IMMUNE RESPONSE TO CHEMICALLY MODIFIED FLAGELLIN

III. Enhanced Cell-Mediated Immunity During High and Low Zone Antibody Tolerance to Flagellin

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Recent studies in this laboratory have indicated that humoral and cell-mediated immunity may be intimately related (1–3). Using a series of acetoacetylated derivatives of flagellin, it was observed that increasing acetoacetylation steadily destroyed the ability of flagellin to initiate antibody formation but enhanced the capacity of the molecule to induce flagellin-specific delayed-type hypersensitivity and antibody tolerance. Thus, in this system antibody tolerance in adult rats was accompanied by enhanced cell-mediated immunity. If this relationship represents a general phenomenon, then it would be predicted that all states of antibody tolerance in adult animals should be accompanied by enhanced cell-mediated immunity. Using bacterial flagellin as the antigen, studies were initiated to test this prediction.

It has been previously reported that antibody tolerance to flagellin can be induced in adult rats by multiple injections of a cyanogen bromide (CNBr) digest of flagellin at two widely spaced dose levels (4). This phenomenon is called high and low zone antibody tolerance (5). Intermediate doses of the CNBr digest resulted in very high antibody titers rather than in antibody tolerance. Data presented in this paper reveal that, as predicted, both high and low zone antibody tolerance to flagellin were associated with heightened levels of delayed-type hypersensitivity. Furthermore, when enhancement of the antibody response occurred, suppression of delayed-type hypersensitivity was observed. Thus, an inverse relationship appears to exist between humoral and cell-mediated immunity. However, this relationship is not invariably true as rats from another strain exhibited only a partial inverse relationship. In addition, it was confirmed (3) that administration of antigen to neonatal rats induced tolerance at the level of both humoral and cell-mediated immunity.

Materials and Methods

Animals.—Outbred Wistar rats were obtained from two separate colonies and according to their source were termed strain W or strain J Wistar rats. Strain W Wistar rats were either obtained directly from The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, or bred in Canberra from Wistar breeders supplied by The Walter and Eliza Hall Institute. Strain W rats bred in either Parkville or Canberra expressed similar immune responsiveness. Strain J Wistar rats were obtained from the Wistar rat colony present at The

John Curtin School of Medical Research, Australian National University, Canberra. In studies with both strains, adult (6–10 wk of age) Wistar rats of either sex were used. In the case of strain J Wistar rats, both adult and neonatal rats were used.

Antigens.—Flagellin from *Salmonella adelaide* (strain SW1338; H antigen, fg) was prepared as described previously (6). A CNBr digest of flagellin was prepared as described elsewhere (7). A stock solution of 1 mg/ml was prepared and to avoid antigen losses by adsorption, tenfold dilution steps were made in saline containing 0.1% gelatin. Acetoacetylated flagellin (16.8 acetoacetyl groups/mole, relative antigenic activity $[K_{rel}]$[1] $= 6.8 \times 10^{-3}$) was obtained by a previously described method (1, 2).

Antigens were injected in saline either intraperitoneally (0.1 ml), intradermally into the flanks (50 μl at two separate sites), intravenously into a lateral tail vein (0.5 ml), or subcutaneously into the hind footpads (50 μl/footpad). In some experiments, flagellin was emulsified in Freund's complete adjuvant (FCA), in the ratio of 3 vol of antigen to 1 vol of adjuvant, and injected intradermally (50 μl/site).

Antibody Estimations.—Antibody to flagellin was estimated by hemagglutination using sheep erythrocytes sensitized with *S. adelaide* polymerized flagellin by a chromic chloride procedure (8).

Assay for Delayed-Type Hypersensitivity.—Hypersensitivity reactions were determined by measuring the increase in footpad thickness after the injection of antigen in saline into the hind footpads. Flagellin-specific reactions were elicited by injecting 0.5–100 μg (50 μl) of flagellin in saline into the right hind footpads and saline (50 μl) alone into the left hind footpads. Footpad thickness was measured at 3, 6, 24, and 48 hr after challenge and specific footpad swelling was determined by subtracting the thickness of the left hind footpad from the right. Footpad thickness was measured by a dial caliper gauge AO2T (Schnelltaster, H. C. Kröplin GmbH, Schluchtern, Hessen, Germany) which had 0.1-mm graduations. Compared with control animals no significant immediate-type hypersensitivity (3 hr footpad swelling) was observed in any of the experiments reported in this paper. It was also found that all the delayed responses detected, although still being prominent at 48 hr, peaked at the 24 hr time point. Thus, only 24-hr footpad swellings are reported in the figures and tables.

