A MECHANISM FOR THE INDUCTION OF IMMUNOLOGICAL TOLERANCE BY ANTIGEN FEEDING: ANTIGEN-ANTIBODY COMPLEXES*

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Oral or intragastric administration of antigens such as foreign proteins or erythrocytes is known to cause the appearance of circulating antibodies which frequently are predominantly of the IgA class (1). Some of this antibody is made locally in the immunized gut wall (2, 3), but an additional, as yet unspecified, fraction originates in extraintestinal lymphoid tissues, particularly the spleen. In this organ, many PFC, chiefly of the IgA class, can be identified 1 wk after a 4 day course of intragastric immunization with sheep red blood cells (SRBC) (4). Reasons for attributing this preference for IgA to the colonization of the spleen and lymph nodes by IgA-committed cells triggered by antigen in the gut wall have been set forth in recent reviews (1, 5).

While exploring the immunologic memory of this type of immune response, we encountered a second striking feature of the systemic effects of enteric (mucosal?) immunization. When our standard 4-day intragastric SRBC course given to mice was repeated 2–3 wk after a similar priming course, i.e. at a time when the primary spleen response had subsided, the expected booster effect was not obtained; it was in fact replaced by a complete absence of response, and it took several months for systemic reactivity to renewed intragastric challenge to be restored to the level of an ordinary primary response (4). We termed this phenomenon "negative immunologic memory" (5).

The relevance of these intriguing findings with respect to work on oral vaccination need not be pointed out. It was therefore clearly important to gain insight into its mechanism, with the hope that this would suggest means of avoiding or manipulating the phenomenon at will.

In our previous work, hyporesponsiveness had been assessed by giving the challenging dose of antigen intragastrically. Therefore the possibility remained that coproantibody induced by the primary immunization had interfered with

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the absorption of the challenging antigen. We have in fact established its ability to do so (6). In the present study we will, however, demonstrate that intragastric immunization also caused hyporesponsiveness to parenteral challenge, and that one is therefore dealing with a genuine form of tolerance. It will also be shown that a tolerogenic factor present in the serum of intragastrically immunized animals and presumably consisting of immune complexes with IgA as the antibody, is responsible for the effect.

Materials and Methods

Animals. Conventional BALB/c/Cen mice of both sexes, raised at the laboratory, were used at the age of 3 mo. They received water ad lib., and were fed UAR mouse food (Villemoisson/Orge, France).

Antigens. SRBC and horse red blood cells (HRBC)[1] were obtained from the Institut Pasteur, Paris, France, and were washed three times with phosphate-buffer saline before use.

Immunization and Challenge Schedules

INTRAGASTRIC IMMUNIZATION OR CHALLENGE. Intragastric immunization or challenge was performed by giving mice 4×10^9 SRBC by intragastric intubation after 8 h of fasting, the procedure being repeated on 4 consecutive days.

PARENTERAL IMMUNIZATION OR CHALLENGE. Parenteral immunization or challenge consisted of a single-intraperitoneal (i.p.) injection of 2×10^8 SRBC (in some cases HRBC).

Antimouse Sera. Rabbit antisera specific for mouse IgA and IgG (without subclass distinctions) were a gift from Dr. H. Bazin, Experimental Immunology Unit, University of Louvain, Brussels, Belgium.

Serum Antibody Response. Carotid blood from ether-anesthetized mice was allowed to clot at room temperature and the serum was inactivated at 56°C for 30 min. Mouse anti-SRBC antibodies of IgM nature were hemolytic and could therefore be titrated directly, whereas indirect hemolysis by added antimouse IgA or antimouse IgG had to be employed for anti-SRBC of the other Ig classes. A microtitration apparatus was used (Microtiter, Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.).

