IMMUNOLOGIC TOLERANCE AFTER SPECIFIC IMMUNIZATION*

By MARIANNE M. DORNER,‡ M.D., and JONATHAN W. UHR, M.D.

(From the Irvington House Institute and the Department of Medicine, New York University School of Medicine, New York)

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Specific immunologic unresponsiveness (tolerance) can be induced by injection of newborns or adults with large doses of antigen (1–7). One interpretation of these findings is that tolerance is induced before an immune response is initiated. Almost all experiments on the induction of tolerance, however, utilize initially non-immunized animals. It was the purpose of this study to determine the effect of immunization upon the subsequent induction of tolerance to the specific antigen. For this purpose, it was necessary to use an immunization system which satisfied the following criteria:

1. The antigen regularly stimulates the development of a population of immunized cells which persists and which can perform an easily measurable immunologic function. Ideally, the immunization procedure should stimulate the production of relatively small amounts of excess serum antibody so that the latter will not interfere with subsequent attempts to induce tolerance with large doses of antigen (6).

2. Specific tolerance can be induced in non-immunized adult animals by a larger dose of antigen than that used for immunization.

3. The quantity of persisting circulating antigen can be determined to exclude the possibility that "absence" of immunologic responsiveness might actually represent an immune response masked by excess antigen.

These criteria appeared to be satisfied by immunizing rabbits with crystalline bovine serum albumin (BSA). This relatively purified protein usually stimulates an easily detectable immune response in adult rabbits; the protein can be trace-labeled with I^{131} so that its immune elimination can be used as a semiquantitative technique for measuring serum antibody response; only small amounts of excess antibody remain in the circulation following immune elimination; tolerance can be induced to BSA in adult non-immunized rabbits; and the amount of BSA left in the circulation can be determined quantitatively. Using

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‡ Present address: Columbia Presbyterian Medical Center, New York.

this system, it has been shown that rabbits that have previously shown a primary antibody response and are prepared for a secondary antibody response to BSA can subsequently be made tolerant to BSA.

Materials and Methods

Antigens.—Crystalline bovine serum albumin (BSA) (Lot No. W 69312) and bovine gamma globulin (BGG) (Lot No. S 30008) were obtained from Armour Pharmaceutical Co., Chicago, and crystalline human serum albumin (HSA) (Lot No. 45FO4) and horse ferritin (Lot No. F78) from Pentex, Inc., Kankakee, Illinois.

Iodination of BSA.—BSA was trace-labeled with I^{131} by the method described by Helmkamp *et al.* (8). Carrier-free I^{131} obtained from Oak Ridge, Tennessee, was brought to pH 8.0 with borate buffer and 1 N HCl. Sufficient Na_2SO_2 was added to destroy H_2O_2 produced by beta irradiation and the excess sulfite was subsequently oxidized by aerating the solution in a boiling water bath for 15 minutes. Sufficient amounts of a 0.0069 M ICl solution to bind 4 atoms of I/molecule BSA were added to the cooled I^{131} solution, and the mixture was rapidly added to the protein solution. The efficiency of iodination was approximately 30 per cent on each of the 3 occasions that this procedure was performed. The solution was dialyzed in the cold against 0.15 N saline until at least 98 per cent of the I^{131} was precipitable in 10 per cent trichloracetic acid. The final concentration of protein was determined by Folin-Ciocalteu (9) or micro-Kjeldahl technique (10). The I^{131} activity was determined in a well-type scintillation counter.

Immunization and I^{131}-BSA (I^*-BSA) Elimination.—1 to 50 mg of BSA, usually trace-labeled with I^{131} were injected intravenously into 2 kg rabbits that had previously received potassium iodide. When trace-labeled antigen was injected, blood was obtained 3 minutes later and thereafter at least every other day from an ear vein. 0.1 ml serum samples were counted in polyvinylpyrrolidone (PVP)-coated planchets in a gas flow windowless counter. The amount of radioactivity in the serum at 3 minutes was considered as 100 per cent and the disappearance of I^* from the blood was calculated on that basis.

Induction of Immunological Unresponsiveness to BSA.—Newborn rabbits were injected with 100 mg BSA intraperitoneally each day for the first 5 days after birth.

