ANTIBODY-MEDIATED SUPPRESSION OF THE IMMUNE RESPONSE IN VITRO

II. A New Approach to the Phenomenon of Immunological Tolerance*

By ERWIN DIENER,‡ Ph.D., and MARC FELDMANN,§ M.B.

(From The Walter and Eliza Hall Institute of Medical Research,‖ Parkville, 3050, Victoria, Australia)

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Some antigens may induce immunological tolerance not only after injection of high doses (high zone tolerance) but also after injection of subimmunogenic doses (low zone tolerance) (1). Although it is generally agreed that the phenomenon of tolerance results from an effect of antigen on immunocompetent cells, very little is known of the underlying mechanism. In this respect, the phenomenon of low zone tolerance is particularly difficult to understand. Striking examples of the induction of low zone tolerance in rats to H antigens of *Salmonella adelaide* have been reported by Shellam and Nossal (2) and by Ada and Parish (3). They have shown that the repeated injection of flagellin into new born rats (2) or of fragment A (separated from the cyanogen-bromide digest of flagellin) into adult rats (3) at doses of less than 1 pg[1] led to significant tolerance to subsequent challenge with the highly immunogenic polymerized flagellin. It was calculated by the authors (2, 3) that, under the conditions used, the probability of direct interaction between antigen and immunocompetent cells in the animal at any given time during low zone tolerance induction was extremely small. From their studies of antigen localization, Ada and Parish have postulated that this probability could be increased if the assumption was made that, during low zone tolerance induction, antigen might be concentrated in distinct areas such as lymphoid follicles, provided that these areas represent sites in the pathway of the lymphoid cell circulatory system (3). However, the local antigen concentration reached in a lymphoid follicle of a lymph node draining the injection site during the induction of low zone tolerance in adult animals is still far below the dose necessary to induce high zone tolerance. At the cellular level, it is necessary to postulate

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‡ Present Address: Medical Center, University of Alberta, Transplantation Unit, Edmonton, Alberta, Canada.

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[1] $1\ g = 10^3\ mg = 10^6\ \mu g = 10^9 ng = 10^{12} pg$.

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two different induction pathways for high and low zone tolerance. Nossal (4) and Marchalonis and Gledhill (5) have suggested that an immunocompetent cell could be rendered tolerant by interacting with either a very large or an extremely small number of antigen molecules, while interaction with an intermediate concentration of antigen would lead to immunity. Although experimental findings are in accord with this hypothesis in terms of the over-all humoral response, it is difficult to envisage a mechanism by which a cell is enabled to sense accurately the number of antigen molecules interacting with its surface.

Further light may be shed on this problem by our recent finding (6) that a concentration of antigen below the threshold required for high zone tolerance induction is highly efficient in inducing immunological tolerance in vitro, provided it is presented to immunocompetent cells in the presence of a low concentration of specific antibody.

It should be stressed that, under the conditions used, the described phenomenon fulfills the criteria of immunological tolerance. Further studies reported in this paper show that such antibody-mediated tolerance is dependent on the ratio between antigen and antibody used for its induction. Evidence is presented which shows that a shift in this ratio in favor of the antibody leads to the "peripheral" phenomenon of immunosuppression, commonly known as antibody-mediated feedback inhibition, which is not analogous to immunological tolerance.

As cited earlier, Ada and Parish (3) have demonstrated that adult rats may be rendered tolerant to polymerized flagellin of *S.adelaide*, provided they are injected with repeated low doses of fragment A. We have investigated the immunogenic and tolerance-inducing properties of fragment A in vitro. Results presented here indicate that fragment A itself is incapable of inducing either tolerance or immunity in vitro. However, short exposure of mouse spleen cells to fragment A in the presence of an appropriate concentration of specific antibody leads to tolerance induction, as tested by subsequent in vitro challenge with polymerized flagellin. The significance of these findings is discussed with relevance to the phenomenon of low zone tolerance. An attempt is made to reconstruct the mode of tolerance-inducing interaction that may occur between antigen, antibody, and the surface of immunocompetent cells.

Materials and Methods

Mice.—CBA mice of both sexes, 70–110 days of age were used throughout. Mice were killed by cervical dislocation and the spleen excised under aseptic conditions.