It was found that the hind feet of adult rats ranged in thickness from 4 to 5 mm. Footpad thicknesses were measured to the nearest 0.05 of a mm. Repeated measurements (6–10 times) of the same footpad gave standard errors ranging from ±0.01 to ±0.03 mm. The left and right hind feet of normal rats had very similar thicknesses (±0.05 mm).

Statistical Methods.—Standard errors of the means and P values were calculated using the Student's t test.

RESULTS

Humoral and Cell-Mediated Immune Responses Induced in Adult Wistar Rats by Different Doses Given Daily of a Cyanogen Bromide Digest of Flagellin.—Experiments were carried out to determine the ability of different doses of CNBr-digested flagellin to induce (*a*) antibody production to flagellin, (*b*) delayed-type hypersensitivity to flagellin, and (*c*) immunological tolerance to flagellin both at the humoral and cell-mediated level. These determinations were made in two separate strains of Wistar rats (strains W and J).

The experimental design is presented in Fig. 1. Adult rats were injected intraperitoneally daily for 27 days with different doses of a CNBr digest of flagellin.

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[1] *Abbreviations used in this paper:* FCA, Freund's complete adjuvant; K_{rel}, relative antigenic activity.

On day 28, animals were bled and their antibody titers estimated. Also, at this time point, the levels of delayed-type hypersensitivity induced by the different doses of the CNBr digest were determined by eliciting rats with 100 μg of flagellin in saline into the right hind footpads (see Materials and Methods). This high dose (100 μg) of flagellin was used, as not only did it efficiently elicit delayed hypersensitivity, but it also provoked a good antibody response to flagellin and therefore tested animals for antibody tolerance. Antibody titers were measured at weekly intervals after flagellin challenge (up to day 56). The levels of delayed-type hypersensitivity were again determined in animals on day 56 by the injection of 0.5 μg of flagellin in saline into the right hind footpads. A dose of 0.5 μg of flagellin elicited hypersensitivity reactions just as efficiently as 100 μg of flagellin (i.e., day 28 challenge) (3).

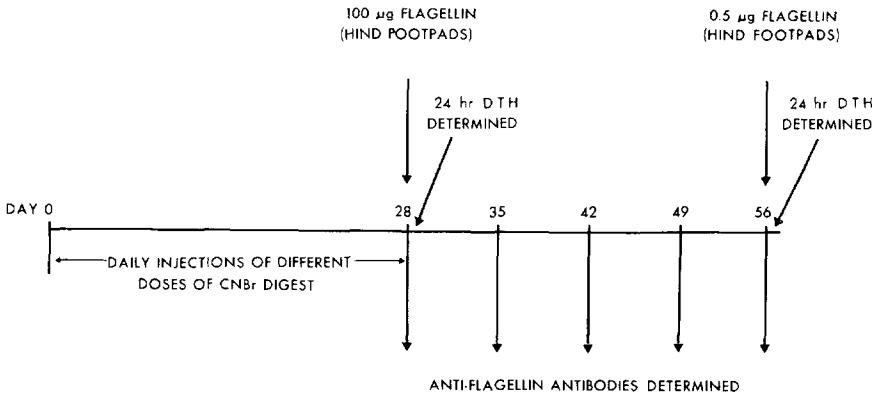

FIG. 1. An experimental design to test the capacity of CNBr-digested flagellin to induce antibody formation, delayed-type hypersensitivity (DTH), and immunological tolerance. The same experimental design was used for both strain W and strain J Wistar rats.

Immune responses in strain W Wistar rats: Adult, strain W, Wistar rats (seven per group) were injected intraperitoneally daily for 27 days with amounts of a CNBr digest of flagellin varying in tenfold dilution steps from 100 μg to 10 fg (fg = femtogram). The experimental protocol was as described above (Fig. 1).

