Clin. exp. Immunol. (1971) **9**, 437–447.

REVIEW

RECENT OBSERVATIONS AND CONCEPTS IN IMMUNOLOGICAL UNRESPONSIVENESS AND AUTOIMMUNITY*

W. O. WEIGLE†

Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

(*Received* 8 *April* 1971)

There are a variety of situations that result in an immunological unresponsive state. However, the specific unresponsive state resulting from a prior exposure to the antigen is of particular importance in understanding the mechanisms involved in both antibody production and autoimmunity. The best example of antigen-directed immunological unresponsiveness is the unresponsive state we have to our own body constituents. In all probability, this unresponsive state develops early in life as a result of direct contact between self components and receptor sites present on the surface of antigen reactive cells. Since first hypothesized by Burnet (1959), a large number of investigators have demonstrated that specific immunological unresponsiveness could be induced to a variety of antigens during early life before acquisition of immunocompetence. It since has been shown that, under selective conditions, a similar unresponsive state can be induced in adult, immunocompetent animals. The induction in adults requires that the antigen be injected under conditions which fail to result in an immune response. Either the immune response can be temporarily abolished with irradiation or immunosuppressive drugs or the antigen can be injected in a non-immunogenic form. The best example of the latter is the induction of unresponsiveness in mice to heterologous γ-globulins (Dresser, 1962). Adult mice respond well to either aggregated preparations of human γ-globulin (HGG) or HGG incorporated into Freund's adjuvant but do not respond to monomeric (deaggregated) preparations of HGG. When deaggregated (ultracentrifuged) preparations are injected into certain strains of mice, these mice develop a complete unresponsive state of long duration to subsequent injections of aggregated HGG. Deaggregated HGG lends itself extremely well to the studies of immunological unresponsiveness, since like other heterologous proteins following injection, it persists in the circulation and equilibrates throughout the intra- and extravascular fluid spaces coming in contact

* Publication No. 489 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation. The work was supported by United States Public Health Service Grant AI-07007, American Cancer Society Grant T-519 and Atomic Energy Commission Contract AT(04-3)-410.

† Supported by United States Public Health Services Research Career Award No. 5-K6-GM-6936.

Correspondence: Dr William O. Weigle, Department of Experimental Pathology, Scripps Clinic and Research Foundation, 476 Prospect Street, La Jolla, California 92037, U.S.A.

with all the antigen reactive cells. In addition, the cellular events involved in both the responsive and unresponsive states can be explored with a modification (Golub *et al.*, 1968) of the Jerne haemolytic plaque assay (Jerne & Nordin, 1963).

CELLULAR SITES OF IMMUNOLOGICAL UNRESPONSIVENESS

It has been demonstrated that the humoral antibody response in mice to sheep red blood cells (SRBC) (Claman, Chaperon & Triplett, 1966; Miller & Mitchell, 1967), bovine serum albumin (BSA) (Taylor, 1968) and HGG (Habicht, Chiller & Weigle, 1969) requires a collaborative role between thymus and bone marrow cells. The immunocompetence of irradiated recipients could be restored by either spleen cells or a combination of thymus and bone marrow cells, but not by either thymus or bone marrow cells alone. Both cell types are also involved in the induction of immunological unresponsiveness (Chiller, Habicht & Weigle, 1970). Neither thymus nor bone marrow cells from A/J mice injected 21 days previously with deaggregated HGG (tolerogen) could participate in the reconstitution of irradiated (840 r)-syngeneic recipients (Table 1). These observations demonstrated that both

TABLE 1. Collaboration between thymus and bone marrow cells from normal and unresponsive mice in antibody production to HGG

Treatment	Indirect PFC/spleen
nT×nBM	2508
uT×nBM	0
nT×uBM	0
uT×uBM	0

n, Cells from normal donors; u, cells from unresponsive donors; T, thymus cells; BM, bone marrow cells.