Antigens.—Purified flagellin (H antigen) was prepared from *Salmonella adelaide* by the method of Ada, Nossal, Pye, and Abbott (7). Sterile polymerized flagellin (POL)[2] was obtained by filtration of flagellin through a Millipore membrane of 0.45 μ pore size before polymerization. Antigen was diluted in double-distilled water containing 0.1% fetal calf serum and kept at $-20°C$. Cyanogen-bromide digests of flagellin to obtain fragment A were prepared by the method of Parish and Ada (8). Sheep erythrocytes (SRC) were collected in Alsever's solution and kept at 4°C. They were washed three times in normal saline and diluted in tissue culture medium to a concentration of 8×10^6 cells per culture.

[2] *Abbreviations used in this paper:* POL, polymerized flagellin; AFC, antibody-forming cell, MON, monomeric flagellin; SRC, sheep red cells.

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Bacteria.—*Salmonella derby* (H antigen fg; O antigens 1, 4, 12) served as the indicator strain for detecting antibody-forming cells to flagellar antigens of *Salmonella adelaide* (H antigen fg; O antigen 35).

Tissue Culture.—The method used was based on that described by Marbrook (9) and modified by Diener and Armstrong (10). A spleen cell suspension containing 15×10^6 cells in 1 ml of medium was placed in a glass tube sealed off by a dialysis membrane and suspended from the stopper of an Erlenmeyer type flask containing tissue culture medium. Cultures were placed in a humidified incubator at 37°C, with a gas flow of 10% CO_2, 7% O_2, and 83% N_2. Preincubation of cells at a concentration of 15×10^6 cells per ml for time periods of no longer than 6 hr was carried out in Falcon 2001 plastic tubes. The pH of the cell suspension was adjusted prior to incubation to 7.2 in a gas flow consisting of 10% CO_2, 7% O_2, and 83% N_2.

Tissue Culture Medium.—Eagle's minimal essential medium was used, as described by Diener and Armstrong (10), with the exception that it contained 5% fetal calf serum and was supplemented with 20 mg of L-asparagine per liter.

Assay for the Enumeration of Antibody-Forming Cells.—Cells were collected from tissue cultures, washed twice, and asayed for the number of antibody-forming cells (AFC). AFC to POL were assayed by the adherence-colony method of Diener (11). AFC to sheep erythrocytes were enumerated by Cunningham's modification (12) of the hemolysin plaque assay of Jerne and Nordin (13).

Immune Sera.—Immune sera to *Salmonella adelaide* H antigens were prepared as described in detail by Feldmann and Diener (6). For use in tissue culture, immune sera were diluted in tissue culture medium and sterilized by passage through a Millipore filter of 0.45 μ pore size.

Titration of Immune Sera.—Antibody to polymerized flagellin was assayed by the immobilization method of Ada, Nossal, Pye, and Abbott (7). Titration was carried out by serial two-fold dilutions of antiserum in saline. The end point was taken as 90% immobilization of motile bacteria of the indicator strain.

RESULTS

Analysis of Different Parameters of the Induction of Antibody-Mediated Tolerance to Polymerized Flagellin.—An understanding of the tolerance phenomenon as put into effect in vitro by the short exposure of lymphoid cells to antigen and antibody (6) requires a quantitative analysis of the different parameters involved. These parameters are: concentration of antigen and antibody and the duration of exposure of cells to antigen and antibody. Experiments were carried out to test the degree of tolerance induced when cells were preincubated for 6 hr at 37°C in vitro with a fixed concentration of antigen but with different concentrations of antibody. Such pretreatment of spleen cells was carried out at an antigen concentration of 20 ng/ml of polymerized flagellin and at titers of antibody against polymerized flagellin ranging from 10^{-2} to 10^3.[3] After preincubation, the cells were washed 4 times and subsequently cultured for 4 days in the presence of an immunogenic concentration of polymerized flagellin (20 ng/ml). To control for antigen-specificity, 8×10^6 sheep erythrocytes (SRC) were added

[3] Immobilization titers as described in Materials and Methods.