Rabbits weighing approximately 2.5 kg were injected intravenously with 200 mg BSA daily for a period of 21 days (high doses of BSA, HDB).

Other Tests for Serum Antigen or Antibody.—The BSA content of serum from BSA-immunized rabbits was determined qualitatively by double Preer agar diffusion (11) or by quantitative precipitation according to the method of Gitlin (12). For the latter purpose, a quantitative precipitation curve was first determined for a hyperimmune rabbit antiserum to BSA to serve as a standard. This antiserum, containing 2.1 mg anti-BSA/ml, gave a single line of precipitation by Ouchterlony double diffusion-in-agar analysis (13) with crystalline or crude BSA, or whole bovine serum in varying dilutions. 0.1 ml of this antiserum was added to 0.15 ml of the BSA-containing serum, and the amount of protein of the precipitate was determined. The amount of antigen in the precipitate could then be calculated by reference to the standard quantitative precipitation curve.

Microhemagglutination was carried out by the method of Boyden (14) using a Takatsky microtitrator. An aliquot of a 0.25 per cent antigen-coated red cell suspension was added dropwise to the test serum diluted previously in the microhemagglutination plates. The test serum was diluted with saline containing heat-inactivated rabbit serum (1:100), and sedimented patterns were read after incubation for 16 to 20 hours at room temperature. For absorption of sera, 350 μg antigen was added and the mixture was incubated at 37°C for 30 minutes and 4°C for 24 hours. The precipitate was removed and the procedure was repeated on the supernatant two times using 150 μg of antigen each time. The final addition of antigen resulted in little or no precipitation.

RESULTS

The Primary and Secondary Antibody Response to BSA.—It was the purpose of the first experiment to determine whether 10 mg of BSA could stimulate as a routine a primary antibody response in adult rabbits. As can be seen in Fig. 1, following injection of 10 mg I^{131}-BSA (I*-BSA), all of 5 rabbits demonstrated an immune elimination with onset on day 6 to day 10. After 8 weeks the same rabbits were again injected with 10 mg of I*-BSA. In contrast to the first response, the onset of immune elimination occurred on day 3 in 4 rabbits and on day 7 in 1. Thus, injection of 10 mg of I*-BSA in adult rabbits induces a primary antibody response and also prepares for a secondary antibody response. It was also shown that a dose as low as 1 mg I*-BSA was eliminated in an immune fashion by all of 5 rabbits tested.

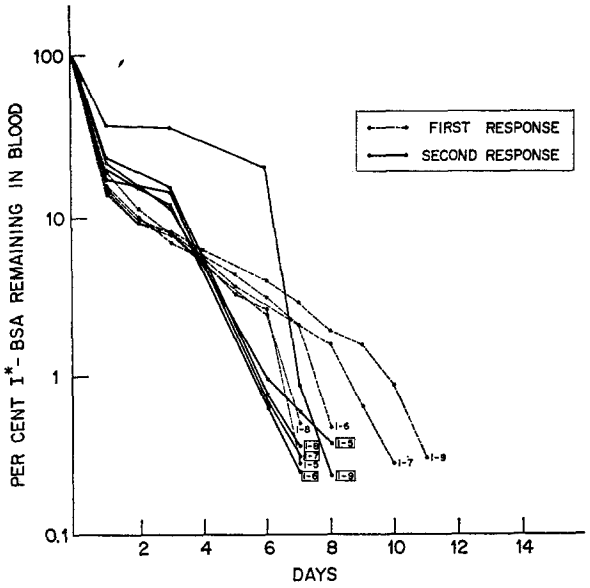

FIG. 1. The primary and secondary antibody response to BSA. 10 mg of I*-BSA was injected into 5 rabbits twice at an 8 week interval.

The Induction of Specific Tolerance by High Doses of BSA (HDB) in BSA-Immunized Rabbits.—In these experiments, 5 rabbits were injected with 10 mg I*-BSA and immune elimination was demonstrated. Following the elimination of BSA, the rabbits received HDB, and 3 weeks after this regimen was terminated, the rabbits were challenged with 1 mg of I*-BSA.

