ANTIGEN-INDUCED DECREASE OF SALICYLIC ACID ABSORPTION FROM THE SMALL INTESTINE IN ACTIVELY IMMUNIZED RATS

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Rats were intraperitoneally immunized with bovine γ -globulin (BGG) emulsified in incomplete Freund's adjuvant. Intestinal absorption of salicylic acid from the rat small intestine was examined by means of *in situ* recirculation technique. The disappearance of salicylic acid from the luminal solution was significantly decreased in rats challenged intravenously with BGG compared to saline as control. A significant decrease in absorption of salicylic acid was noted in rats immunized twice and three times, but not in rats immunized only once. The effect was maintained for at least 5 weeks after the third immunization, whereas it disappeared within 10 weeks. In normal rats, the decreased absorption of salicylic acid by the intravenous challenge with BGG was hardly observed at all. These findings suggest that the intestinal absorption of salicylic acid may be affected by systemic anaphylaxis in actively immunized rats.

Keywords — intestinal absorption; rat small intestine; salicylic acid; systemic anaphylaxis; active immunization; bovine γ -globulin; *in situ* recirculation technique

INTRODUCTION

The intestinal absorption of intact macromolecules and the ability of such molecules to induce both a local and a systemic immune response has been demonstrated under natural and experimental conditions.¹⁻⁴⁾ Walker *et al.* have shown the mechanism whereby immunization interferes with the intestinal absorption of macromolecules.⁵⁻⁹⁾ Also, Bloch *et al.* reported the enhanced uptake of immunoreactive bovine serum albumin (BSA) during intestinal or mild systemic anaphylaxis in rats.^{10,11)} In contrast to the well-studied immunological effect on the intestinal transport of macromolecules, few studies have been done to investigate the immunological consideration on the intestinal transport of low molecular weight organic compounds.

The present study was designed to investigate the effect of intravenous challenge with bovine γ -globulin (BGG) on the intestinal absorption of salicylic acid in rats immunized with BGG.

MATERIALS AND METHODS

Materials—Salicylic acid and BGG (Cohn fraction II, Sigma Chemical Co.) were of reagent grade. All other reagents used in these experiments were of the finest grade available.

Preparation of Drug Solution—The isotonic buffer solution of pH 6.5 was prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4 . Salicylic acid was dissolved in this buffer solution at the concentration of 1 mM for absorption experiments.

Animals and Immunization—Male Wistar albino rats ranging in weight from 150 to 200 g were used in these studies and maintained on a diet free of BGG. Rats were immunized according to the following schedule: 1 mg of BGG dissolved in 0.25 ml of saline (0.9% w/v) was emulsified with an equal volume of incomplete Freund's adjuvant (Difco Laboratory) and injected into peritoneal cavity under light ether anesthesia. The same dose of antigen and adju-

vant was administered intraperitoneally at 10 days intervals thereafter. Animals were immunized once to three times and absorption experiments were carried out one week after the last immunization.

Absorption Experiments—The procedure of the *in situ* absorption experiment in the rat small intestine was the same as that reported in a previous paper.¹²⁾ Animals were anesthetized with pentobarbital, given intraperitoneally, and the small intestine was cannulated for *in situ* recirculation. The entire length of the small intestine, from the pylorus to the ileo-cecal junction, was used for the absorption experiments. The bile duct was ligated in all experiments. BGG dissolved in 0.25 ml of saline was administered into a right femoral vein for 5 min. Immediately, 40 ml of drug solution kept at 37°C, was recirculat-

ed through the intestine at a rate of 5 ml/min using a peristaltic pump. At the end of an experimental period, the perfused solution in the small intestine was withdrawn as completely as possible, and the intestinal lumen was washed with pH 6.5 buffer solution. The washings were combined with the perfused solution and made up to 100 ml with pH 6.5 buffer solution. The amount that disappeared from the lumen was calculated as the difference between the amount of the drug in the initial and the final solutions. Results were compared statistically by Student's t-test.