Fig. 2 presents the antibody titers and delayed-type hypersensitivity responses (24-hr footpad swellings) of rats after 27 daily injections of different doses of the CNBr digest of flagellin. Significant delayed-type hypersensitivity was induced by doses of the CNBr digest ranging from 10 fg to 1 ng/day and 1 to 100 μg/day, the strongest delayed responses being induced by 100 fg/day and 100 μg/day (Fig. 2). It is noteworthy that doses of antigen as low as 10 fg/day could induce significant delayed-type hypersensitivity. In contrast, only the larger doses of antigen (100 ng–100 μg/day) produced detectable antibody. This result is at variance with an earlier study in strain W Wistar rats, where it

was demonstrated that doses of the CNBr digest as low as 10 pg could induce detectable primary antibody (4). This variation is probably due to the presence of variable amounts of undigested flagellin in different preparations of the CNBr digest of flagellin.

The rats which had been pretreated with the CNBr digest of flagellin (see Fig. 2) were challenged with 100 μg of flagellin. The immune status of these animals 4 wk after flagellin challenge is presented in Fig. 3. The antibody responses showed two zones of antibody tolerance separated by a region where

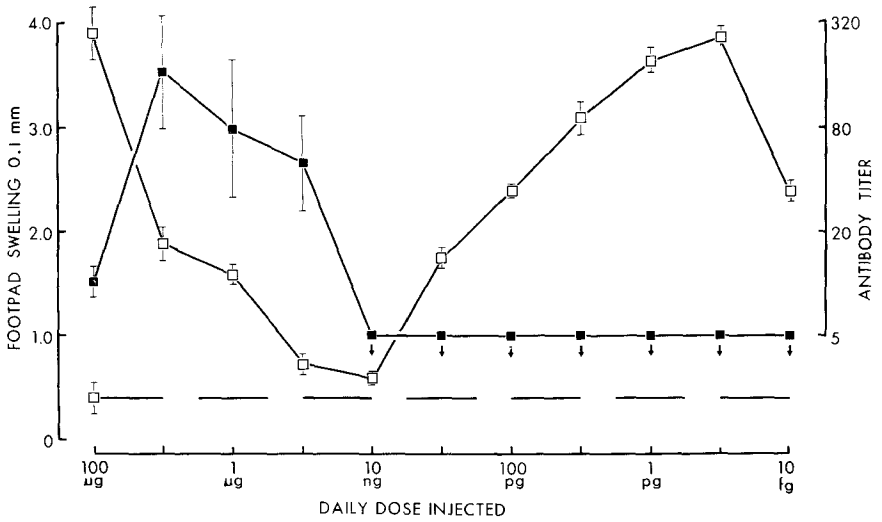

FIG. 2. Antibody titers (■—■) and delayed-type hypersensitivity responses (□—□) of strain W Wistar rats which had been injected intraperitoneally daily for 28 days with varying amounts of a CNBr digest of flagellin. Antibody titers were estimated on day 28 and delayed-type hypersensitivity was elicited on the same day by the injection of 100 μg of flagellin in saline into the hind footpads. The dotted line(- - -) represents the 24-hr footpad swelling of control rats which were elicited with 100 μg of flagellin in saline. Vertical bars represent standard errors of the means.

enhanced antibody formation occurred (Fig. 3). A dose of 100 μg/day of the digest induced high zone antibody tolerance ($0.05 > P > 0.01$) whereas optimum low zone tolerance was induced by 100 fg/day ($0.05 > P > 0.01$). These results confirm earlier antibody tolerance studies in strain W Wistar rats (4). In contrast, both high and low zone antibody tolerance were accompanied by enhanced delayed-type hypersensitivity. Furthermore, significant suppression of delayed-type hypersensitivity was observed ($0.01 > P > 0.001$) when an enhanced antibody response existed (i.e., 10 and 100 ng/day treatments). Thus, in this rat strain there appears to be a striking "mirror-image" relationship between humoral and cell-mediated immunity.

Immune responses in strain J Wistar rats: The experimental protocol was

identical to that described in the preceding section (see Fig. 1), although the 10 fg dose of the CNBr digest of flagellin was not included in this experiment. Fig. 4 presents the antibody titers and delayed-type hypersensitivity responses of strain J Wistar rats which had been pretreated with different doses of the CNBr digest of flagellin and then challenged with 100 μg of flagellin in saline.