Plaque-Forming Cell (PFC) Assays. PFC were enumerated after mixing SRBC or HRBC with spleen cells according to a previously described modification (4) of the fluid-medium procedure. The final mixtures contained 0.1 ml of spleen cells and 0.9 ml of a SRBC or HRBC suspension of appropriate concentration. This system was convenient for the enumeration of direct PFC assumed to produce IgM antibodies. For the assay of IgA or IgG PFC, antiserum specific for mouse IgA or IgG was added in optimal amounts, and the net numbers of IgA or IgG PFC were computed from the difference between counts on these "indirect" slides and those from the "direct" slides with no added antiserum.

PFC Inhibition Assays. Serum from normal or immunized mice was tested for inhibitory activity towards PFC from i.p. immunized mice by incubating 25×10^6 spleen cells for 30 min at 37°C with 0.5 ml of the serum to be assayed or, for the control, with 0.5 ml of Eagle's medium containing 20% fetal calf serum (FCS). The cells were then centrifuged at 200 *g* for 5 min at room temperature and resuspended in 0.5 ml of Eagle's medium plus 20% FCS, after which they were plated in the usual manner for the incubation. The viability of the spleen cells, as assessed by the trypan blue exclusion test, was about 95%.

Detection and Removal of Circulating Antigen-Antibody (AgAb) Complexes. The presence of AgAb complexes in the serum from mice immunized with SRBC or in normal control sera was detected, and their concentration semiquantitatively assessed, by two tests based on the ability of such complexes to inhibit the agglutination of polystyrene ("latex") particles coated with human IgG by rabbit rheumatoid factor (RF) or the C1q component of rabbit complement (C), respectively.

[1] *Abbreviations used in this paper:* AgAb, antigen-antibody; BSA, bovine serum albumin; FCS, fetal calf serum; HRBC, horse red blood cells; HSA, human serum albumin; PFC, plaque-forming cells; RF, rheumatoid factors.

TABLE I
Comparative Effects of Enteric vs. Parenteral Priming on the Spleen PFC Response to a Single Intraperitoneal Dose of SRBC*

	No previous priming, group 1	Intragastric priming‡		i.p. priming§ 2 wk before, group 3		
		2 wk before, group 2A	3 mo before, group 2B			
No. of animals per test	5	5	3	3		
days after single i.p. dose of 2 × 10⁸ SRBC	*IgM PFC/10⁶ spleen cells* ‖	*IgM PFC/10⁶ spleen cells* ‖	*IgM PFC/10⁶ spleen cells* ‖	*PFC/10⁶ spleen cells*		
				IgM	*IgA*	*IgG*
0	1	3	1	ND¶	ND	ND
2	17	1	9	68	50	135
4	256	32	162	ND	ND	ND
5	94	9	53	ND	ND	ND
6	69	1	30	ND	ND	ND
7	62	1	ND	30	27	157
11	35	2	14	ND	ND	ND
15	20	1	1	5	8	28

* Averages of counts from the spleens of the number of animals indicated.
‡ 1.6×10^{10} SRBC divided over four daily doses of 4×10^9 SRBC.
§ Single IP dose of 2×10^8 SRBC.
‖ No IgA or IgG PFC were found.
¶ ND, not done.

Further details on this technique will be given in a forthcoming publication.[2] Soluble AgAb complexes were eliminated by absorbing the serum on Sepharose-bound rabbit rheumatoid factor according to a method to be described elsewhere.[3]

Results

Effect of Intragastric Preimmunization on the Response to Parenterally Administered Antigen. The response of mice given a single i.p. injection of 2×10^8 SRBC is shown in Table I.