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to each culture along with polymerized flagellin. Cultures were tested for the number of AFC to both flagellar antigens and SRC. The results in Fig. 1 show that there exists an optimal ratio between the concentration of antigen and antibody which is able to induce tolerance during the 6 hr preincubation of cells. Any change in this ratio, either in favor of the antigen or the antibody renders

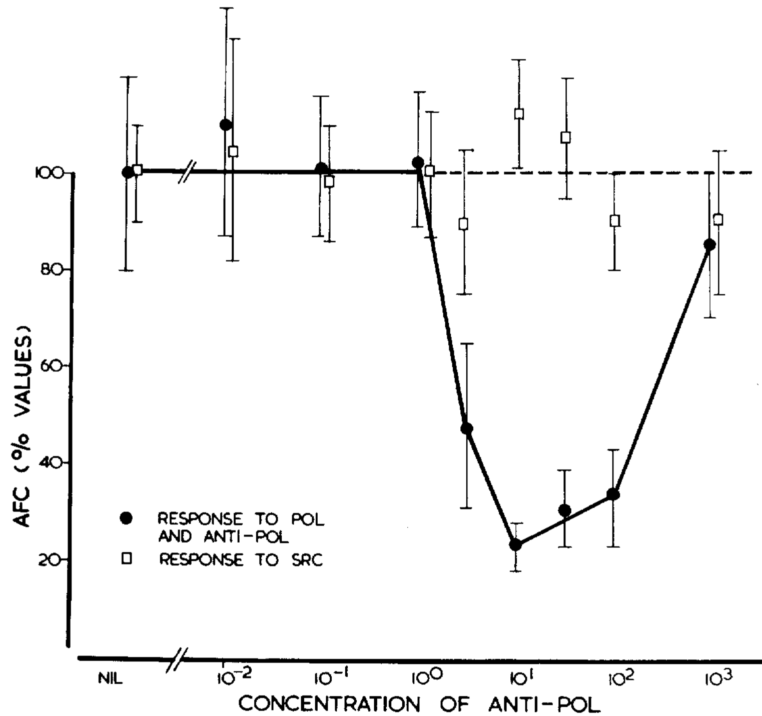

FIG. 1. Capacity of an immunogenic concentration of polymerized flagellin (20 ng/ml) to induce tolerance in the presence of varying concentrations of antibody to polymerized flagellin. Spleen cells were incubated in vitro for 6 hr in the presence of antigen and antibody, followed by challenge in vitro for 4 days with polymerized flagellin (20 ng/ml). Each value comprises the arithmetic mean of 4–8 cultures. Vertical bars represent the standard errors of the mean. Anti-POL, antibody to polymerized flagellin.

the treatment of lymphoid cells for tolerance induction ineffective. The significance of these findings will be discussed later.

The Tolerance-Inducing Capacity of Polymerized Flagellin, Flagellin, and of Fragment A of Flagellin.—It was reported in previous papers that antigen concentrations of polymerized flagellin in the range between 2 ng and 500 ng/ml induced a primary immune response in vitro (14). Concentrations of this antigen higher than 1 μg/ml were reported to induce immunological tolerance (10).

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Furthermore, it was found that the induction of tolerance was complete within a minimal exposure time (3–6 hr) of lymphoid cells in vitro to 30 μg of polymerized flagellin (10).

In vivo work has led to the theory that monomeric antigens should be more effective in their capacity to induce tolerance than their polymeric forms (15). Experiments were therefore carried out to test whether this theory also applies to the in vitro phenomenon of tolerance. Dispersed mouse spleen cells were ex-

TABLE I

Tolerance-Inducing Capacity In Vitro of Polymerized Flagellin (POL), Flagellin-Monomer (MON) and Fragment A

Preincubation for 6 hr in vitro with	Cultured for 4 days in vitro with	AFC/ culture
	ng	
POL:	POL:	
20 ng	20	960 ± 98
30 μg	20	23 ± 15*
MON:	POL:	
200 pg	20	890 + 48
2 ng	20	885 ± 72
20 "	20	715 ± 143
30 μg	20	270 ± 316‡
A:‖	POL:	
20 pg	20	835 ± 209
200 "	20	770 ± 132
2 ng	20	750 ± 242
20 "	20	940 ± 123
300 "	20	655 ± 244
30 μg	20	750 ± 250

