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IMMUNOLOGICAL TOLERANCE TO MICROBIAL ANTIGENS*

Editor and Conference Chairman
HERMAN FRIEDMAN

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Part III. Persistent Tolerant Infection to LCM and Other Nononcogenic Viruses

Part IV. Immunological Tolerance to and Suppression by Oncogenic Viruses

Part V. Significance of Immunological Tolerance to Microorganisms and Their Components

INTRODUCTORY REMARKS

Herman Friedman

Albert Einstein Medical Center, Philadelphia, Pa.

The major purpose for the organization of this conference, sponsored by The New York Academy of Sciences, was to assemble at one meeting distinguished investigators from seemingly unrelated areas of immunology and microbiology who are interested in fundamental aspects of immunologic tolerance as applied to infectious organisms, including bacteria and viruses. Immunologic tolerance is a phenomenon of equal fascination to both the investigator of fundamental biologic responses and to the practicing laboratory worker and clinician. It is widely known that administration of a foreign antigen to an individual can either induce synthesis of antibody with specificity for that antigen or, in contrast, *prevent* the immune response to a subsequent and normally immunizing dose of the same antigen. Such induced immunologic unresponsiveness was once separated into several distinct categories, including the now classic designation of acquired immunologic tolerance to tissue antigens, immune paralysis to bacterial polysaccharides, and immunologic unresponsiveness to serum proteins, defined polypeptides, or chemical haptens.

Recent studies in many laboratories have resulted in newer concepts, as well as development of newer techniques that reveal that immune tolerance or paralysis, regardless of category, is much more complex than the mere absence of an immune response. As pointed out by many investigators, it is now apparent that tolerance not only is an important component of the immune mechanism, but also may be an exceedingly versatile tool for analysis of various aspects of immunology, as well as of biology in general. Thus, an understanding of the nature and mechanism of immunologic tolerance has direct relevance to many fields. There are many areas of applied and clinical microbiology that will benefit by a more complete understanding of unresponsiveness from the viewpoint of the host-parasite relationship.

It should be noted that the study of microbial infections and immunity leads to one of the first descriptions of immune unresponsiveness or paralysis. Investigation of the immune response to pneumococcal polysaccharides, as described in the first section of this monograph, revealed that an "excess" dose of antigen could actually result in induction of unresponsiveness rather than in immunity. In addition, the classic work of Traub in the 1930's established the concept of persistent tolerant infection in mice infected *in utero* with a virus. The advent of transplantation surgery as a potential means for restoring useful life to an individual with an irreversibly damaged vital organ such as a kidney has obviously stimulated the current widespread interest in immunologic tolerance. The pioneering studies of Medawar and his colleagues, which established, for the first time, methods for inducing acquired tolerance to transplantation antigens need no restatement. Much of the current interest in tolerance stems from the model system of transplantation immunity. However, many of the major advances concerning the basic mechanisms involved in tolerance induction are derived from work with serum proteins, chemical haptens, and other relatively well-defined antigens. Nevertheless, studies concerning immunity and tolerance to microbial antigens have been quite fruitful during the last decade.

Studies on immunologic tolerance to microbial antigens may be divided roughly into two broad categories—one dealing with microbial antigens as a convenient "tool" and very sensitive model system, the other based on an interest in the relationship between susceptibility to infection and prior tolerance induction. It was the general goal of this conference to bring together investigators from both areas of interest. The first two sections in this monograph deal with more basic topics of the mechanism of immunity and tolerance as related to bacterial models. For example, the first section is concerned with paralysis to pneumococcal polysaccharides, especially the renewed interest in "central" vs. "peripheral" inhibition mechanisms. The succeeding section presents discussions of the basic mechanism of tolerance as assessed on the cellular level, using highly sensitive immunoplaque methods with gram-negative bacteria.