thymus and bone marrow cells can be made unresponsive and that both probably contain specific receptor sites for antigen. Furthermore, they suggest that only one of the two cell types needs to be unresponsive in order that the animal is unresponsive. Mitchell & Miller (1970), using similar reconstitution experiments, recently observed that spleen cells and thoracic duct lymphocytes, but not thymus or bone marrow cells, became unresponsive in mice injected with cyclophosphamide and SRBC. As suggested by the authors, the failure of induction of unresponsiveness in the thymus may have been the failure of penetration of the thymus by the antigens. One would not expect a sufficient concentration of antigens from SRBC to persist in the circulation and equilibrate into the extravascular fluid spaces of either the thymus or bone marrow. Gershon & Kondo (1970) were able to circumvent this problem by inducing unresponsiveness in bone marrow cells after they were transferred to thymectomized and irradiated mice. These data indicated that an interaction between thymus and bone marrow cells was a prerequisite for the induction of unresponsiveness in bone marrow cells to SRBC. In contradiction to the observations of Mitchell & Miller,

Playfair (1969) reported unresponsiveness in bone marrow cells in mice injected with SRBC and cyclophosphamide. However, (NZB × BALB/c) F_1 hybrids were used in these studies. There is some question whether the immune response in NZB mice is normal. Taylor (1968) found only the thymus cells unresponsive in adult mice receiving multiple injections of BSA; however, as will be discussed later, his results can be explained by the difference in the kinetics of the induction of unresponsiveness in thymus and bone marrow cells.

In addition to the thymus and bone marrow cells, macrophages are undoubtedly involved in the immune response to many, if not most antigens. However, they play a nonspecific role. Macrophages fix small amounts of antigen to their surface, which act as effective immunogen when the macrophages are injected into normal mice (Unanue & Askonas, 1968). This role is nonspecific in that macrophages from immunologically unresponsive, as well as normal mice, are effective (Kolsch & Mitchison, 1968).

The thymus cell has been shown to be a site of unresponsiveness in delayed hypersensitivity in the rat (Isakovic, Smith & Waksman, 1965). Although bone marrow cells are necessary for manipulation of the skin reaction (Lubaroff & Waksman, 1968) they are nonspecific in that, in reconstitution experiments, they are effective when their source is an unresponsive donor (J. D. Feldman, personal communication).

CELLULAR KINETICS OF INDUCTION

The establishment of immunological unresponsiveness in mice to deaggregated HGG requires approximately 4 days for completion (Golub & Weigle, 1967), but 70, 85 and 92% of the cells are unresponsive after 6, 24 and 48 hrs, respectively (Chiller & Weigle, 1970). Since thymus and bone marrow cells fail to reconstitute the immunocompetence of irradiated mice to aggregated HGG if only one of the cell types is unresponsive, it was possible to study the kinetics of the induction of unresponsiveness separately in thymus and bone marrow cells (Chiller, Habicht & Weigle, 1970). Thymus and bone marrow cells were removed from A/J mice at various times after injection of 2·5 mg deaggregated HGG and the ability of either the thymus or bone marrow cells, when injected with their normal counterpart, to reconstitute irradiated recipients was tested. These experiments demonstrated that thymus cells become unresponsive very rapidly, reflecting the kinetics of induction in the intact spleen, whereas the induction in the bone marrow cells is much slower, requiring 15–21 days before it is complete (Fig. 1). The spontaneous loss of the unresponsive state is also much slower in the thymus than the bone marrow cells. By day 49, following injection of the tolerogen, the bone marrow cells completely return to the responsive state, while the thymus cells remain unresponsive for at least 100 days. There are several explanations that can account for the difference in the rate of induction of unresponsiveness in thymus and bone marrow cells. First, the difference may be quantitative, i.e. bone marrow cells all may require much larger amounts of tolerogen. The time required for induction may be that required for optimal uptake of tolerogen by the bone marrow cells. Second, the induction of unresponsiveness in bone marrow cells may be dependent on thymus cells. There are some recent data which suggest that bone marrow cells do not become unresponsive in the absence of thymus cells (Gershon & Kondo, 1970). In becoming unresponsive, the thymus may release a humoral factor that influences the induction of unresponsiveness in bone marrow cells, or thymus–bone marrow cell interaction may have to occur for induction of