Each value represents the geometric mean of 8–10 cultures ± the standard deviation.
* $P < 0.0001$ compared with 20 ng POL.
‡ $P < 0.02$ compared with 20 ng POL.
‖ A, fragment A of the cyanogen-bromide digest of flagellin.

posed for 6 hr at 37°C to various concentrations of flagellin (mol wt 40,000) and to the fragment A (mol wt 18,000). After this treatment, the cells were washed 4 times and cultured in vitro for 4 days in the presence of an immunogenic dose (20 ng/ml) of polymerized flagellin. Critical tests had previously shown that the washing procedure applied to the cells was adequate to ensure that insufficient antigen to induce tolerance was transferred from pretreated cells to the final tissue culture (10). After 4 days of culture, the cells were harvested and assayed for the number of AFC to *Salmonella* H antigens. Results in Table I show that, at comparable concentrations among the different forms of the antigen (weight for weight basis), polymerized flagellin was most efficient in inducing tolerance.

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While the tolerance-inducing capacity of flagellin was significantly less than that of polymerized flagellin, fragment A completely failed to induce tolerance within the wide dosage range tested. It is of importance to note that this absence of the capacity to induce tolerance in vitro coincides with a negligible degree of immunogenicity (Table II).

The Tolerance-Inducing Capacity of Fragment A of Flagellin.—The finding that fragment A is deficient in its capacity to induce tolerance in vitro is in contrast to the fact that in vivo fragment A is a far more potent tolerance-inducing agent than flagellin or polymerized flagellin. The discovery of antibody-mediated tolerance induced with polymerized flagellin (6) prompted us to test whether fragment A could induce tolerance to polymer in the presence of anti-

TABLE II

Immunogenicity of Polymerized Flagellin (POL), Flagellin-Monomer (MON) and Fragment A

Cultured for 4 days in vitro with	AFC/culture
POL:	
20 ng	3200 ± 107
MON:	
20 ng	715 ± 143*
A:‡	
2 ng	7 ± 3*
20 "	3 ± 1*
200 "	6 ± 5*
20 μg	8 ± 5*

Each value represents the geometric mean of 8–10 cultures ± the standard deviations.
* $P < 0.0001$ compared with the response to POL.
‡ A, fragment A of the cyanogen-bromide digest of flagellin.

polymer antibody. As in experiments using polymer, spleen cells were preincubated with fragment A, together with the relevant antibody for 6 hr, washed 4 times, and cultured in the presence of an immunogenic concentration of polymerized flagellin (20 ng) for 4 days. To test for specificity of an immunosuppressive effect by the pretreatment of the cells, the cultures were supplemented with 8×10^6 SRC, together with the flagellar antigen. The range of different concentrations of fragment A used for preincubation was 200 pg, 2 ng, 20 ng, and 200 ng/ml. These antigen concentrations were tested at two different titers of antibody in the tissue culture fluid, i.e., 10 and 10^2 for each group.[4] Cultures were tested for the number of AFC to flagellar antigens as well as to SRC.

Results are presented in Fig. 2. It was found that within the antigen dose range tested, no tolerance was induced with fragment A when the antibody titer

[4] Titers of 10 and 100 immobilization units as described in Materials and Methods.

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during preincubation was kept at a concentration of 10. Immunological tolerance was however induced during a 6 hr preincubation period in the presence of 20 ng and 200 ng/ml of fragment A and antibody at a titer of 10^2. The specificity of the phenomenon was verified by the fact that normal immune responses to SRC were obtained in the absence of an immune response to