In contrast to strain W rats, antibody tolerance to flagellin was not induced in strain J Wistar rats by any dose of the CNBr digest of flagellin (Fig. 4). Furthermore, the inverse relationship between humoral and cell-mediated

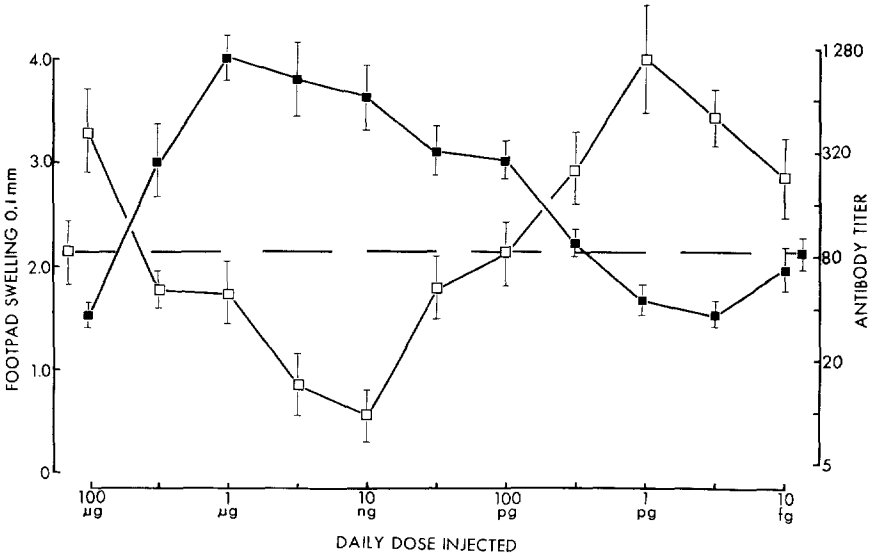

FIG. 3. Antibody titers (■—■) and delayed-type hypersensitivity responses (□—□) of strain W Wistar rats injected daily for 28 days with varying amounts of the CNBr digest of flagellin (Fig. 2) and then challenged with 100 μg of flagellin in saline. The antibody titers represent the mean of the 7, 14, 21, and 28 day postchallenge titers. Delayed-type hypersensitivity was elicited 28 days after the flagellin challenge. The dotted line (---) represents the antibody and delayed hypersensitivity responses of control rats which were injected only with 100 μg of flagellin in saline. Vertical bars represent standard errors of the means.

immunity, seen so clearly with the W rat strain, was not so clear-cut with the J strain. For example, with certain doses of the CNBr digest (10 and 100 ng/day), both humoral and cell-mediated immunity were higher than control animals. Despite these results, suppression of delayed-type hypersensitivity was associated with heightened antibody titers (i.e. 1 μg/day dose of digest), whereas the lowest antibody responses were accompanied by high delayed reactions (i.e., 1 pg–1 ng/day doses).

Comparison of the Humoral and Cell-Mediated Immune Responses to Flagellin in Strain J and Strain W Wistar Rats.—Experimental data presented in the preceding section clearly demonstrated that strain J and strain W Wistar rats

differed in their immunological responsiveness to flagellin. Experiments were carried out to further investigate these differences in responsiveness. Groups of adult strain J and strain W Wistar rats (seven to eight per group) were injected into the flanks with 1 μg of either flagellin or acetoacetyl-flagellin (16.8 acetoacetyl groups/mole, $K_{rel} = 6.8 \times 10^{-3}$) (2) in saline. 35 days later delayed-type hypersensitivity and secondary antibody responses were elicited by the injection of 1 μg of flagellin in saline into the right hind footpads. Control groups of rats which had been injected with saline alone were also challenged with flagellin