The third and fourth sections of this monograph deal with the more "practical" aspects of immunologic unresponsiveness to viral antigens, including nononcogenic, as well as tumor-inducing, viruses. A number of investigators believe that suppression of the immune response, either specific (tolerance) or nonspecific, is an important and perhaps necessary component of tumor induction by viruses. Furthermore, the effects of tolerance to viruses such as LCM, rubella, influenza, and others are discussed.

The final section includes a panel discussion among investigators representing basic microbiology, virology, and oncology, who summarize pertinent facts in their respective fields and point out areas in which further progress may be made by newer experiments and studies.

It was a pleasure to have so many eminent persons in the fields of microbiology, immunology, and virology present at this conference, both as participants and in the audience, especially Dr. Ludwik Gross, one of the early pioneers in the field of virus-induced leukemia, who first suggested a possible relationship among age, tolerance, and susceptibility to virus infection. We are also grateful to our colleagues from France, England, Sweden, and Australia who found it possible to attend. Regretfully, Dr. Erich Traub, who first described tolerance to LCM virus, was unable to participate in this conference.

PART I. IMMUNOLOGICAL PARALYSIS TO PNEUMOCOCCAL POLYSACCHARIDES—A MODEL SYSTEM

INTRODUCTORY REMARKS TO SESSION 1

Merrill W. Chase

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I would like to direct your attention to two points worth keeping in mind during the presentations that you will hear at this conference.

These points have come to the fore during our work on the induction of tolerance in adult guinea pigs to allergenic chemicals. Several persons have made contributions—Drs. J. R. Battisto, Thelma H. Carter, Egon Macher, and Roy E. Ritts. Other laboratories have also contributed to this area of inquiry, particularly those of Drs. Coe and Salvin [1] and, in Europe, Drs. De Weck and Frey.[2]

First, just as tolerance can be established against immunoglobulin synthesis, tolerance is encountered in the area of "cell-mediated immunity" (or rather, "cell-mediated hypersensitivity," which I think is a more precise phrase). Both aspects should be kept in mind. Thus if one establishes tolerance of both kinds in the same animal and, by appropriate use of highly antigenic hapten-carrier complexes, breaks through the barrier toward antibody formation, it is found that the barrier to acquiring delayed hypersensitivity is only dented.

The second point that I would offer is that, in cell-mediated hypersensitivity at least, *partial* tolerance can become established such that an animal showing real but low-grade responses remains fixed at that level despite attempts to push the sensitivity upward. In this instance one must exclude using mycobacterial adjuvant unless it has been employed in first-phase sensitization.

Partial tolerance is less widely known as a phenomenon and contrasts with the rather uniform tolerance that is established by feeding an allergenic chemical such as DNCB or picryl chloride over a series of days,[3, 4] or by making the first injection while the immunologic response of the animal is held down by administering cyclophosphamide.[1] Partial tolerance is encountered chiefly when less efficient methods of induction are used, such as pretreatment by intravenous administration.

Recently, Dr. E. Macher carried out further informative experiments that emphasize the reality of partial tolerance owing to the use of large groups.[5, 6] In the very center of a guinea pig's ear, volumes of 10 μl were injected intradermally to deposit minimal sensitizing doses of labeled DNCB or picryl chloride. Ears were excised at various times to study retention of chemical. Although 90% escaped from the ear within 24 hours (and by blood vessels, not by the lymphatics), cell-mediated hypersensitivity was found to be established only by the small portion fixed in the ear. Consequently, the earless animals lacking cell-mediated hypersensitivity could be studied to see what effect might be caused by the large, nonsensitizing amount of allergen that had left the ear before the time of excision. The operated animals, having no resultant contact sensitivity despite the injection, had to receive an active course of sensitizing injections of the chemical. The animals of the normal control group responded well, and 56% of the experimental group were found to be in the expected tolerant range, not acquiring contact reactivity at any moment. Most of the remaining 44% showed variable, intermediate levels of contact sensitivity. Sub-

sequent attempts at sensitizing such animals of intermediate reactivity would hardly budge them from their original state of partial tolerance. Evidently, certain of the lymphocytes became committed, but a type of homeostasis had been established. It was then found to be unnecessary to inject and later excise the ear. Alternatively, the calculated amount of run-off from the ear could be simply injected i.v., with the same effect. Or, subsensitizing doses could be injected i.d. without any attempt at site excision: the same results were secured —from rather uniform to stages of partial tolerance.