In control animals (group 1), i.e. without previous contact with the antigen, the splenic PFC response was virtually all of the IgM class and peaked at day 4 with 256 PFC/10^6 spleen cells. Animals having received four intragastric doses of 4×10^9 SRBC each, on days −14 through −11 before the i.p. injection of 2×10^8 SRBC (group 2A) likewise showed a splenic PFC response of the IgM class with a maximum at day 4, but the magnitude of this response was eight times smaller than in the control group. If a similar 4-day intragastric preimmunization course was given three months before the i.p. injection of 2×10^8 SRBC (group 2B), the response resembled that seen in the controls, although the PFC peak on day 4 still averaged only 162 PFC/10^6 spleen cells. Group 3 is added as a control to show that parenteral priming with a comparable total dose of antigen set the stage for a vigorous secondary response. No hemolytic antibody activity,

[2] Lurhuma, A., C. L. Cambiaso, P. L. Masson, and J. F. Heremans. 1975. Detection of circulating antigen-antibody complexes by their inhibitory effect on the agglutination of IgG-coated particles by rheumatoid factor or C1q. Manuscript submitted for publication.

[3] Cambiaso, C. L., P. L. Masson, and J. F. Heremans. The use of insolubilized rheumatoid factor and C1q for isolating immune complexes. Manuscript in preparation.

TABLE II

Effect of Intraperitoneal Priming on the Spleen PFC Response to a 4 day Intragastric Immunization with SRBC*

	No previous priming, group 4			i.p. priming 2 wk before‡, group 5		
No. of animals per test	5			3		
days after onset of intragastric SRBC§	*PFC/10⁶ spleen cells*			*PFC/10⁶ spleen cells*		
	IgM	*IgA*	*IgA*	*IgM*	*IgA*	*IgG*
0	1	0	0	25	0	0
6	3	1	0	13	2	0
8	22	25	3	13	23	7
9	23	55	11	15	56	13
10	17	18	4	9	14	3
13	4	4	0	2	3	0
17	1	0	0	1	1	0

* Averages of counts from the spleens of the number of animals indicated.

‡ Single IP dose of 2×10^8 SRBC.

§ 1.6×10^{10} SRBC divided over 4 daily doses of 4×10^9 SRBC.

whether direct (IgM) or indirect (IgG or IgA), could be detected in the serum of nonimmunized mice. In five animals of group 1, direct (IgM) hemolytic activity was found at titers of 1/4,096, 1/2,048, 1/2,048, 1/512, and 1/256, respectively, on day 4. In five animals of Group 2A, the IgM antibody titers found on day 4 were, respectively, 1/128, 1/128, 1/64, 1/32, and 1/16, in agreement with the depression of their IgM PFC counts.

Effect of Parenteral Preimmunization on the Response to Intragastrically Administered Antigen. The splenic PFC response of mice given a 4 day course of intragastric immunization with 4×10^9 SRBC as the daily dose is shown in Table II.

The control series (group 4) having experienced no previous contact with the antigen, is taken from a previous publication (4). In these animals the splenic PFC response extended over all three immunoglobulin classes but predominated in the IgA class and reached a maximum on day 9.

Parenteral preimmunization with a single i.p. dose of 2×10^8 SRBC on day −14 before the onset of the intragastric course (group 5) significantly changed this pattern of response in only one respect, namely by raising the starting baseline level of IgM PFC, from virtually none, to 25 PFC/10^6 spleen cells. This was obviously a carry-over from the primary response to the i.p. injection of antigen, since IgM PFC again declined to half that number within 1 wk. For the rest, the response was predominantly IgA and peaked at day 9, exactly as in the control group.

Tolerogenic Effect In Vivo of Serum from Mice Intragastrically Immunized with SRBC. Mice were given the standard course of four intragastric doses of 4×10^9 SRBC at daily intervals, and their serum was collected on day 14 after the first dose. These sera exhibited neither direct IgM nor indirect IgG hemolytic activity, but contained IgA anti-SRBC antibodies at titers ranging from 1/1 to

TABLE III

*Effect of IP Injection of Serum from Intragastrically Immunized Mice Upon the Subsequent Splenic PFC Response to Intragastric SRBC Antigen**

