**, 1327.

MÖLLER, E. (1965) Antagonistic effects of humoral isoantibodies on the *in vitro* cytotoxicity of immune lymphoid cells. *J. exp. Med.* **122**, 11.

MÖLLER, G., Ed. (1975a) Suppressor T lymphocytes. *Transplant. Rev.* **26**, 1.

MÖLLER, G., Ed. (1975b) Antibody suppression of gene products. *Transplant. Rev.* **27**, 1.

MÖLLER, G. & MÖLLER, E. (1962) Studies *in vitro* and *in vivo* of the cytotoxic and enhancing effect of humoral isoantibodies. *Ann. N.Y. Acad. Sci.* **99**, 504.

MOSIER, D.E. & JOHNSON, B.M. (1975) Ontogeny of mouse lymphocyte function. II. Development of the ability to produce antibody is modulated by T lymphocytes. *J. exp. Med.* **141**, 216.

NAMBA, Y., JEGASOTHY, B.V. & WAKSMAN, B.H. (1977) Regulatory substances produced by lymphocytes. V. Production of inhibitor of DNA synthesis (IDS) by proliferating T-lymphocytes. *J. Immunol.* **118**, (In press).

NAMBA, Y. & WAKSMAN, B.H. (1975a) Regulatory substances produced by lymphocytes. I. Inhibitor of DNA synthesis in the rat. *Inflammation*, **1**, 5.

NAMBA, Y. & WAKSMAN, B.H. (1975b) Regulatory substances produced by lymphocytes. II. Lymphotoxin in the rat. *J. Immunol.* **115**, 1018.

NAMBA, Y. & WAKSMAN, B.H. (1976) Regulatory substances produced by lymphocytes. III. Evidence that lymphotoxin and proliferation inhibitory factor are identical and differ from the inhibitor of DNA synthesis. *J. Immunol.* **116**, 1140.

NAMBA, Y. & WAKSMAN, B.H. (1977) Regulatory substances produced by lymphocytes. IV. Further characterization of the inhibitor of DNA synthesis. *Immunochemistry*, **14**, 143.

NOSSAL, G.J.V. & PIKE, B.L. (1975) Evidence for the clonal abortion theory of B-lymphocyte tolerance. *J. exp. Med.* **141**, 904.

OWEN, R.D. (1945) Immunogenetic consequences of vascular anastomoses between bovine twins. *Science*, **102**, 400.

PENFOLD, P.L., GREENBERG, A.H. & ROITT, I.M. (1976) Characteristics of the effector cells mediating cytotoxicity against antibody-coated target cells. III. Ultrastructural studies. *Clin. exp. Immunol.* **23**, 91.

PIERCE, C.W. & KAPP, J.A. (1976) Regulation of immune responses by suppressor T cells. *Contemp. Topics Immunobiol.* **5**, 91.

PIERCE, C.W., SOLLIDAY, S.M. & ASOFSKY, R. (1972) Immune responses *in vitro*. IV. Suppression of primary γM, γG, and γA plaque-forming cell responses in mouse spleen cell cultures by class specific antibody to mouse immunoglobulins. *J. exp. Med.* **135**, 675.

RAFF, M.C. & CANTOR, H. (1971) Subpopulations of thymus cells and thymus-derived lymphocytes. *Progress in Immunology* (ed. by B. Amos), p. 83, Academic Press, New York.

RAFF, M.C., OWEN, J.J.T., COOPER, M.D., LAWTON, A.R. III, MEGSON, M. & GATHINGS, W.E. (1975) Differences in susceptibility of mature and immature B lymphocytes to anti-immunoglobulin-induced immunoglobulin suppression *in vitro*. Possible implications for B-cell tolerance to self. *J. exp. Med.* **142**, 1052.

ROELANTS, G.E., MAYOR, K.S., HÄGG, L.-B. & LOOR, F. (1976) Immature T-lineage lymphocytes in athymic mice. Presence of TL, lifespan and homeostatic regulation. *Europ. J. Immunol.* **6**, 75.

RUUSKANEN, O. (1975) Subpopulations of guinea-pig thymocytes. Different distribution patterns of alkaline phosphatase positive and negative autologous thymocytes. *Cell. Immunol.* **15**, 246.

SCHLESINGER, M. & ISRAEL, E. (1975) The recovery of spleen-seeking and lymph node-seeking thymus subpopulations following cortisol administration. *Cell Immunol.* **18**, 144.

SCHRADER, J.W. (1975) Effector cell blockade. II. A demonstration of the reversible masking of an immune response by blockade of antibody-forming cells. *Europ. J. Immunol.* **5**, 808.

SCOTT, D.W. & WAKSMAN, B.H. (1968) Tolerance *in vitro*: Suppression of immune responsiveness to bovine γ-globulin after injection of antigen into intact lymphoid organs. *J. Immunol.* **100**, 912.

SCOTT, D.W. & WAKSMAN, B.H. (1969) Mechanism of immunologic tolerance. I. Induction of tolerance to bovine γ-globulin by injection of antigen into intact organs *in vitro*. *J. Immunol.* **102**, 347.

SHEARER, G.M., GARBARINO, C.A. & CUDKOWICZ, G. (1976) *In vitro* induction of F_1 hybrid anti-parent cell-mediated cytotoxicity. *J. Immunol.* **117**, 754.