As can be seen in Fig. 2, all of the 5 rabbits that had previously shown an immune elimination of BSA now failed to demonstrate an immune elimination during the 14 days of observation. These same 5 animals were retested with 10 mg I*-BSA 2 months later at which time only 1 of the 5 animals had remained

tolerant. The serum of this animal was further tested for antibody by hemagglutination and for antigen by double Preer agar diffusion 2 months later and both tests were negative.

This experiment indicates that tolerance can be induced in a population of cells that has the capacity for secondary antibody responsiveness.

The Effect of Different Doses of BSA for Primary Immunization on the Subsequent Induction of Specific Tolerance Following HDB.—If tolerance induction in immune cells is more difficult than in non-immunized cells, induction of

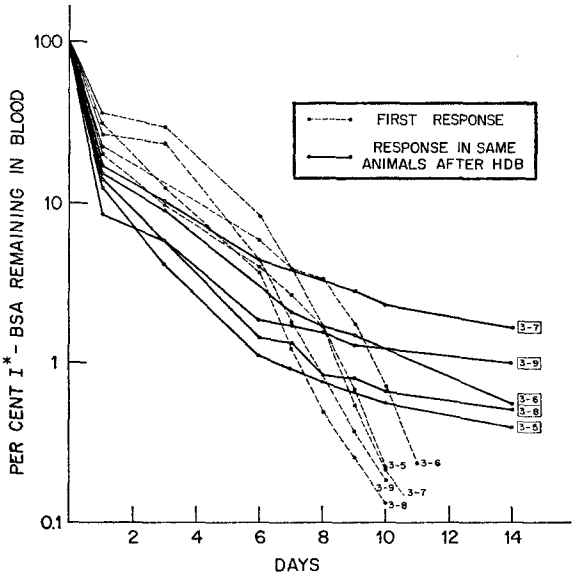

FIG. 2. The effect of HDB on the elimination of I*-BSA. I*-BSA was injected into 5 rabbits before and 3 weeks after HDB.

tolerance should be more difficult to achieve in immunized compared to non-immunized animals, and the difficulty should increase as the magnitude of the immune response increases. In order to investigate this possibility, groups of 3 rabbits received either 50, 10, or 1 mg of BSA for primary immunization; 1 group was not injected. Three weeks later, all the animals received the HDB. Three weeks after the termination of HDB, all animals and an additional group of 5 normal animals were tested with 1 mg of I*-BSA. As can be seen in Fig. 3, 7 of the 11 animals that received the HDB were unresponsive during the period of observation; the remaining 4 animals eliminated I*-BSA in an accelerated immune fashion. In contrast, all 5 normal animals challenged with I*-BSA eliminated the BSA in a primary immune fashion. There was no significant difference between the incidence of unresponsiveness in the 4 groups that had received the HDB. The amount of BSA in the circulation of 2 unresponsive and

1 normal rabbit 5 minutes after injection of 10 mg I*-BSA was determined by quantitative precipitation. The results indicated that unresponsive rabbits at this time had 115 and 175 μg of BSA/ml serum compared to 105 μg/ml for the normal. These results were consistent with calculations based on the amount of BSA injected during the HDB and the half-life of BSA in the circulation of rabbits (5.5 days) (15). If the immune cells which persisted following primary immunization had been unaffected by the HDB, then the secondary antibody

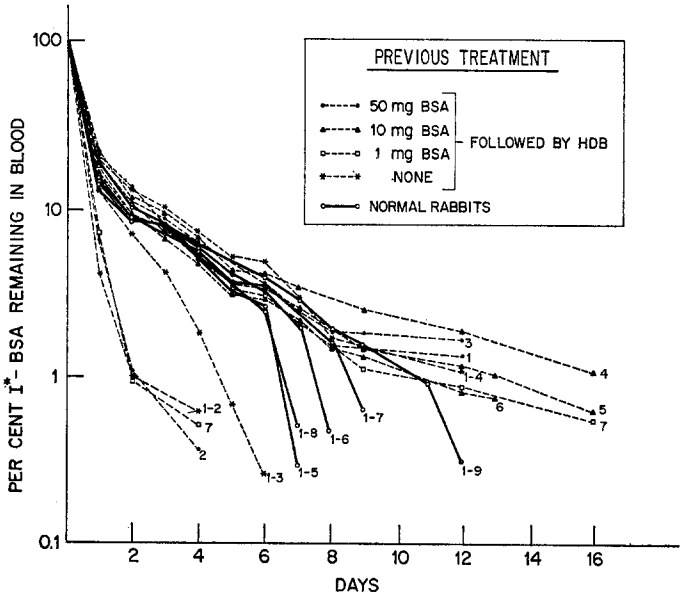

FIG. 3. The effect of different doses of BSA for primary immunization on the subsequent induction of specific tolerance following HDB. Rabbits were immunized with different doses of BSA followed by HDB, and 3 weeks later received 1 mg I*-BSA.