Animal no.	Not pretreated (controls), group 6				One i.p. injection‡ of serum from mice intragastrically immunized with four doses of 4 × 10^9 SRBC 2 wk before, group 7			
	PFC/10^6 spleen cells		*Serum antibody reciprocal titers*		*PFC/10^6 spleen cells*		*Serum antibody reciprocal titers*	
	IgM	*IgA*	*IgM*	*IgA*	*IgM*	*IgA*	*IgM*	*IgA*
1	21	53	—	4	0	2	—	—
2	12	60	—	8	0	1	—	2
3	17	28	—	4	0	0	—	—
4	5	60	—	4	0	2	—	1
5	16	33	—	8	0	2	—	1
Mean ± SD	14.2 ± 6.1	53.4 ± 15.2	—	5.6 ± 2.2	0	1.4 ± 0.9	—	0.8 ± 0.8

* Tested on day 9 after the first of four intragastric doses of 4 × 10^9 SRBC, i.e., at the peak of this type of response.
‡ 0.5 ml of pooled immune serum given 8 h before the first intragastric dose.
§ —, no detectable hemolytic activity.

1/8, and demonstrable by the indirect hemolysis assay. These sera were pooled and the IgA anti-SRBC titer was adjusted to 1/2 by appropriate dilution with normal mouse serum.

When 0.5 ml of this immune serum from enterically stimulated mice was injected intraperitoneally to recipient mice 8 h before the onset of a 4 day intragastric course with 1.6 × 10^{10} SRBC (group 7, Table III) or 8 h before a single i.p. injection of 2 × 10^8 SRBC (group 9A, Table IV), the subsequent splenic PFC responses and serum antibody titers after both types of antigenic stimulation were considerably depressed. In these experiments, the controls were mice similarly treated with antigen but having received no i.p. pretreatment with immune serum (group 6, Table III; and group 8, Table IV). In both cases the recipients' responses were assessed at the time they should normally have reached their maximum, viz. on day 9 for the intragastric group and on day 4 for the i.p. group.

When the whole antigenic load of 1.6 × 10^{10} SRBC was given to the serum donors in a single intragastric dose instead of being divided over four consecutive days, and their serum was collected within 1 day instead of 2 wk later, no tolerogenic effects were obtained by injecting it i.p. to recipients challenged i.p. 8 h later (group 9B, Table IV). The pooled serum used in this experiment possessed neither direct IgM nor indirect IgG or IgA hemolytic activity.

Tolerogenic Effect In Vitro of Serum from Mice Intragastrically Immunized with SRBC. The report by Schrader and Nossal (7) on the capacity of tolerogenic material to block the activity of PFC in vitro prompted us to verify whether similar inhibiting activity would be displayed by the tolerogen which the preceding in vivo experiments had shown to exist in the serum of intragastrically immunized mice.

For this purpose mice were given an i.p. priming with 2 × 10^8 SRBC together with 2 × 10^8 HRBC, and their spleens were tested for direct PFC of both specificities, 4 days after the injection. The spleen cells were exposed for 30 min to various media before being washed and plated for the Jerne plaque assay, as

TABLE IV

*Effect of i.p. Injection of Serum from Intragastrically Immunized Mice Upon the Subsequent Splenic PFC Response to Intraperitoneal SRBC Antigen**

Animal no.	Not pretreated (controls), group 8		One i.p. injection‡ of serum from mice having received intragastrically:			
			4 doses of 4 × 10^9 SRBC 2 wk before, group 9A§		1 dose of 1.6 × 10^{10} SRBC 1 day before, group 9B	
	IgM PFC/10^6 spleen cells	*IgM serum antibody reciprocal titers*	*IgM PFC/10^6 spleen cells*	*IgM serum antibody reciprocal titers*	*IgM PFC/10^6 spleen cells*	*IgM serum antibody reciprocal titers*
1	102	256	5	4	180	1,024
2	200	512	0	2	190	256
3	562	4,096	0	2	310	256
4	170	1,024	0	2	250	512
5	170	2,048	3	2	150	256
Mean ± SD	240.8 ± 183.1	1,587 ± 1,561	1.6 ± 2.7	2.5 ± 0.9	246.0 ± 63.9	461 ± 334