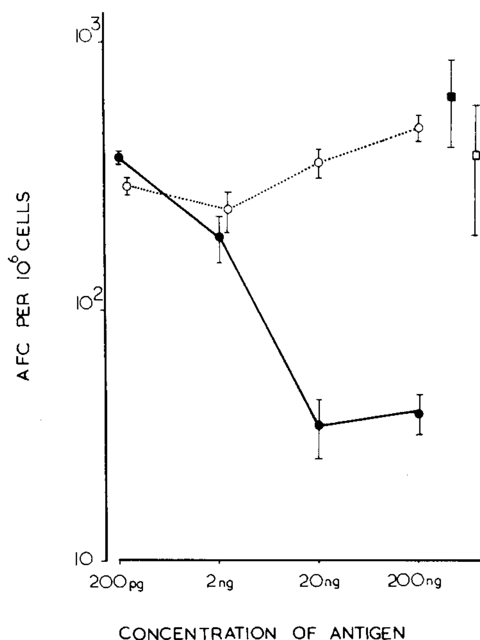

FIG. 2. Capacity of fragment A of flagellin to induce tolerance in vitro in the presence of antibody against polymerized flagellin at a titer of 10^2. Spleen cells were incubated in vitro for 6 hr in the presence of antigen and antibody, followed by challenge in vitro for 4 days with polymerized flagellin (20 ng/ml). Each value comprises the geometric mean of 8–20 cultures. Vertical bars represent the standard deviations. ●——●, immune responses after preincubation with fragment A in the presence of antibody;○——○, immune responses after preincubation with fragment A only; □, immune response after preincubation with antibody only; ■, immune response after preincubation with sheep erythrocytes in the presence of fragment A and antibody.

polymerized flagellin. Preliminary experiments were carried out to investigate whether the degree of tolerance induced during the preincubation of cells with fragment A and antibody was time-dependent. It was found in a first series of experiments, that tolerance could be induced with 2 ng/ml of fragment A and an antibody titer of 10, provided the time of preincubation of cells with antigen and antibody was extended from 6 hr to 12–18 hr. Thus, the number of AFC to polymerized flagellin obtained in the group treated with 2 ng of fragment A in

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the presence of antibody was 80 ± 112 (geometric mean ± SD), while the group treated with 2 ng of fragment A only was 680 ± 250 AFC per culture ($P < 0.01$).

DISCUSSION

The data presented in this paper augment those of an earlier publication in which we reported for the first time the induction, in vitro and in vivo, of immunological tolerance mediated by low doses of antigen in the presence of specific antibody (6). In close analogy to the in vitro induction of tolerance to *Salmonella adelaide* H antigens by the brief exposure of dispersed mouse spleen cells to high concentrations of a polymerized form of these antigens (10, 14), tolerance may also be induced by the in vitro treatment of spleen cells for 6 hr with an immunogenic dose of the antigen together with low concentrations of the relevant antibody.

The first series of experiments reported here was carried out to determine the influence of different concentrations of antibody, in the presence of a fixed dose of antigen, on the degree of tolerance induced in vitro. This led to the important finding that there exists an optimal antigen: antibody ratio which ensures the establishment of tolerance. Any change in this ratio in favor of either the concentration of the antigen or the antibody prevents the induction of tolerance. It is important to remember that, an antibody concentration too high to favor tolerance induction under the described experimental conditions of preincubation of cells with antigen and antibody for only 6 hr, prior to culture and antigenic challenge, is readily immunosuppressive when present during the entire culture period (6). This leads to the conclusion that antibody-mediated immune suppression must be effective either at the central level (i. e. the level of the immunocompetent cells), in which case it represents the induction of immunological tolerance, or, alternatively, at the peripheral level by altering the antigenic stimulus that is necessary for immune performance. Which of these two mechanisms operates depends on the ratio between antigen and antibody. The separation of the two events is most readily achieved under in vitro conditions. In vivo, central and peripheral mechanisms may be concomitant. It is thus assumed that most experimental work on antibody-mediated immune suppression in vivo has so far been carried out under conditions which favor mechanisms acting at the peripheral, rather than at the central level. A possible exception in this respect has been reported by Hanna and Francis (16). These authors state, in agreement with previous work by Hege and Cole (17), that the spleen cells of animals primed with sheep erythrocytes 2–4 wk previously responded to a secondary antigenic challenge after transfer into a syngeneic irradiated recipient with a hemolytic plaque-forming cell response below that of the primary response. Furthermore, Hanna and Francis demonstrated that this suppression was partially due to a lack of specific immunocompetent progenitor cells in the

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primed animal. It is, in view of our findings, most likely that such loss of immunocompetent cells was due to tolerance mediated by the combined action of antibody derived from the primary response and antigen introduced by the secondary challenge.