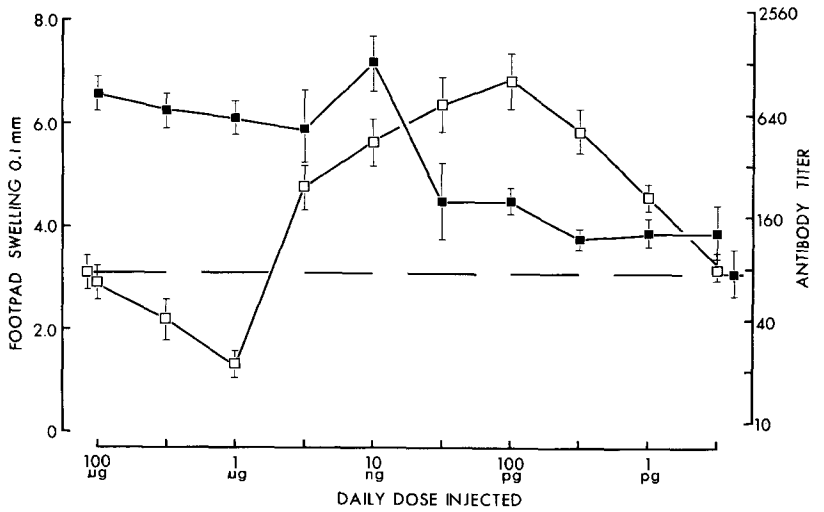

FIG. 4. Antibody titers (■—■) and delayed-type hypersensitivity responses (□—□) of strain J Wistar rats injected daily for 28 days with varying amounts of the CNBr digest of flagellin and then challenged with 100 μg of flagellin in saline. The antibody titers represent the mean of the 7, 14, 21, and 28 day postchallenge titers. Delayed-type hypersensitivity was elicited 28 days after the flagellin challenge. The dotted line (---) represents the antibody and delayed hypersensitivity responses of control rats which were injected only with 100 μg of flagellin in saline. Vertical bars represent standard errors of the means.

at this time. Footpad swelling was determined as described in the preceding section. Antibody titers were measured at weekly intervals during the experiment.

Table I compares the immune responses to flagellin of strain J and strain W Wistar rats. From this comparison the following observations were made: (*a*) The primary antibody responses of the two rat strains were similar. At all time points both strains produced similar primary antibody titers to flagellin. Furthermore, both strains failed to produce any primary antibodies when immunized with acetoacetyl-flagellin. (*b*) Both rat strains expressed comparable levels of delayed-type hypersensitivity when injected with either flagellin or acetoacetyl-flagellin. (*c*) In contrast, strain J rats gave a significantly higher

and more prolonged secondary antibody response to flagellin than strain W rats. (*d*) Acetoacetyl-flagellin induced antibody tolerance to flagellin in strain W Wistar rats but failed to produce antibody tolerance in strain J animals.

In addition, multiple doses of a cyanogen bromide digest of flagellin induced higher levels of delayed-type hypersensitivity in strain J rats (peak response = 6.9) than in strain W rats (peak response = 4.0) (cf. Figs. 3 and 4).

From these results it was concluded that strain J Wistar rats give a higher immune response to flagellin than do strain W rats. This difference became evident when animals were treated with multiple doses of antigen.

Ability of Cyanogen Bromide-Digested Flagellin to Induce Immunological

TABLE I

Comparison of the Immune Responses to Flagellin Produced by Strain J and Strain W Wistar Rats

Strain of Wistar rats	Priming antigen (1 μg in saline)	Peak primary antibody (35 day)	Delayed-type hypersensitivity*	Challenge (1 μg in saline)	Secondary antibody titers		
					7 day	14 day	28 day
Strain J	Flagellin	100	2.3 ± 0.5‡	Flagellin	8700	13,300‖	3840‖
	Acetoacetyl-flagellin§	<2.5	4.2 ± 0.4	"	<2.5	76‖	112‖
	Nil	<2.5	0.4 ± 0.2	"	2.5	20	160
Strain W	Flagellin	160	2.3 ± 0.5	Flagellin	3580	1540‖	540‖
	Acetoacetyl-flagellin§	<2.5	3.7 ± 0.3	"	<2.5	<2.5‖	30‖
	Nil	<2.5	0.3 ± 0.2	"	<2.5	30	240

Rats were primed in the flanks and delayed-type hypersensitivity and secondary antibody responses were elicited in the hind footpads with flagellin (1 μg) 35 days later.

* Footpad swellings (0.1 mm) 24 hr after eliciting dose of flagellin.

‡ Standard error of mean.

§ 16.8 Acetoacetyl groups/mole, $K_{rel} = 6.8 \times 10^{-3}$ (2).

‖ Significant difference between strain J and strain W response ($P < 0.01$).