response of these cells to reinjection of I*-BSA should have resulted in an immune elimination detected well within the 16 days of observation in all animals. These same 7 tolerant animals were challenged 3 months later with 20 mg of I*-BSA and all were tolerant during the 14 days of observation. After an additional 6 weeks, sera were obtained from 4 animals and studied for antibody to BSA by hemagglutination. All 4 were negative: 2 of these sera were also tested for BSA by double Preer agar diffusion and both were negative. (10 μg of BSA/ml was easily detected by this method.)

The specificity of tolerance was demonstrated by injecting 4 tolerant animals and 2 controls with bovine gamma globulin. The primary hemagglutinating antibody response to BGG was similar in the 2 groups (1:640–2512 in BSA-tolerant rabbits; 1:1280 in the 2 controls).

This experiment failed to show a dose-response effect of the antigen used for primary immunization on the subsequent incidence of tolerance but did indicate that the tolerance induced in previously immunized animals can be long-lived.

Table I summarizes all experiments involving induction of tolerance. All of 4 newborns that received 500 mg BSA neonatally and were subsequently tested at 3 to 4 months of age, and 10 of 28 adult rabbits that received HDB were made tolerant. There was an unexplained variability in the incidence of tolerance between similar experiments which may reflect in part the dose of I*BSA used for challenge. Thus, in one experiment using 1 mg I*BSA for the challenge,

TABLE I

Summary of Experiments on Induction of Tolerance

Animal No.	Age	Amount of protein injected before HDB*	Tolerance induced by HDB‡
4-1, 4-3, 4-4, 4-6	Newborn	None	4/4
1, 2, 3	Adult	50 mg BSA	2/3
4, 5, 6	"	10 mg BSA	3/3
7, 9	"	1 mg BSA	1/2
1-2, 1-3, 1-4	"	None	1/3
3-5 to 3-9	"	10 mg I^{131}-BSA	1/5
5-6 to 6-2	"	10 mg Ferritin	1/8
6-3 to 6-8	"	None	1/6

* HDB, high doses of BSA.

‡ No. of animals made tolerant/No. of animals injected with HDB.

7 of 11 animals appeared tolerant, in contrast to a second experiment using 10 mg I*BSA for challenge in which only 3 of 18 animals appeared tolerant. There was no evidence, however, that a previous antibody response to BSA or to an unrelated antigen (ferritin) inhibited the subsequent induction of tolerance to BSA.

Attempts to Terminate Tolerance by Immunization with a Cross-Reacting Protein followed by BSA Immunization.—It was the purpose of this experiment to terminate tolerance in BSA-tolerant animals that had previously been immunized to BSA and to determine whether the antibody response to BSA would be a primary 19S or secondary 7S response. Weigle (16) has previously reported that acquired tolerance to BSA induced by neonatal injection of rabbits with BSA was terminated following immunization with serum albumins which cross-react with BSA. Accordingly, 4 adult tolerant rabbits and 5 normal adult rabbits were immunized with 20 mg of HSA and 7 weeks later with 20 mg of BSA. Both immunizing injections were administered in complete Freund's adjuvant into all 4 foot-pads.