* Tested on day 4 after the i.p. dose of 2 × 10^8 SRBC, i.e., at the peak of this type of response.
‡ 0.5 ml of serum given 8 h before the i.p. dose.
§ Same immune serum as given to group 7, Table III.

described under Materials and Methods. The results (exp. 1, Table V) show that, with reference to the control using Eagle's medium enriched with FCS, normal mouse serum had no blocking effect whatever. In contrast, marked inhibition of anti-SRBC but not of anti-HRBC PFC activity occurred when the cells had been exposed to serum from mice intragastrically immunized with SRBC 2 wk before. Also in analogy with the in vivo findings, this blocking activity was absent from serum collected 1 day after the total immunizing dose (1.6 × 10^{10} SRBC) was given as a single intragastric dose.

As mentioned in describing the in vivo tolerization experiments, the serum pool from animals immunized intragastrically 2 wk before contained IgA antibodies to SRBC. No direct test was available to assay this blocking serum for the simultaneous presence of antigen, but it was thought that, by that time, circulating SRBC material might at best persist only in trace amounts and would therefore occur in combination with antibody. The assay for immune complexes (cf. Materials and Methods) proved negative when C1q was used as the reagent, but was positive at a 1/8 dilution with RF. A serum pool from nonimmunized mice was also negative with C1q, whereas its inhibition titer with RF was 1/2. When the blocking serum was absorbed with insolubilized RF (cf. Materials and Methods), its inhibition titer with RF dropped to the control value of 1/2. This absorbed serum was then tested again for in vitro inhibitory activity towards direct PFC elicited by i.p. immunization. As shown in the lower part of Table V, all such activity was abolished by the removal of material retained by RF.

Discussion

Systemic Unresponsiveness after Immunization by the Digestive Route is not due to Impaired Absorption of Antigen Caused by Coproantibody, but is a Genuine Form of Tolerance. Reports from different laboratories (6, 8, 9) indicate that immunization by the digestive route leads to impaired enteric absorp-

TABLE V
Specific Paralyzing Effect In Vitro of Serum from Intragastrically Immunized Mice Upon Splenic PFC from Parenterally Primed Animals, and Removal of the Inhibitory Material by Absorption with Rheumatoid Factor

Spleen cells exposed for 30 min (37°C) to:	Direct PFC/10^6 spleen cells on day 4 after one i.p. priming with 2×10^8 SRBC and 2×10^8 HRBC	
	Anti-SRBC*	Anti-HRBC‡
	mean ± SD	*mean ± SD*
Exp. 1		
Eagle's + 20% FCS	180 ± 47	238 ± 119
Normal mouse serum	190 ± 10	227 ± 114
2-wk postgastric immunization serum§	32 ± 8	224 ± 117
1-day postgastric immunization serum‖	182 ± 29	ND
Exp. 2		
Eagle's + 20% FCS	225 ± 68	
2-wk postgastric immunization serum absorbed on RF	222 ± 74	

* Mean ± SD from 7.
‡ Mean ± SD from 3.
§ 1.6×10^{10} SRBC divided over four doses of 4×10^9 SRBC.
‖ 1.6×10^{10} SRBC in a single dose.

tion of macromolecular antigens. More specifically we have been able to show that in rats having received a single dose of 200 mg of human serum albumin (HSA) by gastric intubation, the capacity of the gut to transfer intact albumin molecules from the gut contents into the blood was decreased to less that 50% of its normal level by the 2nd wk after the immunization, and that secretory IgA coproantibody, present in the enteric lumen at that time, was responsible for the effect (6).

A priori, therefore, impaired uptake of antigen might have been a factor in the previously reported hyporesponsiveness to SRBC caused by intragastric immunization and revealed upon rechallenge by the digestive route (4). Here we show, however, that similar unresponsiveness is found when the enteric portal of entry is bypassed by injecting the challenging dose of antigen i.p. (group 2A, Table I). One is therefore dealing with tolerance, not with lack of uptake of antigen.