Analytical Methods—Salicylic acid in luminal solution: Seven ml of chloroform and 0.1 ml of concentrated HCl were added to 3 ml of sample solution. The mixture was shaken for 15 min and then centrifuged for 10 min at 2500 r.p.m. After aspirating the aqueous phase, 5 ml

FIG. 1. Effect of Intravenous Challenge with BGG on the Intestinal Absorption of Salicylic Acid in 15 min[A] and in 30 min[B] in Rats immunized Three Times with BGG

0.5 mg of BGG dissolved in 0.25 ml of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments. Vertical bars indicate $\pm$ S.D.

of the organic phase was added to 5 ml of 0.1 N NaOH. The mixture was shaken for 10 min and then centrifuged for 10 min at 2500 r.p.m. The optical density of the aqueous phase was determined spectrophotometrically at 295 nm.

RESULTS

The absorption of salicylic acid from the rat small intestine was examined at pH 6.5 using the *in situ* recirculation technique. The protocol of the immunization with BGG and the absorption experiments are shown in each Figure. The results of absorption experiments in rats immunized intraperitoneally three times with BGG are summarized in Fig. 1. The disappearance of salicylic acid from the luminal solution in 15 min and in 30 min were significantly decreased in rats challenged intravenously with BGG compared to saline as control. To exclude the possibility that the decreased absorption of salicylic

acid is not due to systemic anaphylaxis, but solely due to the intravenous injection of BGG, absorption experiments were carried out in normal rats. The results are given in Fig. 2. In normal rats, the decreased absorption of salicylic acid by the intravenous challenge with BGG was hardly observed at all. From the results described above, it is reasonable to consider that systemic anaphylaxis results in the decrease of salicylic acid absorption from the rat small intestine.

The effect of dose of the intravenous challenge on the intestinal absorption of salicylic acid was shown in Fig. 3. In rats challenged intravenously with 0.1 mg of BGG, salicylic acid absorption was not affected at all, although it decreased significantly by the intravenous challenge with 0.5 mg and 1.0 mg of BGG. Consequently, 0.5 mg of BGG was injected as the intravenous challenge in additional experiments.

FIG. 2. Effect of Intravenous Challenge with BGG on the Intestinal Absorption of Salicylic Acid in 15 min[A] and in 30 min[B] in Normal Rats

0.5 mg of BGG dissolved in 0.25 ml of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments. Vertical bars indicate $\pm$ S.D. NS means not significantly different from each control.

Fig. 4 shows the effect of the number of immunization on the intestinal absorption of salicylic acid. In rats immunized twice and three times with BGG, a significant decrease in salicylic acid absorption was observed by the intravenous challenge with BGG. In contrast, rats immunized only once with BGG showed no significant decrease in salicylic acid absorption by the intravenous challenge with BGG compared to control.

In order to examine the duration after the last immunization in the decreased absorption of salicylic acid, experiments were carried out 1 week, 5 weeks, and 10 weeks after the third immunization in rats immunized three times with BGG. The results are shown in Fig. 5. Salicylic acid absorption was significantly decreased 1 week and 5 weeks after the last im-

FIG. 3. Effect of Dose of the Intravenous Challenge with BGG on the Intestinal Absorption of Salicylic Acid in 15 min in Rats immunized Three Times with BGG

BGG dissolved in 0.25 ml of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments. Vertical bars indicate $\pm$ S.D. NS means not significantly different from the control.

munization in rats challenged intravenously with BGG compared to control, whereas the effect disappeared within 10 weeks.