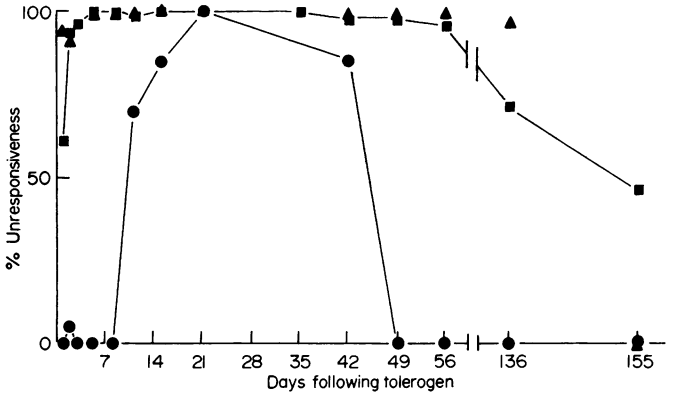

FIG. 1. Kinetics of the induction of unresponsiveness in thymus and bone marrow cells. ■, Thymus; ●, Bone marrow; ▲, DHGG injected donor. DHGG deaggregated human γ-globulin. Modified from Chiller *et al.* (1971).

tolerance as it does for induction of immunity. Third, unresponsiveness in thymus cells may involve only interaction with the antigen, while in the bone marrow cells it may involve an active process requiring a latent period. Although this active process may be the synthesis of an effective number of antibody molecules which act as receptor sites, it is unlikely that it involves the synthesis of detectable antibody for export. Neither direct or indirect plaque-forming cells to HGG can be detected in the spleen of adult A/J mice during a 20-day period following the injection of a tolerogenic dose of deaggregated HGG (Chiller & Weigle, 1971). Similarly, antibody producing cells to BSA cannot be detected in either the spleen, thymus or appendix during a 22-day period following a tolerogenic injection of BSA into newborn rabbits (Chiller, Romball & Weigle, 1971). Although these data do not rule out the possibility that circulating antibody plays a role in the induction of immunological unresponsiveness, they do not support a mandatory requirement of antibody formation. Others, using different types of antigen, have observed antibody formation either preceding or during the induction of unresponsiveness. However, it may be that the establishment of an unresponsive state occurs despite a transient production of antibody rather than because of antibody formation. Sterzl (1966) suggested that the transient production of antibody to *Salmonella*, followed by an unresponsive state in rabbits, may be the result of exhaustive differentiation of competent cells into short-lived antibody producing cells. Conversely, Rowley & Fitch (1965) suggested that the role played by antibody, produced during the induction of unresponsiveness in neonatal rats to SRBC, was that of feedback inhibition, resulting in a direct suppression of immunocompetent cells. With certain doses of pneumococcal polysaccharides, an inhibition of specific plaque-forming cells can be obtained in the spleens of adult mice, but rosette-forming cells can be detected (Howard, Christie & Courtenay, 1970; Sjoberg & Moller, 1970). A direct effect of antibody was demonstrated by Diener & Feldman (1970), who found that a specific immunosuppression occurred *in vitro* when both antibody and antigen were incubated together with spleen cells. They suggested that the role of antigen may be to focus antibody on the cells. It has recently been suggested by Bretscher & Cohen (1970) that the unresponsive state was the result of an absence of 'carrier antibody'. If an absence of 'carrier effect' is responsible for unresponsiveness, it seems more likely that the carrier effect is represented by receptor sites on antigen reactive

cells and that these cells are absent in unresponsive animals. The role of antibody in the *in vitro* induction of unresponsiveness may be that of presenting the antigen in a proper physical state to the receptors of the cells involved.

The *in vitro* induction of unresponsiveness with thymus independent antigens is much more rapid than would be expected from the kinetics of induction of unresponsiveness to HGG. The *in vitro* induction of unresponsiveness (partial) to the lipopolysaccharide of *E. coli* (Britton, 1969) and flagellin (Diener & Armstrong, 1969) takes only a few hours. This would not be expected if only bone marrow cells were involved. There are several possible explanations for the short time period needed for induction of unresponsiveness to these thymus independent antigens. First, the response to these antigens may involve a collaboration between the bone marrow cell and a cell type other than the thymus cell. Second, the bone marrow cells may contain a few thymus-derived cells which are more effective in their interaction with certain antigens than with others. Third, as the result of absence of thymus dependency, there is a shorter induction period that has evolved as a result of necessity to survive.