Previous work on in vitro tolerance has shown that unresponsiveness to H antigens of *S. adelaide* flagella may be induced by exposing dispersed mouse spleen cells to relatively high doses of polymerized flagellin (10, 14). Subsequent experiments revealed that, using this antigenic form, both the induction of immunity as well as of tolerance in vitro may be achieved in the absence of phagocytic cells, and must, therefore, result from a direct encounter of immunocompetent cells with the antigen (18). These findings are regarded as a basic supposition for the following interpretations.

Much evidence from in vivo experiments on tolerance has led to the generally accepted theory that the smaller the antigen molecule, the stronger is its potency to render immunocompetent cells tolerant upon direct contact (22). This is in contradiction to our findings which show that polymerized flagellin is more potent in its ability to induce tolerance in vitro than is flagellin monomer (mol wt 40,000). An even smaller unit, fragment A of flagellin (mol wt 18,000) which contains all the antigenic determinants that can be recognized on flagellin monomer (19) entirely failed to induce tolerance in vitro over the wide dosage range tested. This may be analogous to the situation described by Mitchison where a low molecular weight fraction of bovine serum albumin (BSA) that was able to induce tolerance in vivo failed to show this property when tested in vitro (20). Like BSA, fragment A in the *Salmonella* antigen system is highly tolerogenic in vivo at concentrations far below those required to induce immunity. The paradox of this situation remains unresolved unless one assumes that these antigens owe their tolerance-inducing capacity to the collaboration of mechanisms that are operative in vivo only. It is felt that this communication offers a solution to the problem. Fragment A, which fails to induce tolerance when presented alone to lymphoid cells in vitro, becomes highly potent in its tolerance-inducing capacity, provided it is presented in combination with specific antibody. Similarly, with the situation described for antibody-mediated tolerance using polymerized flagellin, the tolerance-inducing capacity of fragment A depends on the maintenance of a certain antigen: antibody ratio. The following attempt to reconstruct these mechanisms is based on the justifiable assumption that antigen-reactive cells possess antibody molecules at their surfaces which act as antigen receptors. These receptors may not be randomly distributed over the entire cell surface; instead, they seem to be located in small patchy areas as suggested by Mandel, Byrt, and Ada (21). Upon exposure of an appropriate antigen-reactive cell to a mixture of antigen and antibody, antigen combines with the cell's recognition sites. Antigen molecules attached to the cell surface have still a large number of exposed antigenic determinants that have

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not combined with recognition antibody. Free antibody in the cell's environment will thus attach to these determinants and in turn will attract more antigen. This antigen-antibody focusing process must eventually lead to the formation of a lattice of antigen-antibody complexes on top of antigen recognition sites on the cell. Furthermore, such a process must establish interlinkage of recognition sites or even recognition areas across the cell surface by antigen-antibody bridges. It is suggested that such interlinkage of recognition units provides the stimulus that renders the cell nonreactive to a further encounter with the relevant antigen.

Ada and Parish (3) have suggested that both immunological tolerance and antibody-mediated suppression may be caused by antigen localized in lymphoid follicles. The experimental results described above and previously (6) are in accordance with this hypothesis. We have suggested that in vitro immune complexes may form on the surface of immunocompetent cells. This is similar to the situation in vivo where antibody adherent to the dendritic processes of the reticular cells of lymphoid follicles may enable the antigen to form a similar immunosuppressive lattice. This concept may explain the tolerogenic effect of antigen and antibody that we have recently described in vivo (6).

This interpretation of our results on in vitro induced tolerance does not, at the same time, provide an understanding of mechanisms responsible for immune induction. Obviously, both phenomena involve the interaction of immunocompetent cells with antigen. It is felt that one of the two phenomena, i.e. immunologic tolerance, depends on mechanisms that involve the interlinkage of a critical number of antigen recognition sites, either by a large number of antigen molecules of suitable size or by a combined action of antigen and antibody. It should be stressed that we do not imply that an interlinkage frequency below the critical threshold for tolerance is per se responsible for immune induction. All it means is that the cell is still able to participate in the generation of an immune response, whatever the mechanism of its induction may be.

The above model is suited to explain the observed differences in the tolerance-inducing capacity among the different forms of *Salmonella* H antigens.