Two final observations may be provocative: 1) Tolerance has not been conferred adoptively by lymphoid cells of well-tolerant animals, and 2) tolerant animals of Wright's Family XIII have not been rendered responsive when normal lymphoid cells of the same strain are transferred.

These opening remarks are offered to emphasize the pluralities of tolerance.

This morning's program should present new findings in an area opened by Schiemann in 1927 and 1929,[7,8] and pursued in some detail by Felton and colleagues,[9–12] and later by Stark.[13,14] The following papers will, hopefully, probe into the basic considerations of theory so necessary for our understanding.

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IMMUNITY AND TOLERANCE TO PNEUMOCOCCAL POLYSACCHARIDE *

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Since the classical observations of Felton and coworkers,[1–3] it has been known that immunological unresponsiveness (paralysis) to pneumococcal polysaccharides can be readily induced in adult mice by the injection of an amount of antigen greater than the optimal immunizing dose. These polysaccharide antigens are somewhat unusual with regard to the ease with which unresponsiveness can be induced in adult animals. Furthermore, although readily phagocytized, these antigens appear to be only extremely slowly metabolized or excreted. These characteristics have intrigued numerous workers, with the result that extensive investigation of this experimental model has been carried out. This report summarizes a series of studies on unresponsiveness to pneumococcal polysaccharide in which my colleagues and I have been engaged over the past few years.[4–10] Different portions of the work discussed here were carried out in collaboration with Drs. Baruj Benacerraf, James Howard, Philip Y. Paterson, and William Paul.

The response of adult mice to various doses of pneumococcal polysaccharide is indicated by the data presented in TABLE 1. Animals were injected with Type II pneumococcal polysaccharide solutions (SII) intraperitoneally without adjuvant. At the time indicated the animals were challenged by the i.p. injection of approximately 100 lethal doses of a virulent Type II pneumococcal culture and observed for deaths over the succeeding five days. All nonimmunized mice die following such challenge. Survival indicated that the animal had been stimulated by the preceding injection of antigen to produce antibodies specific for the pneumococcal capsular polysaccharide. Animals receiving large doses of antigen fail to survive challenge even if subsequently given a normally immunizing dose of polysaccharide. It is clear from TABLE 1 that adult mice can be readily rendered unresponsive with antigen doses above 2 μg. Furthermore, it is clear (TABLE 2) that protection can be observed preceding complete paralysis, that is, there is some antibody formation prior to paralysis. This observation may be better understood in terms of data from experiments by Dr. James Howard reported elsewhere in this monograph.[11]

Immune responsiveness following the spontaneous termination of tolerance has been studied by several workers. Upon loss of tolerance to protein antigens mice spontaneously synthesize antibody or become prepared to give a secondary response upon injection of antigen.[12–14] A similar effect can be observed with polysaccharide antigens as illustrated in TABLE 3.

Thus with regard to dose effects in the induction of tolerance and to the appearance of antibody without further injection of antigen upon the spontaneous loss of tolerance, the response to polysaccharide antigens is essentially

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TABLE 1

RESPONSE OF NORMAL ADULT MICE TO TYPE II PNEUMOCOCCAL POLYSACCHARIDE *

Dose SII (μg)	Result of Challenge % Survival (No. Challenged)
25.	13% (15)
10.	23% (26)
2.	33% (24)
0.5	40% (25)
0.05	62% (25)
0.005	48% (25)
0.0005	13% (15)
0	0% (20)

* Mice injected i.p. with SII and challenged with virulent pneumococci i.p. one week later. (From Siskind & Howard.[6])