The slowness of recovery, as revealed by parenteral challenge after 3 mo (group 2B, Table I), has also been noted formerly when the challenge was given intragastrically (4), and is of relevance to the discussion of the mechanisms involved (see below).

At any rate, powerful tolerizing capacity seems to be one of the features inherent to the enteric (mucosal?) mode of administration of antigen. It is therefore surprising that the literature should so rarely have alluded to this phenomenon. It is not clear to what extent it is related to the still unexplained "Sulzberger-Chase phenomenon" (10), by which systemic tolerance is induced in guinea pigs by feeding them chemically active haptens capable of conjugation

with body components or foreign helper antigens from the gut contents. A better parallel exists with the recent report by Thomas and Parrott (1), that systemic hyporesponsiveness to bovine serum albumin (BSA) is found in rats after 2 wk of daily intragastric administration of 25 mg of this antigen (see below).

Parenteral Priming, in Contrast to Intragastric Priming, Appears to have Little Effect on the Systemic Response to Subsequent Intragastric Challenge. Besides its predominant IgA character and its propensity to cause subsequent unresponsiveness, the immune response to intragastric administration of antigen displays yet a third unusual feature, which is evident from Table II.

Parenteral priming with an immunogenic dose of SRBC is of course a classical means to prepare a mouse for a vigorous secondary response to a second immunogenic dose of the same antigen, given parenterally 2 wk later (group 3, Table I). Yet, when challenged by the enteric route (group 2, Table II), the parenterally primed animals behaved as if they had never before encountered the antigen. Except for the understandable carry-over of some IgM PFC from the primary response, their "secondary" response was in all points similar to a primary enterically induced reaction.

A rational explanation is however available if one accepts our previous proposal that splenic PFC responses after enteric administration of antigen are not due to resident cells of the spleen but to immigrants from the stimulated gut wall. Reports on the enteric absorption of HSA in rats (6) and BSA in rats (11) agree that less than 0.1% of an intragastric dose of these antigens reaches the circulation as intact macromolecules. Intragastric challenge is therefore probably insufficient to reveal immunologic memory in spleen cells primed by the parenteral route. Then, any response observed in that organ must reflect the reaction of immigrant cells to contact with antigen in the gut wall. Now, parenteral priming has been shown only minimally to affect the lamina propria of the bowel (12), which is probably neither primed nor tolerized by such a preparation. It is only logical, then, that the "secondary" spleen response to intragastric antigen after parenteral priming, should assume the features of a primary enterically induced reaction.

Tolerance Induced by Intragastric Immunization is Mediated by a Circulating Tolerogen Presumably Consisting of Immune Complexes Containing IgA Antibody. Our data clearly indicate that the specific hyporesponsiveness shown by mice 2 wk after intragastric immunization with SRBC can be transferred passively to virgin recipients by means of serum. This circulating tolerogen has a powerful blocking effect on parenteral (Table IV) as well as enteric (Table III) challenges.

Some idea as to the mode of action of the tolerogen is afforded by the fact that it paralyses, in vitro, the activity of B cells already engaged in antibody production and secretion (Table V). This does not exclude the existence of other, perhaps more important, targets for its action. One would envisage in the first place the possibility of an inhibitory effect on the primary triggering of the B cell; experiments now in progress aim at elucidating this point.

The in vitro effect of the enterically induced tolerogen on pre-existing PFC is evidently analogous to the phenomenon described by Schrader and Nossal (7) as

"effector cell blockade." In their experiments the paralyzing agent was highly multivalent antigen such as dinitrophenylated flagellin, whereas in the present case the relationship between the inducing enterically given antigen (SRBC) and the resulting circulating tolerogen is not immediately apparent. The following reasoning may clarify this question.