ROLE OF ANTIGEN RECEPTORS

It seems most likely that in the induction of either immunological unresponsiveness or an immune response thymus and bone marrow cells interact with the antigen via specific receptors present on these cells. In the immune response, it has been suggested that the thymus and bone marrow-derived cells react with different determinants (Mitchison, 1969; Rajewsky *et al.* 1969). In any event, it appears that the interactions leading to unresponsiveness differ from those leading to immunity. In some cases, the interaction may depend on the quantity and in others the quality (physical form) of the antigen. In the situation where immunogenic forms and quantities of antigen are injected, prolonged interactions in the absence of an immune response (in immunoincompetent animals) might lead to an unresponsive state. In these situations where the interactions result in an unresponsive cell population, it is not known whether the cells are eliminated or merely inhibited. The presence of antigen-reactive cells in unresponsive animals may be an indication that viable, unresponsive cells do exist. Several studies have been carried out with autoradiography where attempts have been made to detect cells reactive with ^{125}I-labelled antigens. Naor & Sulitzeanu (1969) working with BSA observed either no or very few antigen-reactive cells in unresponsive mice. On the other hand, antigen-reactive cells were observed in lymphoid tissue from rats unresponsive to either haemocyanin or a cyanogen bromide fragment of flagellin (Ada, 1970) and, to a lesser extent, mice unresponsive to haemocyanin (Humphrey & Keller, 1970). As mentioned above, unresponsiveness needs to be present in either the thymus or bone marrow-derived cells but not both. Because of the small amounts of antigen injected in these experiments and their inability to persist free in the body fluid, it is likely that unresponsiveness is induced in the thymus-derived but not the bone marrow-derived cells. These antigens, in all probability, are thymus-dependent since Unanue (1970) showed that haemocyanin was thymus-dependent in the mouse and Nossal (1969) demonstrated a dependency for the thymus for flagellin in the rat. Thus, the antigen-reactive cells in an unresponsive animal may be an indication of the presence of immunocompetent cells. It would be of interest to determine if the antigen-reactive cells in unresponsive mice are theta positive.

DOSE RESPONSE

The dose of antigen is very critical in determining whether or not an unresponsive state is induced. Usually, the higher the dose of antigen, the more effective it is in the induction of unresponsiveness. Exceptions to this general rule will be discussed below. In view of the difference in the rate of induction and stability of unresponsiveness in the thymus and bone marrow-derived cell, it is not surprising that the dose of antigen required to render these cells unresponsive also differs. The thymus cells of A/J mice become unresponsive with 2·5, 0·5 and 0·1 mg of deaggregated HGG (Table 2), whereas the bone marrow cells do not become unresponsive with the two lower doses (Chiller, Habicht & Weigle, 1970). This result suggests that, with low levels of either foreign or self antigen, unresponsiveness is only in the thymus-derived cells.

TABLE 2. The effect of dose of deaggregated HGG (tolerogen) on the induction of unresponsiveness in thymus and bone marrow cells to HGG in adult A/J mice*

Dose of tolerogen injected (mg)	% unresponsiveness in:	
	Thymus	Bone marrow
0·1	96	9
0·5	99	56
2·5	99	70

* 11 days after injection of tolerogen.

In certain situations, a 'high-low zone tolerance' has been observed where high and low doses of antigen produce unresponsiveness, while intermediate doses produce an immune response. This phenomenon has been reported with multiple doses of both BSA in adult mice (Mitchison, 1964) and flagellin in neonatal rats (Shellam & Nossal, 1968). In the case of flagellin, injections of ultralow doses were given (1×10^{-15} g) and the injections extended into a time when the rats were immunologically competent. A 'high-low zone tolerance' apparently also occurs with BSA in adult rabbits (Thorbecke & Benacerraf, 1967). It is possible that high-low zone tolerance is the result of special circumstances where the host is able to respond and the antigen contains both immunogenic and tolerogenic forms of the antigen. At various concentrations, the immunogenic and tolerogenic forms may compete differently, leading to unresponsiveness in some cases and immunity in others. In situations where the preparation contains only immunogen or the animal is unable to respond, a 'high-low zone' phenomenon is not observed, e.g. unresponsiveness to deaggregated HGG in adult mice (Golub & Weigle, 1969) and unresponsiveness to BSA in neonatal rabbits (Weigle, 1971). In any event, Mitchison (1971) observed that unresponsiveness induced in mice with low doses of BSA, but not with high doses could be reversed with thymus cells previously stimulated with BSA.