Tolerance in Neonatal Rats.—Cyanogen bromide-digested flagellin was injected into strain J Wistar rats in amounts of 1 μg, three times weekly, beginning within 24 hr of birth. Following this injection schedule, animals were challenged into the flanks with either 1 μg of flagellin in FCA or 1 μg of acetoacetyl-flagellin in FCA (16.8 acetoacetyl groups/mole, $K_{rel} = 6.8 \times 10^{-3}$) (2). Control groups of rats which had not been pretreated with the CNBr digest were similarly challenged. 4 wk after the FCA challenges, delayed-type hypersensitivity was elicited by the injection of 0.5 μg of flagellin in saline into the right hind footpads. Footpad swelling was determined as described earlier. Antibody titers were measured before FCA challenge and at weekly intervals for 4 wk post-challenge. The prechallenge and 28-day postchallenge titers are presented in Table II.

No antibody was detected in the rats after 8 wk of injection with CNBr-digested flagellin (Table II). Pretreatment of rats from birth with CNBr-digested flagellin completely suppressed their antibody response to a subsequent challenge of flagellin. Similarly, pretreatment with the CNBr digest induced complete "cellular immunity tolerance" as these animals were unable to produce detectable delayed-type hypersensitivity against either flagellin or acetoacetyl-flagellin. Thus, CNBr-digested flagellin simultaneously induces tolerance in neonatal rats at the level of both humoral and cell-mediated immunity. In contrast, the same preparation in adult rats produces immunological tolerance only at either the humoral or cell-mediated level.

Desensitization of Delayed-Type Hypersensitivity to Flagellin.—To our knowledge, no other laboratory has demonstrated that suppression of antibody formation can be accompanied by heightened levels of delayed-type hypersensitivity

TABLE II

Induction of Tolerance in Neonatal Rats by Cyanogen Bromide-Digested Flagellin

Initial course of injections*	Antibody titer before second injection	Second injection (1 μg in FCA)	Antibody titer (28 days)	Delayed-type hypersensitivity (28 day)
1 μg CNBr digest	<2.5	Flagellin	<2.5	0.25 ± 0.2‡
NIL	<2.5	"	5120	3.5 ± 0.3
1 μg CNBr digest	<2.5	Acetoacetyl-flagellin§	<2.5	1.0 ± 0.2
NIL	<2.5	"	<2.5	7.0 ± 0.5

* Strain J Wistar rats injected three times weekly from birth for 8 wk before challenge.

‡ Represents 24 hr footpad swelling (0.1 mm) after elicitation with 0.5 μg of flagellin in saline. Standard errors of means are included.

§ 16.8 acetoacetyl groups/mole, $K_{rel} = 6.8 \times 10^{-3}$ (2).

(for further details see Discussion). It may well be, however, that in earlier studies, desensitization of delayed-type hypersensitivity by persisting antigen could have occurred. To test this possibility, an experiment was carried out to determine whether flagellin could desensitize animals which were already hypersensitive to flagellin.

Adult, strain J, Wistar rats were sensitized in the flanks with 1 μg of acetoacetyl-flagellin (16.8 acetoacetyl groups/mole, $K_{rel} = 6.8 \times 10^{-3}$) (2) in FCA and 10 days later delayed-type hypersensitivity was elicited by the injection of 0.5 μg of flagellin in saline into the right hind footpads. Desensitizing doses of flagellin (1 μg–1 mg in 0.5 ml saline) were injected intravenously at the same time as the eliciting antigen. Footpad swelling was determined as described in the Materials and Methods. Control rats were injected into the flanks with FCA-saline and elicited with flagellin 10 days later. It was found that animals hypersensitive to flagellin could be readily desensitized by the intravenous injection of flagellin (Fig. 5). As little as 1 μg of flagellin produced partial de-

sensitization and doses of 10 μg or more completely eliminated the delayed response.

DISCUSSION

It was originally demonstrated by Mitchison (5) and Dresser (9, 10) that antibody tolerance could be induced in adult animals by the injection of antigen at two different dose levels. Intermediate doses of antigen usually resulted in