TABLE 2

ANTIBODY FORMATION BY MICE AFTER PARALYZING DOSES OF SII PRIOR TO IMMUNOLOGICAL UNRESPONSIVENESS *

Prep SII	Dose (μg)	Time of Challenge % Survival (No. Challenged)	
		Day 5	Day 9
1	25	15% (74)	5% (44)
1	10	20% (45)	8% (40)
		Day 4	Day 7
2	25	20% (20)	0% (20)

* Animals injected i.p. with antigen and challenged with virulent pneumococci on the day indicated i.p. (From Siskind & Howard.[6])

TABLE 3

LOSS OF TOLERANCE TO SII *

Dose SII (μg)	Days of Age when Challenged †		
	27–35	50–69	100–135
5.0	2% (59)	—	47% (32)
0.5	28% (65)	60% (5)	77% (30)
0.05	79% (48)	79% (56)	47% (30)

* Newborn mice were injected i.p. with SII and challenged with virulent peumococci i.p. at the indicated age. (From Siskind *et al.*[4])

† % survival (number challenged).

TABLE 4

EFFECT OF AGE UPON THE RESPONSE OF MICE TO 0.5 μg SII *

Age Ag Injection (Days)	Result of Challenge % Survival (No. Challenged)
1	36% (42)
2	26% (34)
3–5	31% (88)
10–11	38% (55)
12–14	24% (91)
21–22	27% (52)
26–28	36% (28)

* Mice injected i.p. with SII at age indicated and challenged with virulent pneumococci i.p. 30–35 days later. (From Siskind *et al.*[4])

the same as the response to protein antigens. In several important aspects, however, the behaviors of these two classes of antigens are, at least on the surface, quite distinct, as illustrated by the following observations.

It generally has been found that tolerance is more readily induced in neonatal, as compared with adult, animals.[15] As illustrated in TABLE 4, the neonatal state does not appear significantly to facilitate paralysis induction to pneumococcal polysaccharide.

It has been shown that a number of protein antigens consist of a phagocytizable, highly immunogenic fraction and a nonphagocytizable tolerogenic fraction.[16–17] Such a nonphagocytizable fraction of a pneumococcal polysaccharide preparation has been looked for without success. As illustrated in TABLE 5, it has not been possible to demonstrate a nonphagocytizable tolerogenic fraction of pneumococcal polysaccharide comparable to that demonstrated by similar techniques with protein antigens. The relatively nonphagocytizable antigen remaining in the circulation is capable of immunizing recipient mice.

TABLE 5

IMMUNOGENICITY OF SERUM FROM SII PARALYZED MICE *

Injection	Result of Challenge % Survival (No. Challenged)
1 ml serum	80% (10)
1 ml 1:25 serum	100% (10)
1 ml 1:625 serum	60% (10)
1 ml 1:3125 serum	20% (10)
0.5 μ SII	85% (20)
0	5% (38)

* Twenty donor mice given 500 μg SII i.p., were bled 10 days later and dilutions of their serum injected i.p. into normal mice that were challenged with virulent pneumococci i.p. two weeks later. (From Howard & Siskind.[9])

This is true despite the fact that polysaccharide antigens are cleared from the circulation in a distinctly heterogenous manner (see FIGURE 1).

Mitchison [18] has shown that repeated injections of subimmunogenic doses of a soluble protein antigen will induce immunologic tolerance. As indicated in TABLE 6, no comparable low dose zone of tolerance induction could be demonstrated with pneumococcal polysaccharide. This was true even with sublethally irradiated mice.[9] According to Mitchison,[19] low dose tolerance can be more readily detected in irradiated animals.