TERMINATION OF IMMUNOLOGICAL UNRESPONSIVENESS

It has been demonstrated by a number of workers that immunological unresponsiveness is terminated following immunization with cross-reacting antigens (cited in Weigle, 1967). Although the unresponsive state to BSA induced in rabbits by neonatal injections is stable, it is readily terminated following immunization with either certain preparations of altered BSA (Weigle, 1962) or certain cross-reacting albumins (Weigle, 1961). After the termination of the unresponsive state, the rabbit will respond to a subsequent injection of BSA. However, none of the antibody is specific for BSA since it can all be absorbed with the terminating antigen and continued injection of BSA results in a return to the unresponsive state. More recently, it has been shown that there is no significant difference in the immune response to cross-reacting albumins in normal rabbits and rabbits rendered unresponsive by neonatal injections of BSA (Table 3) (Benjamin & Weigle, 1970). Both the quantitative and qualitative

TABLE 3. Mean antigen-binding capacities* of sera from normal and BSA-unresponsive rabbits after two courses of various soluble albumins

Terminating antigen	Status	No. of animals	Antigen tested				
			BSA	PSA	HSA	ESA	GPSA
PSA	Unresponsive	19	11·6	188·8	3·3	7·0	9·4
	Normal	12	11·7	249·1	3·7	9·2	8·0
HSA	Unresponsive	26	9·1	8·3	200·8	8·9	11·5
	Normal	26	7·8	6·9	142·7	7·6	10·0
GPSA	Unresponsive	9	7·1	32·6	15·7	10·8	324·4
	Normal	14	8·7	33·7	14·2	12·7	289·3
ESA	Unresponsive	15	6·6	18·7	15·1	170·2	11·9
	Normal	26	5·6	14·4	10·9	139·4	8·3

* μg antigen N bound to the globulin (precipitated with 50% saturated $(NH_4)_2SO_4$) present in 1·0 ml of serum.

response to the BSA-related determinants were the same in both groups. These results strongly indicate that the unresponsive rabbit has a normal complement of precursor cells to the tolerated antigen. The presence of a normal complement of precursor cells to BSA in a rabbit unresponsive to BSA could only be explained if the site of unresponsiveness was at some cell type other than the precursor cell. All of the events involved in the termination of immunological unresponsiveness to BSA, following injections of cross-reacting albumins, could be explained if the thymus-derived cells, but not the bone marrow-derived cells, were unresponsive. It was shown in adult mice that only one cell type had to be unresponsive in order that the mouse be unresponsive. In addition, in view of the rapidity of the spontaneous loss of unresponsiveness in the bone marrow cells of the mouse, an unresponsive state in the bone marrow-derived cells would not be expected at the time of injection of the cross-reacting albumins (90 days after neonatal injections). The response to a subsequent injection of BSA would be expected if the memory cells could be stimulated by BSA. The

failure of such an injection to cause the production of antibody to determinants on BSA-unrelated to those on the cross-reacting antigen would also be anticipated since the failure of BSA to react with thymus-derived cells would preclude the recruitment of additional precursor cells with specificity for BSA. In the absence of recruitment of additional precursor cells, continued injections of BSA would cause an exhaustive differentiation of the memory cells and the rabbit would be expected to return to the unresponsive state.

The absence of unresponsiveness in bone marrow cells would also explain the ability of injections of DNP-BSA to terminate unresponsiveness induced with small injections of BSA, but not with large injections (Paul *et al.*, 1969). In these experiments, rabbits were injected periodically for 46 days starting on the day of birth with small doses of BSA. An additional group received injections of 100 mg of BSA on days 50 and 53. The former group, but not the latter, lost their unresponsive state following immunization with DNP-BSA. It it most likely that the injections of small doses of BSA induced unresponsiveness in only the thymus cells and it was not until the large doses were injected that unresponsiveness was induced in the bone marrow-derived cells. A similar explanation can be offered for the normal response to DNP following immunization with DNP-horse γ-globulin of adult rabbits rendered unresponsive by neonatal injections of DNP-BSA (Weigle, 1965).