(*a*) *Polymerized Flagellin.*—The ability of polymerized flagellin to act as an immunogen or a tolerogen in vitro is determined by its concentration. Polymerized flagellin consists of elongated rod-like entities up to 15,000 A in length (7). It is clear that polymerized flagellin could therefore cause interlinkage of antigen recognition units at the surface of an immunocompetent cell. An increase of the antigen concentration could thus lead to an increase in the frequency of such interlinkage. Once a critical degree of interlinkage is established, the cell would be rendered unresponsive. This process is facilitated by the introduction of antibody by the mechanism described in the previous paragraph. Now, less antigen is required to achieve the same degree of interlinkage of antigen recognition sites than is required with a tolerance-inducing dose of antigen only.

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(*b*) *Monomer and Fragment A*.—Monomeric units of the flagellar H antigens were only marginally tolerogenic in vitro relative to comparable tolerance-inducing concentrations of the polymeric form. A further decrease in molecular size of the antigen completely abolished its tolerance-inducing capacity, as was demonstrated with fragment A of flagellin. This is inconsistent with the model proposed by Bretscher and Cohn (15), suggesting that the binding of a single antigen molecule with one cell receptor on the antigen-reactive cell surface should induce tolerance. We have interpreted the lack of tolerogenicity of fragment A as the failure of fragment A to cause interlinkage of antigen recognition sites, due to its small size. Again, it is antibody that is necessary to provide the link in order to bridge the distances between recognition units at the cell surface.

It was to be expected that, under the conditions used, i.e. exposure of lymphoid cells to antigen and antibody for 6 hr, the same antigen cencentrations, when compared on a weight for weight basis for polymer and for the much smaller fragment A, would require different concentrations of antibody to induce comparable degrees of tolerance. Thus the induction of tolerance with fragment A required 10 times more antibody than was needed to achieve the same degree of unresponsiveness when the much larger entity of polymerized flagellin was used for tolerance induction.

The question now remains as to the relevance of antibody-mediated tolerance in vitro in view of the in vivo phenomenon known as low zone tolerance. We have shown (6), that antibody-mediated tolerance to polymerized flagellin may be induced within 6 hr, using immunogenic concentrations of the antigen. With an equivalent dose of fragment A (on a weight for weight basis), antibody-mediated tolerance could be induced within a time comparable to that required for tolerance induction with polymerized flagellin. However, to induce tolerance with an even smaller amount of fragment A (2 ng and 200 pg) the induction time for tolerance had to be extended from 6 hr to 16 hr. This latter finding, though yet preliminary, suggests that an antigen at a concentration that is well below that required to induce immunity, could be tolerogenic, provided enough time is allowed for its interaction with antibody and immunocompetent cells.

An adaptation of our in vitro model to the phenomenon of in vivo tolerance provides a suggestion as to the origin of the antibody that is required for tolerance induction. The most obvious answer derives from the well known fact that antibody may often be produced during the induction of high zone tolerance as the result of concomitant immunity (22). This phenomenon has also been reported by Ada and Parish to occur during the induction of low zone tolerance to polymerized flagellin by means of fragment A (19). Thus it seems likely that low zone tolerance in vivo depends on the initial production of antibody. Under some circumstances, natural antibody may fulfill the same purpose. In this context, one could think of the possibility that an antigen-reactive cell's own recognition antibody, when produced and secreted at a rate high enough to

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accumulate in the cell's microenvironment, may serve as a mediator of tolerance upon interaction with small amounts of antigen. The possibility arises, that such a mechanism could be operative in the maintenance of tolerance to self antigens.

SUMMARY

Immunological tolerance to H antigens of *Salmonella adelaide* may be induced in vitro by the exposure of mouse spleen cells for 6 hr to an immunogenic dose of polymerized flagellin in the presence of low concentrations of specific antibody. Such antibody-mediated tolerance requires an optimal antigen: antibody ratio for its induction. A shift in this ratio in favor of the antibody concentration results in failure of tolerance induction and leads to immune suppression commonly known as antibody-mediated feedback inhibition which is not analogous to immunological tolerance.

Fragment A of flagellin fails to induce immunological tolerance in vitro. Tolerance to polymerized flagellin may however be induced in vitro, provided the spleen cells are exposed to fragment A in the presence of specific antibody for 6 hr. The results are discussed in the light of current theories of the mechanism of tolerance induction.

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