Table II summarizes the hemagglutinating antibody responses to HSA and BSA in these animals. As can be seen, HSA stimulated substantial anti-HSA titers and relatively low anti-BSA titers at 2 weeks in both groups. Antibody activity was abolished by treatment with 0.1 M 2-mercaptoethanol suggesting that the antibody was γ-1M. Antibody determinations to HSA and BSA were repeated after absorption of representative sera with HSA or BSA. The results indicated that the low anti-BSA titers represented cross-reacting antibody to

TABLE II

Hemagglutinating Antibody Responses after Immunization with HSA Followed by BSA in BSA-Tolerant Animals

Group	Animal No.	Titer of hemagglutinating antibodies following injection of HSA*		Titer of hemagglutinating antibodies following injection of BSA*	
		BSA	HSA	BSA	HSA
Tolerant‡	1	320	2560	160	320,000
	3	40	640	320	320,000
	4	320	1280	320	320,000
	5	2560	10240	320	320,000
Controls	6-9	720	320	10,240	320,000
	7-0	80	5120	2560	160,000
	7 1	160	5120	10,240	320,000
	7-2	160	640	10,240	10,240
	7-3	160	640	10,240	40,960

* Antibody titer stated as reciprocal of highest dilution of antiserum that gave hemagglutination.

‡ Rabbits were previously immunized with either 10 or 50 mg BSA before induction of tolerance.

HSA. After immunization with BSA, high antibody titers appeared to BSA in control animals which by absorption studies were shown to be in large part specific to BSA. Antibody activity was not affected by treatment with 0.1 M 2-mercaptoethanol suggesting that the antibody was of 7S type. In contrast, the adult unresponsive animals made little or no antibody specific to BSA.

Thus, this experiment did not allow evaluation of the quality of the antibody response after termination of tolerance but it did indicate that the tolerant state in previously immunized animals is not easily terminated by vigorous immunization procedures.

DISCUSSION

Based on the observations of Owen (17) of blood chimaeras in dizygotic bovine twins, Burnet and Fenner (18) first suggested that a recognition system

must develop during embryonic life which allows the mature immune mechanism to distinguish between "self" and "not self" molecules and thus form antibodies only against the latter. These authors predicted that if a foreign protein was injected into embryos or newborns before maturation of the recognition system occurred, a state of immunologic unresponsiveness (tolerance) to that antigen would develop so that the foreign protein would thereafter be treated as an autologous one. This prediction was confirmed by the classical experiments of Billingham *et al.* (1) in which a state of specific immunologic tolerance was induced in mice by injecting them during the neonatal period with allogeneic spleen cells. These results confirmed that a recognition system existed and suggested that immunological tolerance involves "fooling" the recognition process by the introduction of foreign proteins before development of the recognition system.

It soon became apparent however that induction of immunologic tolerance was not limited to the embryonic or neonatal period. Dixon and Maurer (19) showed that daily infusions of large amounts of heterologous serum proteins for many weeks eventually induced a transient state of tolerance in adult rabbits followed by a period of accelerated antibody formation. Parabiosis (20–23) between adult mice resulted in partial tolerance to transplantation antigens if parabiosis lasted for a sufficient length of time. Chase (24) induced tolerance to picryl chloride in adult guinea pigs following oral administration for several weeks of the allergen. Finally, reexamination of Felton's "immunologic paralysis" (25) by Sercarz and Coons (26) and by Siskind *et al.* (27) suggested that this form of immunologic unresponsiveness also represents a state of immunological tolerance rather than the masking of antibody formation by excess antigen. All these studies indicate that prolonged exposure to excess antigen induces a state of tolerance in adult animals that appears similar to tolerance induced in newborn animals.

There are also several reported studies in which an immune response preceded the induction of tolerance. Felton *et al.* (28) stated that previously immunized mice required 8 times more polysaccharide to induce unresponsiveness than non-immunized mice and Dresser (29) observed that prior bovine γ-globulin immunization of mice impeded the induction of tolerance to BGG but that partial tolerance could be induced by administration of large doses of BGG. Of particular relevance are the studies of Simonsen (30). He showed that in the graft *versus* host runting syndrome, splenic enlargement occurs as a routine and is particularly pronounced at 10 days if adult parenteral spleen cells are injected into neonatal F_1 hybrid recipients. At 19 days, however, the recipient spleen appears atrophied and assay of its cells by their injection into neonatal F_1 hybrids indicates that the donor cell element of these spleens is unreactive to host antigens but can react to other antigens. Simonsen concludes that excessive antigenic stimulation induces a state of exhaustion in immunized cells re-

sulting in tolerance. Sercarz and Coons (31) also observed unresponsiveness in immunized mice after secondary challenge, but unresponsiveness was transient. Mitchison in a study of tolerance (32) to BSA in mice also concludes that there is a transient phase of immune response in the early stages of the tolerance-inducing regimen, since mice not made tolerant by such injections and later immunized, develop higher antibody levels than control immunized mice. None of these above studies indicates that a population of specifically stimulated immune cells (in contrast to non-stimulated but immunologically competent cells) would have been present at the time of immunological testing, and that prolonged administration of antigen has induced *long-lived* unresponsiveness (*i.e.* tolerance) in that population.