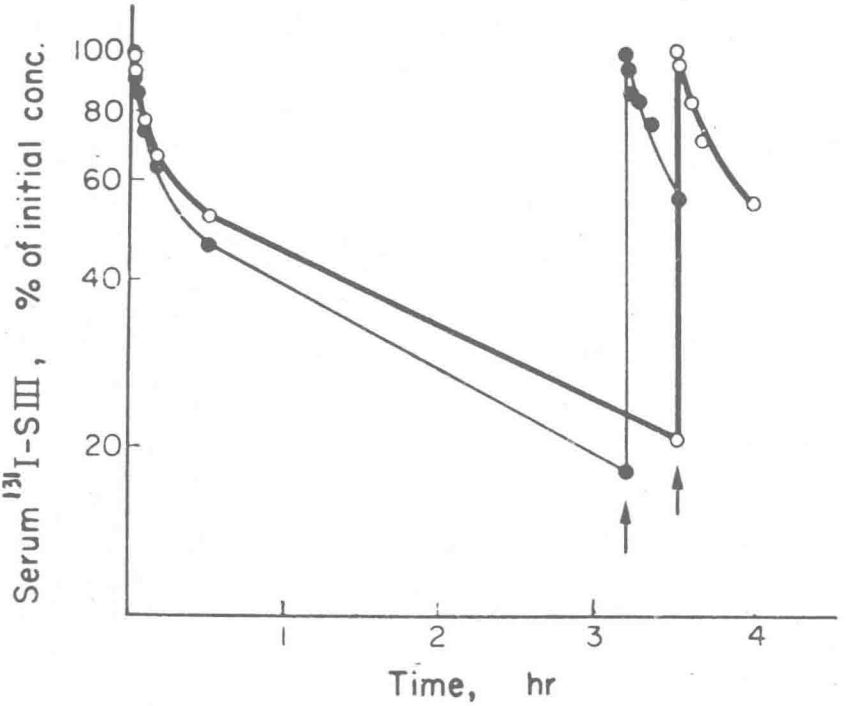

FIGURE 1. Clearance of SIII from the circulation of normal mice injected i.v. with 0.1 μg iodinated SIII at time 0 and again at the point indicated by the arrows.[7] (Reproduced by permission of J. Immunochemistry.)

Aggregation of a number of antigens by electrostatic coupling to methylated bovine serum albumin has been shown to augment their ability to stimulate antibody synthesis.[20] However, as indicated in TABLE 7, aggregation of pneumococcal polysaccharide in this manner did not significantly alter the dose response curve of mice to this antigen.

It is generally more difficult to induce unresponsiveness to protein antigens in previously immunized animals.[21] However, as indicated in TABLE 8, prior immunization with pneumococcal polysaccharide did not significantly alter a subsequent attempt to induce paralysis.

TABLE 6

EFFECT OF REPEATED INJECTIONS OF SMALL DOSES OF SII ON IMMUNOLOGIC STATUS *

Dose SII (μg)	Duration of Injections (Weeks)	Result of Challenge % Survival (No. Challenged)	
		No. Additional Antigen	Immunizing Dose SII
0	—	0% (5)	80% (20)
5×10^{-6}	3	5% (19)	79% (19)
5×10^{-6}	6	0% (17)	95% (18)
0	—	0% (10)	60% (20)
5×10^{-5}	3	26% (19)	63% (19)
5×10^{-5}	6	12% (17)	59% (17)

* Animals received twice weekly injections of SII i.p. for the number of weeks indicated. Half of the mice then received 0.5 μg SII (optimal immunizing dose) i.p. and all are challenged one week later with virulent pneumococci i.p. (From Siskind & Howard.[6])

TABLE 7

EFFECT OF AGGREGATION OF SII WITH METHYLATED BOVINE SERUM ALBUMIN ON THE IMMUNE RESPONSE OF MICE *

Dose SII (μg)	Result of Challenge % Survival (No. Challenged)	
	Soluble SII	SII-MBSA complex
400.	0% (9)	9% (11)
25.	10% (30)	10% (29)
10.	13% (30)	14% (29)
0.5	52% (29)	57% (30)
0.005	24% (29)	25% (28)

* Mice injected with antigen i.v. and challenged with virulent pneumococci i.p. one week later. (From Siskind *et al.*[7])