AUTOIMMUNITY

The events involved in certain experimental autoimmune diseases appear to be similar to those involved in the termination of acquired immunologic unresponsiveness in rabbits to heterologous serum proteins. There is little question that rabbits enjoy an unresponsive state to their own thyroglobulin (Nakamura & Weigle, 1967). Injections of aqueous preparations of either certain heterologous thyroglobulins (Weigle, 1967) or preparations of altered homologous thyroglobulin (Weigle, 1965) result in thyroiditis and the production of circulating antibody to rabbit thyroglobulin. A subsequent injection of rabbit thyroglobulin at a later time results in the production of antibody and a return of thyroiditis, but all the antibody is specific for the related determinants on the cross-reacting thyroglobulin and additional injections of rabbit thyroglobulin result in a return to the unresponsive state. These results could also be explained by unresponsiveness to autologous thyroglobulin in the thymus-derived cell but not in the bone marrow-derived cells. The low level of thyroglobulin present in the body fluids would not be expected to maintain an unresponsive state in bone marrow-derived cells. Thus, a different population of thymus-derived cells could react with the unrelated determinants of the cross-reacting thyroglobulin, permitting the precursor cells in the bone marrow to react with a self determinant. The precursor cell would then be stimulated to differentiate into cells making antibody to native rabbit thyroglobulin. This interpretation is in agreement with the evidence that circulating antibody is the effector of experimental thyroiditis in rabbits (Nakamura & Weigle, 1969). It may be that unresponsiveness at both the thymus and bone marrow-derived cells is required to assure protection against autoimmunity and that the ease with which experimental thyroiditis, aspermatogenesis, uveitis, encephalomyelitis and certain types of glomerulonephritis are induced results from a lack of unresponsiveness in the bone marrow cells as a result of insufficient concentrations in the body fluid of the antigen involved.

It is possible that the lack of unresponsiveness of bone marrow-derived cells may be responsible for certain autoimmune diseases in humans. The events involved in rheumatic

fever are compatible with this hypothesis. These patients contain circulating antibody to heart antigens which cross-react with streptococcal antigens and this disease has been associated with infections with streptococci. It has recently been shown that the white cells in the peripheral blood of these patients can be stimulated to proliferate by the streptococcal antigen, but not by the heart antigen (McLaughlin, Paterson & Wilkes, 1971) suggesting that, despite the presence of cells making antibody to heart antigens, the thymus cells are not responsive to these antigens. It may be that the streptococcal antigens that are unrelated to the heart antigens react with thymus cells specific for them, permitting the 'non-tolerant' bone marrow cells to react with determinants related to those present on the heart antigens.

A similar circumstance may be involved in children with hypopituitary dwarfism given therapeutic injections with growth hormones (Illig, 1970). The immunological consequences of such injections depend on the physiochemical nature of the hormone preparations. Preparations containing aggregated (altered) hormone cause a production of antibody in a high percentage of the patients, while clear preparations resulted in antibody production in only an occasional patient. Children containing antibody, following long-term treatment with the aggregated preparation, lost the antibody when the injections of aggregated preparations were replaced with injections of the clear preparations. This situation resembles the termination of immunological unresponsiveness following injections of altered tolerogen or cross-reacting antigens where unresponsiveness is probably present in only thymus-derived cells. Because of the low level of growth hormones in the body fluid, one would not expect unresponsiveness in the bone marrow-derived cells.

REFERENCES

ADA, G.L. (1969) Antigen binding cells in tolerance. *Transplant. Rev.* **5,** 105.

BENJAMIN, D.C. & WEIGLE, W.O. (1970) The termination of immunologic unresponsiveness to BSA in rabbits. *J. exp. Med.* **132,** 66.

BRETSCHER, P. & COHN, M. (1970) A theory of self-nonself discrimination. *Science,* **169,** 1042.

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.

BURNET, F.M. (1959) *The clonal selection theory of acquired immunity.* Cambridge University Press.

CHILLER, J.M., HABICHT, G.S. & WEIGLE, W.O. (1970) Cellular sites of immunologic unresponsiveness. *Proc. nat. Acad. Sci. (Wash.),* **65,** 551.