DISCUSSION

While the mucosal surface of the intestine provides a relatively efficient barrier to ingested antigens, it is well established that immunoreactive proteins are absorbed by both neonatal and adult mammals, including man.¹³⁾ Oral immunization with a variety of antigens leads to local immunoglobulin A antibody. Continued local feeding of protein antigens in rabbits eventually leads to a serum antibody response which in amount, class, and affinity is indistinguishable from that seen following parenteral immunization.¹⁴⁾

Recently, Walker *et al.* have shown the mechanism whereby immunization interferes with the intestinal absorption of macromolecules.⁵⁻⁹⁾ Antigen was found to become rapidly associated with antibodies present on the surface of the intestine. Formation of antigen-antibody complexes seemed to prevent binding of antigen to intestinal epithelial cells, as well as the subsequent endocytosis of antigen by these cells. In addition, Bloch *et al.* reported the enhanced uptake of immunoreactive BSA during intestinal or mild systemic anaphylaxis.^{10,11)} However, past work on the immunological consideration in the gastrointestinal absorption process of low molecular weight organic compounds has been relatively sparse. In the present study, we investigated the effect of intravenous challenge with antigen in actively immunized rats on the absorption of salicylic acid from the small intestine *in situ*. Salicylic acid was chosen as a model of low molecular weight organic compound due to the ease of assay and well-defined absorptive properties.¹⁵⁻¹⁷⁾ BGG chosen as an antigen is a foreign to the circulation of the rats.

As shown in Fig. 1, the disappearance of salicylic acid from the luminal solution was significantly decreased in rats challenged intravenously with BGG in comparison with saline as

control. This observation is inconsistent with the results of Bloch *et al.* that the intestinal uptake of BSA enhanced during intestinal or mild systemic anaphylaxis in rats. Presumably, it seems that this discrepancy is due to the difference of absorption mechanisms between the macromolecule and salicylic acid.

In Fig. 4, rats immunized only once with BGG showed no significant difference in the intestinal absorption of salicylic acid by the intravenous challenge with BGG compared to control. However, our preliminary experiments demonstrated that the intestinal absorption of salicylic acid was significantly decreased by the

intravenous challenge with ovalbumin in rats immunized once with ovalbumin. From these results, it is reasonable to consider that BGG is relatively less immunogenic than ovalbumin in rats. It is well established that the immunogenicity is dependent on foreignness, molecular size, and chemical complexity of an antigen. In rats immunized twice and three times with BGG, on the other hand, a significant decrease in the intestinal absorption of salicylic acid was observed by the intravenous challenge with BGG.

It is well known that once the immune system makes contact with a given antigen, it usually forgets it. If the same antigen ever enters

FIG. 4. Effect of Intravenous Challenge with BGG on the Intestinal Absorption of Salicylic Acid in 15 min in Rats immunized Once[A], Twice[B], and Three Times[C] with BGG

0.5 mg of BGG dissolved in 0.25 ml of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments. Vertical bars indicate $\pm$ S.D. NS means not significantly different from the control.

the body again, the immune response is much more rapid, intense, and prolonged. However, the intestinal absorption of salicylic acid was significantly decreased by the intravenous challenge with BGG 1 week and 5 weeks, but not 10 weeks, after the last immunization (Fig. 5). Generally, immunoglobulins are constantly being synthesized and catabolized in the body. These data tend to suggest the catabolism of the immunoglobulin.

Anaphylaxis is a manifestation of the immediate hypersensitivity resulting from the *in vivo* interaction of cellular sites with antigen and specific antibody. Combination with antigen sets off a sequence of intracellular reactions resulting in the release of histamine and other vasoactive substances, which produce increased capillary permeability, vasodilation, and smooth muscle contraction. Lake *et al.* have shown the anaphylactic release of intestinal goblet cell

FIG. 5. Effect of Intravenous Challenge with BGG on the Intestinal Absorption of Salicylic Acid in 15 min in Rats immunized Three Times with BGG
Absorption experiments were carried out 1 week[A], 5 weeks[B], and 10 weeks[C] after the last immunization. 0.5 mg of BGG dissolved in 0.25 ml of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments. Vertical bars indicate $\pm$ S.D. NS means not significantly different from the control.

mucus, too.^{18,19)} The mechanism by which systemic anaphylaxis decreases the intestinal absorption of salicylic acid in rats is not completely defined. Salicylic acid is well-absorbable from the rat small intestine and its absorption rate is considered to be affected by blood flow.²⁰⁾ Therefore, some changes in the vascular system induced by the systemic anaphylaxis might influence its absorption rate.

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