SHORTMAN, K. & JACKSON, H. (1974) The differentiation of T-lymphocytes. I. Proliferation, kinetics and interrelationships of subpopulations of mouse thymus cells. *Cell. Immunol.* **12**, 230.

SHORTMAN, K., VON BOEHMER, H., LIPP, J. & HOPPER, K. (1975) Subpopulations of T-lymphocytes. *Transplant. Rev.* **25**, 163.

SMITH, J.B. & EATON, G.J. (1976) Suppressor cells in spleens from 'nude' mice: Their effect on the mitogenic response of B lymphocytes. *J. Immunol.* **117**, 319.

SMITH, S.B., ISAKOVIĆ, K. & WAKSMAN, B.H. (1966) Role of the thymus in tolerance. II. Transfer of specific unresponsiveness to BSA with thymus grafting. *Proc. Soc. exp. Biol. (N.Y.)* **121**, 1005.

STAPLES, P.J., GERY, I. & WAKSMAN, B.H. (1966) Role of the thymus in tolerance. III. Tolerance to bovine gamma globulin after direct injection of antigen into the shielded thymus of irradiated rats. *J. exp. Med.* **124**, 127.

STUTMAN, O. (1975) Humoral thymic factors influencing postthymic cells. *Ann. N.Y. Acad. Sci.* **249**, 89.

SULZBERGER, M.B. (1929) Hypersensitiveness to arsphenamine in guinea-pigs. Experiments in prevention and in desensitization. *Arch. Derm. Syph.* **20**, 669.

TADA, T. (1975) Regulation of reaginic antibody formation in animals. *Progr. Allergy* **19**, 122.

TADA, T., TANIGUCHI, M. & DAVID, C.S. (1977) Suppressive and enhancing T cell factors as I region gene products: properties and the subregion assignment. *Origins of Lymphocyte Diversity, XLI Cold Spring Harbor Symposia on Quantitative Biology*, p. 119, Cold Spring Harbor Laboratory, New York.

TADA, T., TANIGUCHI, M. & TAKEMORI, T. (1975) Properties of primed suppressor T cells and their products. *Transplant. Rev.* **26**, 106.

TADAKUMA, T., KUHNER, A.L., RICH, R.R., DAVID, J.R. & PIERCE, C.W. (1976) Biological expressions of lymphocyte activation. V. Characterization of a soluble immune response suppressor (SIRS) produced by concanavalin A-activated spleen cells. *J. exp. Med.* **117**, 323.

THOMAS, D.W., ROBERTS, W.K. & TALMAGE, D.W. (1975a)

Regulation of the immune response: Production of a soluble suppressor by immune spleen cells *in vitro*. *J. Immunol.* **114**, 1616.

THOMAS, D.W., TALMAGE, D.W. & ROBERTS, W.K. (1975b) Inhibition of tumor cell proliferation: A second role for suppressor cells? *J. Immunol.* **115**, 1366.

TOULLET, F.T. & WAKSMAN, B.H. (1966) Role of the thymus in tolerance. IV. Specific tolerance to homografts in neonatally thymectomized mice grafted with thymus from tolerant donors. *J. Immunol.* **97**, 686.

TRAUB, E. (1936) The epidemiology of lymphocytic choriomeningitis in white mice. *J. exp. Med.* **64**, 183.

VARGA, J.M. DI PASQUALE, A., PAWELEK, J., MCGUIRE, J.S. & LERNER, A.B. (1974) Regulation of melanocyte stimulating hormone action at the receptor level: Discontinuous binding of hormone to synchronized mouse melanoma cells during the cell cycle. *Proc. nat. Acad. Sci.* (*Wash.*) **71**, 1590.

VOJTÍŠKOVÁ, M. & LENGEROVÁ, A. (1965) On the possibility that thymus-mediated alloantigenic stimulation results in tolerance response. *Experientia* **21**, 661.

VOISIN, G.A. (1971) Immunological facilitation, a broadening of the concept of the enhancement phenomenon. *Prog. Allergy* **15**, 328.

VOISIN, G.A. & KINSKY, R. (1962) Protection against runting by specific treatment of newborn mice, followed by increased tolerance. *Ciba Found. Symposium on Transplantation*, (ed. by G.E.W. Wolstenholme and M.P. Cameron) p. 286, Churchill, London.

WAGSHAL, A.B., JEGASOTHY, B.V. & WAKSMAN, B.H. (1977) Cell cycle specificity of inhibitor of DNA synthesis action. *Fed. Proc.* **36**, 1320 (abstract).

WAKSMAN, B.H. & NAMBA, Y. (1976) On soluble mediators of immunologic regulation. *Cell. Immunol.* **21**, 161.

WEISSMAN, I.L. (1977) Thymus and T cell maturation. *Origins of Lymphocyte Diversity, XLI Cold Spring Harbor Sympos. on Quantitative Biology*, p. 9, Cold Spring Harbor Laboratory, New York.

WELLS, H.G. (1911) Studies on the chemistry of anaphylaxis (III). Experiments with isolated proteins, especially those of the hen's egg. *J. infect. Dis.* **9**, 147.