CHILLER, J.M., HABICHT, G.S. & WEIGLE, W.O. (1971) Kinetic differences in unresponsiveness of thymus and bone marrow cells. *Science,* **171,** 813.

CHILLER, J.M., ROMBALL, C.G. & WEIGLE, W.O. (1971) Induction of immunologic unresponsiveness in neonatal and adult rabbits. Differences in the cellular events (*Submitted for publication*).

CHILLER, J.M. & WEIGLE, W.O. (1971) Cellular events during the induction of immunologic unresponsiveness in adult mice. *J. Immunol. (In press.)*

CLAMAN, H.N., CHAPERON, E.A. & TRIPLETT, R.F. (1966). Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. exp. biol. (N.Y.),* **122,** 1167.

DIENER, E. & ARMSTRONG, W.D. (1969) Immunological tolerance *in vitro*. Kinetic studies at the cellular level. *J. exp. Med.* **129,** 591.

DIENER, E. & FELDMAN, M. (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.

DRESSER, D.W. (1962) Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen. *Immunology,* **5,** 378.

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

GOLUB, E.S., MISHELL, R.I., WEIGLE, W.O. & DUTTON, R.W. (1968) A modification of the hemolytic plaque assay for use with protein antigens. *J. Immunol.* **100,** 133.

GOLUB, E.S. & WEIGLE, W.O. (1967) Studies on the induction of immunologic unresponsiveness. II. Kinetics. *J. Immunol.* **99,** 624.

GOLUB, E.S. & WEIGLE, W.O. (1969) Studies on the induction of immunologic unresponsiveness. III. Antigen form and mouse strain variation. *J. Immunol.* **102,** 389.

HABICHT, G.S., CHILLER, J.M. & WEIGLE, W.O. (1970) Absence of plaque-forming cells in animals immunologically unresponsive to protein antigens. *Developmental Aspects of Antibody Formation and Structure* (Ed. by J. Sterzl, M. Riha), p. 893. Academia Publishing House of the Czechoslovakia Academy of Science, Prague.

HOWARD, J.G., CHRISTIE, G.H. & COURTENAY, B.M. (1970) Treadmill neutralization of antibody and central inhibition. *Transplantation,* **10,** 351.

HUMPHREY, J.H. & KELLER, H.U. (1970) Some evidence for specific interaction between immunologically competent cells and antigen. *Developmental Aspects of Antibody Formation and Structure* (Ed. by J. Sterzl and M. Riha), p. 485. Academia Publishing House of the Czechoslovakia Academy of Science, Prague.

ILLIG, R. (1970) Growth hormone antibodies in patients treated with different preparations of human growth hormone (HGH). *J. clin. Endocr.* **31,** 679.

ISAKOVIC, K., SMITH, S.B. & WAKSMAN, B.H. (1965) 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.

JERNE, N.K. & NORDIN, A.A. (1963) Plaque formation in agar by single antibody-producing cells. *Science,* **140,** 405.

KOLSCH, E. & MITCHISON, N.A. (1968) The subcellular distribution of antigen in macrophages. *J. exp. Med.* **128,** 1059.

LUBAROFF, D.M. & WAKSMAN, B.H. (1968) Bone marrow as source of cells in reactions of cellular hypersensitivity. I. Passive transfer of tuberculin sensitivity in syngeneic systems. *J. exp. Med.* **128,** 1425.

MCLAUGHLIN, J.F., PATERSON, P.Y. & WILKES, R.M. (1971) Rheumatic carditis: *In vitro* response of peripheral blood leukocytes to heart and streptococcal antigens. (Submitted for publication).

MILLER, J.F.A.P. & MITCHELL, G.F. (1967) The thymus and the precursors of antigen reactive cells. *Nature (Lond.),* **216,** 659.

MITCHELL, G.F. & MILLER, J.F.A.P. (1970) Cell to cell interaction in the immune response. V. Target cells for tolerance. *J. exp. Med.* **131,** 675.

MITCHISON, N.A. (1964) Induction of immunological paralysis in two zones of dosage. *Proc. roy. Soc. B,* **161,** 275.