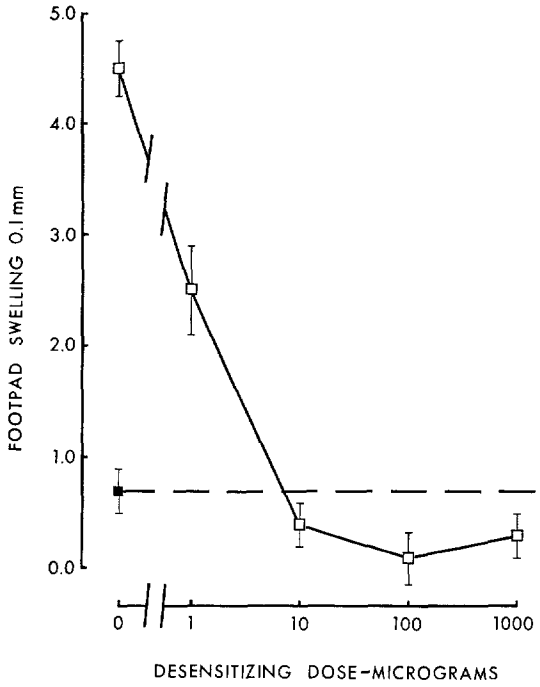

FIG. 5. Desensitization of delayed-type hypersensitivity to flagellin in strain J Wistar rats. For experimental details see text. The dotted line (- - -) represents the response of control animals which had been primed with FCA-saline.

enhanced antibody titers rather than in antibody tolerance. These two regions of antibody tolerance have been termed "high zone" and "low zone" tolerance according to the relative amounts of antigen required to induce the tolerant state (5). More recently it has been reported by several laboratories that high and low zone antibody tolerance can be induced by a range of antigens (11). However, although the antibody responses in these systems have been extensively investigated, the cell-mediated immune responses have been largely neglected.

Data presented in this paper indicate for the first time that both high and low zone antibody tolerance to flagellin may be accompanied by enhanced levels of

delayed-type hypersensitivity. In addition, it was observed that an enhanced antibody response can be associated with suppressed delayed-type hypersensitivity. This mirror-image relationship between humoral and cell-mediated immunity was very striking in strain W Wistar rats but was not quite so clear-cut in strain J animals. From these results it was concluded that frequently, but not invariably, an inverse relationship exists between humoral and cell-mediated immunity in adult rats.

From the data presented in this paper it could be argued that all doses of the CNBr digest of flagellin induced similar levels of delayed-type hypersensitivity but that high levels of serum antibody effectively masked the delayed response by competing with hypersensitive cells for antigen. Two lines of evidence have already been reported suggesting that this interpretation is unlikely: (*a*) The administration of large doses of hyperimmune serum into rats hypersensitive to flagellin failed to mask the elicitation of delayed responsiveness (3). (*b*) Peritoneal cells from rats with high antibody titers to flagellin were unable to transfer delayed-type hypersensitivity (3). If, in fact, antibody had masked delayed hypersensitivity, then these cells should have transferred delayed responsiveness.

Although to our knowledge, no other laboratory has demonstrated such a striking inverse relationship between humoral and cell-mediated immunity, there have been some indications that this phenomenon may exist with other antigens. For example, it has been observed that "antibody tolerance" to tuberculin (12) and lysozyme (E. Benjamini, personal communication) in adult animals was accompanied by normal levels of delayed-type hypersensitivity. On the other hand, numerous investigators (13–22) have reported that pretreatment of adult animals with antigen in saline can suppress delayed hypersensitivity and this suppression is usually accompanied by "control" (13–21) and sometimes by heightened (22) levels of antibody.

In contrast, there have been several reports of "immunological tolerance" occurring in adult animals at the level of both humoral and cell-mediated immunity (13, 16, 17, 19–23). However, in these systems complete tolerance was only induced by the administration of large quantities of antigen which was slowly eliminated and it would be expected that such antigen persisting in the serum would both desensitize delayed-type hypersensitivity and neutralize serum antibodies. In these experiments, it has been particularly useful that flagellin is very rapidly eliminated from the circulation (24) and only minute amounts of antigen are needed to immunize (25). Thus, it is unlikely that delayed-type hypersensitivity or serum antibody would be masked by persisting flagellin. As additional evidence for this interpretation, it was demonstrated that flagellin can desensitize animals which are hypersensitive to flagellin when both desensitizing and eliciting doses of antigen are injected simultaneously (Fig. 5). It should be noted that as little as 1 μg of flagellin induced partial desensitization and doses of 10 μg or more completely abrogated the delayed

response. Similar desensitization results have been reported for several other antigens (26, 27).

Many workers would agree that there is no ideal method for measuring delayed-type hypersensitivity. For example, inhibition of macrophage migration works well with some antigens but not with others. In our experience, footpad swelling has been found to be a reliable and reproducible assay method. It must be pointed out, however, that frequently the increases in footpad thickness are not large and considerable practice is necessary to obtain reproducible results. It may be that the small differences obtained is the reason why others have not observed the relationship described in this paper between antibody production and delayed-type hypersensitivity.