In their study on orally induced tolerance to BSA in rats, Thomas and Parrott (11) noted that antigen was absorbed as native protein, and therefore thought it likely that they were dealing with low-zone tolerance (13), rather than with effects of tolerogenic products such as are known to arise by "deaggregation" (14, 15) or proteolysis (16) of certain antigens. Incidentally, a water-soluble tolerogenic extract from erythrocytes has been described (17). In our experiments, however, the absence of tolerogen in serum collected 24 h after a massive intragastric dose of SRBC (Tables IV and V) speaks against the paralyzing material being either intact, deaggregated, or degraded antigen. Rather it seems that some form of immune response must be set up by the body before the tolerogen can make its appearance in the blood. Both in our case and in the experiment of Thomas and Parrott (11) there existed a serum antibody response before the revelation of the hyporesponsive state. More significant, though, was the finding that the tolerogenic factor could be removed from the serum by a procedure known to eliminate soluble AgAb complexes (exp. 2, Table V).

Immune complexes formed in slight excess of antigen can be powerful tolerogens, possibly because they represent a highly multivalent form of antigen (18, 19). Schrader and Nossal have speculated that complexes of appropriate composition may therefore also be capable of "effector cell blockade." This is precisely what the data reported here imply.

In our system, only the enteric, not the parenteral, induction of a primary response appeared to give rise to circulating tolerogenic immune complexes. Perhaps this was due to the predominantly IgA character of the former response, as opposed to the IgM character of the parenterally induced response. It is interesting that the immune complexes present in the tolerizing immune sera could be detected by means of rheumatoid factor but not by using C1q. Immune complexes containing IgA as the main or sole antibody should behave in exactly that way; a point which we have verified with our latex inhibition test using aggregated monoclonal immunoglobulins of various classes as models.[2]

Conclusion. In summary, then, we propose that enteric immunization may be unusually able to create secondary immunologic hyporesponsiveness because it favors the formation of IgA antibody whose immune complexes with the absorbed antigen are, for a reason yet to be discerned, particularly suited to paralyze B cells.

To a large extent this mucosal type of immune response presumably takes place in the gut wall itself. To some extent it may also "metastasize" to distant lymphoid tissues such as the spleen (1). A third site to be considered is the portal lymphoid tissue of the liver. It is in direct continuity with the lamina propria of the gut and, like the latter, is a preferred site of IgA production (18). In addition it is directly exposed to enterically absorbed antigen carried by the mesenteric venous blood. If the mechanism of tolerization via IgA immune complexes that we propose is correct, a novel explanation may then be given to the production of

systemic hyporesponsiveness by antigen given orally (10, 19) or injected into the portal circulation (20, 21), as well as to the suppression of these effects when the liver is bypassed by a portocaval transposition (19).

Summary

We have previously reported on the induction, in mice, of a systemic (splenic) immune response with IgA as the dominant antibody, as a result of a short (4 day) intragastric immunization course with foreign erythrocytes. This response was followed by a prolonged period of hyporesponsiveness to similarly administered antigen. Here it is shown that this hyporesponsiveness is also manifested towards antigen given intraperitoneally, and that one is therefore dealing with tolerance, not with failure to absorb antigen from the gut.

In contrast, mice primed parenterally and then challenged intragastrically, behaved as if never having any previous contact with the antigen, i.e., with a primary-type splenic response of predominant IgA character. This agrees with our former conclusion that splenic responses to enterically absorbed antigen reflect colonization of the spleen by cells sensitized locally in the gut wall, a site not readily primed by the parenteral route.

Serum from intragastrically immunized mice contained a very active tolerogen. In vivo, it was capable of conferring tolerance to nonimmune recipient mice. In vitro, it paralyzed the activity of antibody-producing cells. Inhibitory sera had weak antibody activity, restricted to the IgA class, and contained immune complexes reacting with rheumatoid factor but not with C1q. Elimination of these complexes by means of insolubilized rheumatoid factor abolished the tolerogenic effect. In conclusion, the enterically induced tolerogen seems to consist of immune complexes with IgA as the antibody.

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