MITCHISON, N.A. (1967) Antigen recognition responsible for the induction *in vitro* of the secondary response *Cold Spring Harb. Symp. quant. Biol.* **32,** 431.

MITCHISON, N.A. (1971). In *Cell Interactions in Immune Responses* (Ed. by O. Maksla, A. M. Cross and T.U. Kosumen), Academic Press, New York.

NAKAMURA, R.M. & WEIGLE, W.O. (1967) *In vivo* behavior of homologous and heterologous thyroglobulin and induction of immunological unresponsiveness to heterologous thyroglobulins. *J. Immunol.* **98,** 653.

NAKAMURA, R.M. & WEIGLE, W.O. (1969) Transfer of experimental autoimmune thyroiditis by serum from thyroidectomized donors. *J. exp. Med.* **130,** 263.

NAOR, D. & SULITZEANU, D. (1969) Binding of ^{125}I-BSA to lymphoid cells of tolerant mice. *Int. Arch. Allergy,* **36,** 112.

NOSSAL, G.J.V. (1969) Antigen. *Immunological Tolerance* (Ed. by M. Landy and W. Braun), p. 135. Academic Press, New York.

PAUL, W.E., THORBECKE, J., SISKIND, G.W. & BENACERRAF, B. (1969) The effect of dose in tolerance induction on the subsequent response to a cross-reactive antigen. *Immunology,* **17,** 85.

PLAYFAIR, J.H.L. (1969) Specific tolerance to sheep erythrocytes in mouse bone marrow cells. *Nature (Lond.),* **222,** 882.

RAJEWSKY, K., SCHIRRMACHER, V., NASE, S. & JERNE, N.F. (1969) The requirement of more than one antigenic determinant for immunogenicity. *J. exp. Med.* **129,** 1131.

ROWLEY, D.A. & FITCH, F.W. (1965) The mechanism of tolerance produced in rats to sheep erythrocytes. II. The plaque-forming cell and antibody response to multiple injections of antigen begun at birth. *J. exp. Med.* **121,** 683.

SHELLAM, G.R. & NOSSAL, G.J.V. (1968) Mechanism of induction of immunological tolerance. IV. The effects of ultra-low doses of flagellin. *Immunology*, **14,** 273.

SJOBERG, O. & MOLLER, E. (1970) Antigen binding cells in tolerant animals. *Nature* (*Lond.*) **228,** 780.

STERZL, J. (1966) Immunological tolerance as the result of terminal differentiation of immunologically competent cells. *Nature* (*Lond.*) **209,** 416.

TAYLOR, R.B. (1968) Immune paralysis of thymus cells by BSA. *Nature* (*Lond.*), **220,** 611.

THORBECKE, G.J. & BENACERRAF, B. (1967) Tolerance in adult rabbits by repeated nonimmunogenic doses of bovine serum albumin. *Immunology*, **13,** 141.

UNANUE, E.R. (1970) Thymus dependency of the immune response to hemocyanin: An evaluation of the role of macrophages in thyroidectomized mice. *J. Immunol.* **105,** 1339.

UNANUE, E.R. & ASKONAS, B.A. (1968) The immune response of mice to antigen in macrophages. *Immunology*, **15,** 287.

WEIGLE, W.O. (1961) The immune response of rabbits tolerant to bovine serum albumin to the injection of other heterologous serum albumins. *J. exp. Med.* **114,** 111.

WEIGLE, W.O. (1962) Termination of acquired immunological tolerance to protein antigens following immunization with altered protein antigens. *J. exp. Med.* **116,** 913.

WEIGLE, W.O. (1965) The immune response of rabbits tolerant to one protein conjugate following the injection of related protein conjugates. *J. Immunol.* **94,** 177.

WEIGLE, W.O. (1965) The production of thyroiditis and antibody following injection of unaltered thyroglobulin without adjuvant into rabbits previously stimulated with altered thyroglobulin. *J. exp. Med.* **122,** 1049.

WEIGLE, W.O. (1967) Natural and acquired immunologic unresponsiveness. *Monographs in Microbiology* World Publishing Co., Cleveland.

WEIGLE, W.O. (1971) Immunological unresponsiveness. *Text on transplantation* (Ed. by J. S. Najarian and R. L. Simmons). Lea & Febiger, Philadelphia.