The studies presented here indicate that immunologic tolerance to a specific antigen can be induced in animals that have had a primary antibody response and are prepared for a secondary antibody response to that antigen. Thus, a population of specifically stimulated immune cells that has proliferated can subsequently be made tolerant. Presumably, such stimulated cells are the "memory" cells, large (33) or small lymphocytes (34) that would have differentiated into antibody-forming cells after specific challenge if tolerance had not been induced. The cellular and molecular events underlying the development of tolerance in this population, however, are not known. The results also fail to demonstrate an influence of these memory cells on the incidence of subsequent induced tolerance. The significance of this negative finding is uncertain, however, because of the small numbers of animals used, the unexplained variability between experiments in the incidence of inducing tolerance, and our ignorance of the mechanism(s) responsible for the induction or failure of induction of tolerance. It can be predicted, however, that an immunization procedure which results in the production of large amounts of serum antibody of high binding affinity will probably hamper the induction of tolerance because such serum antibody can bind to the injected antigen and presumably render it ineffective in tolerance induction, analogous to the "feedback" type of mechanism by which serum antibody inhibits antibody formation (35, 36). Thus, the difference between the relative ease of tolerance induction in our studies in contrast to those previously reported for immune animals may be due to the small amounts of excess antibody in BSA-immunized animals as well as the relatively poor immunogenicity of BSA.

Under what circumstances, do immunologically committed cells develop tolerance? This question has not been answered by the studies presented here nor by prior studies of others, in part because the conditions of induction of tolerance in which an excess of antigen is administered make it difficult to detect the initiation of an immune response. There are 2 findings, however, which, taken together suggest that tolerance in adults is usually initiated after the onset of an immune response: (*a*) Antibody formation can be detected within several

hours after injection of certain kinds of antigens (37–41), and the majority of immunization procedures probably initiate an immune response within several days. (*b*) Induction of tolerance in adults usually requires prolonged exposure to antigen as previously discussed.

It would appear, therefore, that in the induction of tolerance in adult animals some of the cells that participate in the process of tolerance induction are cells that have already responded to specific antigenic stimulation. It is possible of course that tolerance and antibody formation to the same antigenic determinant can proceed *pari passu*, with some cells becoming tolerant and others participating in the immune response. This may be a regular feature of both immunization and tolerance induction with quantitative considerations determining the eventual immunologic status. In any event, our studies indicate that unlimited immunization can be prevented by 2 mechanisms (*a*) excess antigen or (*b*) excess antibody. The latter when present may serve as the first line of defense, tolerance induction as the second.

No analogous information is available concerning the induction of tolerance in embryos or newborns, but immunization of newborn humans as well as sheep embryos has shown that immature members of both species can respond to certain antigens. For example, bacteriophage ϕX 174 can immunize newborn premature infants that weigh as little as 1000 gm (42) and can immunize sheep embryos during the end of the first trimester of gestation (43). These findings suggest that the immune mechanism may mature before the onset of formation of certain self antigens; therefore, an immune response could theoretically be initiated by such antigens before tolerance is induced. On the other hand, the histology of fetal sheep lymphoid tissue presents no evidence for previous immunization (44).

The present findings suggest, therefore, that tolerance induction in stimulated immune cells is an important but probably not exclusive pathway for tolerance induction in adult animals. Our findings also suggest that tolerance induction is another mechanism for preventing continuing proliferation of immune cells chronically exposed to large amounts of antigen. In this sense, immunological tolerance can be viewed as a form of cellular adaptation which serves the purpose of providing the necessary biological information for distinguishing self *versus* not self in a simple fashion: self is constantly present; not self is not.