TABLE 8

EFFECT OF PREVIOUS IMMUNIZATION WITH SII UPON THE RESPONSE OF MICE TO SUBSEQUENT INJECTION OF VARIOUS DOSES OF SII *

Dose SII (μg)	Result of Challenge % Survival (No. Challenged)	
	Normal	Preimmunized
100.	3% (30)	3% (29)
25.	18% (60)	12% (60)
2.	39% (91)	25% (98)
0.05	47% (19)	45% (20)
0.0005	20% (20)	40% (20)
0	0% (30)	48% (104)

* "Preimmunized" animals received 0.5 μg SII i.p. and one week later these animals and additional normal animals received varying doses of SII i.p. All mice were challenged with virulent pneumococci i.p. one week later. (From Siskind & Howard.[6])

The failure to demonstrate a nonphagocytizable tolerogenic fraction and the failure of aggregation or preimmunization to alter the dose response curve both suggest that phagocytosis may not be as important in the handling of these antigens as in that of protein antigens.

It has been shown by Pappenheimer and colleagues [22] that the immune response of rabbits to pneumococcal vaccine is characterized, under appropriate immunization conditions, by a striking lack of heterogeneity. Binding studies on anti-hapten antibodies have generally indicated that they are highly heterogeneous with respect to their affinity for the antigenic determinant.[23–27] Furthermore, a progressive increase in average affinity with time after immunization

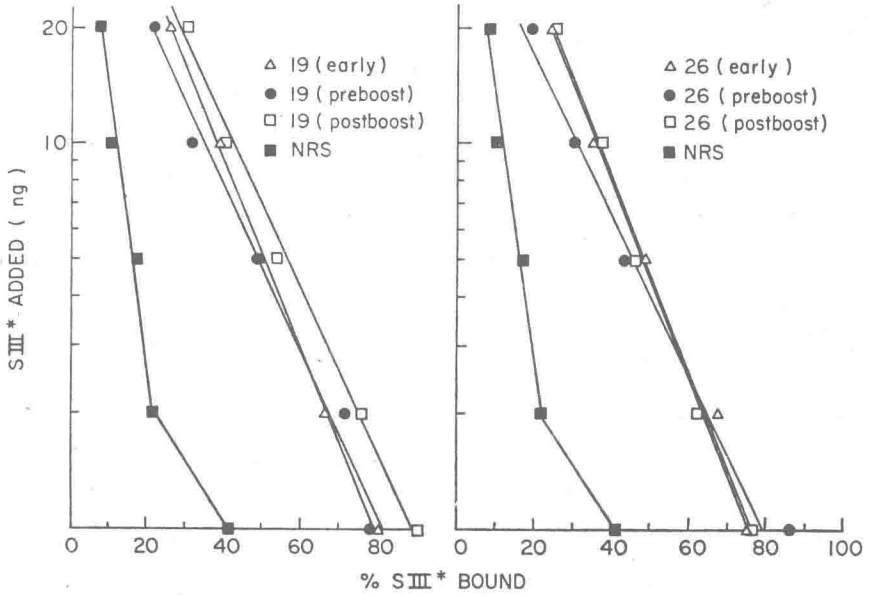

FIGURE 2. Equilibrium binding of varying amounts of iodinated SIII by 0.1 μg anti-SIII. Studies performed on sera early after immunization of rabbits with pneumococcal vaccine, nine months later just prior to boosting with SIII and seven days after boosting.[10] (Reproduced by permission of J. Exp. Med.)

using haptenic determinants has been observed.[23–27] In contrast with these results, studies on polysaccharide antigens [10] have shown only minimal or no increase in affinity with time after immunization (FIGURE 2).

Classically polysaccharide antigens are believed to be unable to elicit a secondary response.[28] We have found [8] that rabbits primed with whole pneumococcal vaccine will give a weak but definite anamnestic response upon boosting with purified pneumococcal polysaccharide late after primary immunization. If such an animal is given a second or third boost with polysaccharide (FIGURE 3), the response after each boost is of progressively lower magnitude. In studies on mice [7] we observed that the antibody response to pneumococcal