In this study two strains of Wistar rats (strains W and J) were investigated which significantly differed in their immunological responsiveness to flagellin. It was found that strain J Wistar rats had a greater potential to respond to flagellin than strain W rats both at the humoral and cell-mediated level. However, these differences in responsiveness only became apparent when rats were injected with multiple doses of antigen. In addition, adult antibody tolerance to flagellin could be induced in strain W rats but not in strain J animals (Table I). These results could be interpreted as indicating that strain J rats possess a greater number of immunocompetent cells to flagellin than strain W rats (28). The additional observation that strain J rats required a 100-fold higher dose of the CNBr digest than strain W rats to both suppress and enhance delayed hypersensitivity was consistent with this interpretation (cf. Figs. 3 and 4). In other words, if immunocompetent cells compete with one another for antigen, then the greater the number of immunocompetent cells present in an animal the higher the dose of antigen required to induce immunological responses.

One of the most remarkable features of this study was the demonstration that as little as 10 fg/day of a CNBr digest of flagellin induced significant delayed-type hypersensitivity in strain W Wistar rats (Fig. 2). During the 28 day course of daily injections, this dose is equivalent to injecting between 10^6 and 10^7 molecules of degraded flagellin. It is likely that substantially less than this amount of antigen would reach the lymphoid system. The mechanism by which such small amounts of antigen can mediate immunological effects is uncertain, although it has been proposed in an earlier publication that antigen localized in lymphoid follicles may play a role (4).

In this paper data were presented confirming the observation that a fundamental difference exists between neonatal and adult tolerance (3). Rats injected with the CNBr digest of flagellin from birth were rendered tolerant to flagellin at the level of both humoral and cell-mediated immunity. In contrast, the CNBr digest in adult animals produced immunological tolerance only at either the humoral or cell-mediated level. This difference between neonatal and adult tolerance has been observed in both strain J (Table II) and strain W (3) Wistar rats.

In an earlier publication a hypothesis was proposed to explain the inverse

relationship between humoral and cell-mediated immunity (3). Briefly, it was postulated that antigen mediated cell-to-cell interaction between B (bursa equivalent) and T (thymus-derived) cells is essential for the induction of antibody formation and suppression of delayed hypersensitivity. In contrast, providing the binding energy of antigen to separate immunocompetent cells reaches a certain threshold, antibody tolerance will be induced in B cells and cell-mediated immunity in T cells. This hypothesis can readily accommodate the observation presented in this paper that both high and low zone antibody tolerance can be accompanied by enhanced cell-mediated immunity. Firstly, it would be anticipated that low doses of the CNBr digest of flagellin would preferentially induce delayed-type hypersensitivity and antibody tolerance, as the probability of small quantities of antigen inducing cell-to-cell interaction is low, whereas this amount of antigen would probably attain the "threshold energy of binding" required to activate cells. Secondly, higher doses of antigen would be expected to favor cell-to-cell interaction and therefore antibody formation. Finally, it would be predicted that very high doses of antigen would produce enhanced cell-mediated immunity and antibody tolerance, as at this dose level antigen molecules would tend to compete with one another for receptors on immunocompetent cells.

SUMMARY

High and low zone antibody tolerance to bacterial flagellin can be induced in adult strain W Wistar rats by multiple injections of a cyanogen bromide (CNBr) digest of flagellin at two widely spaced dose levels. Intermediate doses of the CNBr digest produce enhanced antibody titers to flagellin rather than antibody tolerance. Studies reported in this paper revealed that both high and low zone antibody tolerance to flagellin were accompanied by heightened levels of delayed-type hypersensitivity. Conversely, when enhancement of the antibody response occurred, suppression of delayed hypersensitivity was observed.

This inverse relationship between humoral and cell-mediated immunity was very striking in strain W Wistar rats but was not quite so clear-cut in another strain of Wistar rats (strain J). Strain J rats were resistant to the induction of antibody tolerance and gave higher immunological responses to flagellin than strain W animals. In addition, it was observed that, in contrast to adult tolerance, administration of the CNBr digest to neonatal rats induced complete tolerance at the level of both humoral and cell-mediated immunity.

These findings were discussed in the light of earlier studies with flagellin and provide further evidence for a previously described hypothesis.

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