SUMMARY

Specific immunologic tolerance to bovine serum albumin (BSA) was induced in approximately one-half of the rabbits that had been primarily immunized and were prepared for a secondary antibody response to BSA. The state of tolerance lasted for several months in the majority of rabbits and was not easily terminated by immunization with human serum albumin followed by BSA.

BIBLIOGRAPHY

1. Billingham, R. E., Brent, L., and Medawar, P. B., "Actively acquired tolerance" of foreign cells, *Nature*, 1953, **172,** 603.
2. Hanan, R., and Oyama, J., Inhibition of antibody formation in mature rabbits by contact with the antigen at an early age, *J. Immunol.*, 1954, **73,** 49.
3. Kerr, W. R., and Robertson, M., Passively and actively acquired antibodies for *Trichomonas foetus* in very young calves, *J. Hyg.*, 1954, **52,** 253.
4. Cinader, B., and Dubert, J. M., Specific inhibition of response to purified protein antigens, *Proc. Roy. Soc. London, Series B*, 1956, **146,** 18.
5. Wolfe, H. R., Tempelis, C., Mueller, A., and Reibel, S., Precipitin production in chickens. XVII. The effect of massive injections of bovine serum albumin at hatching on subsequent antibody production, *J. Immunol.*, 1957, **79,** 147.
6. Smith, R. T., and Bridges, R. A., Immunological unresponsiveness in rabbits produced by neonatal injection of defined antigens, *J. Exp. Med.*, 1958, **108,** 227.
7. Bussard, A., Tolérance immunologique provoquée chez le lapin envers certains antigènes de la levûre, *Compt. Rend. Acad. Sc.*, 1957, **245,** 2430.
8. Helmkamp, R. W., Goodland, R. L., Bale, W. F., Spar, J. L., and Mutschler, L. E., High specific activity iodination of gamma-globulin with Iodine^{131} monochloride, *Cancer Research*, 1960, **20,** 1495.
9. Ciocalteu, V., and Folin, O., Tyrosine and tryptophane determinations in proteins, *J. Biol. Chem.*, 1927, **73,** 627.
10. Markham, R., A steam distillation apparatus suitable for micro-kjeldahl analysis, *Biochem. J.*, 1942, **36,** 790.
11. Preer, J. R., A quantitative study of a technique of double diffusion in agar, *J. Immunol.*, 1956, **77,** 52.
12. Gitlin, D., Use of ultraviolet adsorption spectroscopy in the quantitative precipitin reaction, *J. Immunol.*, 1949, **62,** 437.
13. Ouchterlony, D., Diffusion-in-gel methods for immunological analysis, *Progr. Allergy*, 1958, **5,** 1.
14. Boyden, S. V., The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera, *J. Exp. Med.*, 1951, **93,** 107.
15. Smith, R. T., Studies on the mechanism of immune tolerance, *in* Mechanisms of Antibody Formation, (M. Holub and L. Jaroskova, editors), Prague, Czechoslovak Academy of Sciences, 1960, 313.
16. Weigle, W. O., The immune response of rabbits tolerant to bovine serum albumin to the injection of other heterologous serum albumins, *J. Exp. Med.*, 1961, **114,** 111.
17. Owen, R. D., Immunogenetic consequences of vascular anastomosis between bovine twins, *Science*, 1945, **102,** 400.
18. Burnet, F. M., and Fenner, F., The Production of Antibodies, Melbourne, Macmillan & Co., Ltd. 1949.
19. Dixon, F. J., and Maurer, P. H., Immunologic unresponsiveness induced by protein antigens, *J. Exp. Med.*, 1955, **101,** 245.

20. Mariani, T., Martinez, C., Smith, J. M., and Good, R. A., Induction of immunological tolerance to male skin isografts in female mice subsequent to the neonatal period, *Proc. Soc. Exp. Biol. and Med.*, 1959, **101,** 596.
21. Shapiro, F., Martinez, C., and Good, R. A., Homologous skin transplantation from F_1 hybrid mice to parent strains, *Proc. Soc. Exp. Biol. and Med.*, 1959, **101,** 94.
22. Martinez, C., Shapiro, F., and Good, R. A., Essential duration of parabiosis and development of tolerance to skin homografts in man, *Proc. Soc. Exp. Biol. and Med.*, 1960, **104,** 256.
23. Shapiro, F., Martinez, C., Smith, J. M., and Good, R. A., Tolerance of skin homografts induced in adult mice by multiple injections of homologous spleen cells, *Proc. Soc. Exp. Biol. and Med.*, 1961, **106,** 472.
24. Chase, M. W., Inhibition of experimental drug allergy by prior feeding of the sensitizing agent, *Proc. Soc. Exp. Biol. and Med.*, 1946, **61,** 257.
25. Felton, L. D., and Ottinger, B., Pneumococcus polysaccharide as a paralyzing agent on the mechanisms of immunity in white mice, *J. Bact.*, 1942, **43,** 94.
26. Sercarz, E., and Coons, A. H., Specific inhibition of antibody formation during immunological paralysis and unresponsiveness, *Nature*, 1959, **184,** 1080.
27. Siskind, G. W., Paterson, P. Y., and Thomas, L., Induction of unresponsiveness and immunity in newborn and adult mice and pneumococcal polysaccharide, *J. Immunol.*, 1963, **90,** 929.
28. Felton, L. D., Kauffman, G., Prescott, B., and Atlinger, B., Studies on mechanism of immunological paralysis induced in mice by pneumococcal polysaccharides, *J. Immunol.*, 1955, **74,** 17.
29. Dresser, D. W., Specific inhibition of antibody production. I. Protein-overloading paralysis, *Immunology*, 1962, **5,** 161.
30. Simonsen, M., On the acquisition of tolerance by adult cells, *Ann. New York Acad. Sc.*, 1960, **87,** 382.
31. Sercarz, E., and Coons, A. H., The exhaustion of specific antibody producing capacity during a secondary response, *in* Mechanisms of Immunological Tolerance, (M. Hăsek, A. Lengerová, and M. Vojtišková, editors), Prague, Czechoslovak Academy of Sciences, 1960, 73.
32. Mitchison, N. A., Long-term processes in paralysis, *in* Mechanisms of Immunological Tolerance, (M. Hăsek, A. Lengerová, and M. Vojtišková, editors), Prague, Czechoslovak Academy of Sciences, 1961, 245.
33. Nossal, G. J. V., and Makela, O., Autoradiographic studies on the immune responses. I. The kinetics of plasma cell proliferation, *J. Exp. Med.*, 1962, **115,** 209.
34. Gowans, J. L., The fate of parental strain small lymphocytes in F_1 hybrid rats, *Ann. New York Acad. Sc.*, 1962, **99,** 432.
35. Uhr, J. W., and Baumann, J., Antibody formation. I. The suppression of antibody formation by passively administered antibody, *J. Exp. Med.*, 1961, **113,** 935.
36. Uhr, J. W., and Baumann, J. B., Antibody formation. II. The specific anamnestic antibody response, *J. Exp. Med.*, 1961, **113,** 959.
37. Uhr, J. W., Finkelstein, M. S., and Baumann, J. B., Antibody formation. III. The primary and secondary antibody response to bacteriophage ϕX 174 in guinea pigs, *J. Exp. Med.*, 1962, **115,** 655.

38. Svehag, S. E., and Mandel, B., The production and properties of poliovirus neutralizing antibody of rabbit origin, *Virology*, 1962, **18**, 508.
39. Basch, R. S., personal communication.
40. Bauer, D. C., and Stavitsky, A. B., On the different molecular forms of antibody synthesized by rabbits during the early response to a single injection of protein and cellular antigens, *Proc. Nat. Acad. Sc.*, 1961, **47**, 1667.
41. Nossal, G. J. V., Mitchell, J., and McDonald, W., Autoradiographic studies on the immune response. IV. Single cell studies on the primary response, *Australian J. Exp. Biol. and Med. Sc.*, 1963, **41**, 423.
42. Uhr, J. W., Dancis, J., Franklin, E. C., Finkelstein, M. S., and Lewis, E. W., The antibody response to bacteriophage ϕX 174 in newborn premature infants, *J. Clin. Inv.*, 1962, **41**, 1509.
43. Silverstein, A. M., Uhr, J. W., and Kraner, K. L., Fetal response to antigenic stimulus. II. Antibody production by the fetal lamb, *J. Exp. Med.*, 1963, **117**, 799.
44. Silverstein, A. M., and Lukes, R. L., unpublished results.