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Reagin Synthesis in Inbred Strains of Rats

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Summary. Reaginic antibody synthesis after intraperitoneal immunization with ovalbumin in aluminium hydroxide gel ($Al(OH)_3$) has been evaluated in BN, F344 and ACI inbred strains of rats and compared to that in outbred Wistar rats. Regardless of the dose of antigen employed (0·1 mg, 0·01 mg, 0·001 mg), BN inbred and Wistar outbred rats produced comparable amounts of reaginic antibody 9 days after immunization and these reagins were detectable for at least 70 days. In addition mean serum reagin titres could be boosted by subsequent administration of antigen. There was no statistically significant difference in the response of males as compared to females. The use of $Al(OH)_3$ gel as an adjuvant yielded reaginic antibody titres in these animals that were comparable to those induced with antigen administered with saline extracts of *Bordetella pertussis* organisms. In contrast ACI inbred rats failed to generate detectable reaginic antibodies when immunized either singly or repetitively with the same doses of antigen in $Al(OH)_3$ gel as used in the BN and Wistar rats. Wistar, BN, and ACI strains produced IgGa-like antibodies after a single immunization; however, peak serum titres were somewhat greater in the Wistar and BN, as compared to the ACI strain. Haemagglutinating antibody titres were comparable and greater than 1:1000 in all the strains studied within 35 days after immunization. These studies have demonstrated another animal model to explore the genetic mechanisms involved in the synthesis of reaginic antibody.

INTRODUCTION

Clinical studies in man have suggested that reaginic antibody synthesis is in part influenced by genetic factors (Sherman, 1971; Levine, Stember and Fotino, 1973). Levine and co-workers have reported that several inbred strains of female mice produce high titres of reaginic antibody after immunization with low doses of hapten–protein conjugates, whereas other inbred mice strains synthesize less reaginic antibody when the same immunizing antigen is employed (Levine and Vaz, 1970; Vaz, Vaz and Levine, 1971b; Levine and Chang, 1971; Vaz, Phillips-Quagliata, Levine and Vaz 1971a). Utilizing inbred strains of rats which provide the advantage of larger size and greater longevity than mice for long-term studies, Gill and co-workers have delineated several of the genetic mechanisms involved in non-reaginic antibody synthesis (Gill, Kunz, Stechschulte and Austen, 1970; Gill and Kunz, 1971; Gill, Enderle, Germain and Ladoulis, 1971). Reaginic antibody can be readily induced in outbred rats, but requires that an adjuvant such as parasitic infection *Bordetella pertussis*, or aluminum hydroxide

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accompany the administration of antigen for the development of detectable amounts of reagins (Mota, 1964; Orr, Riley and Doe, 1972; Orange and Austen, 1969). Employing outbred rats, Austen and co-workers have shown that a reagin-like antibody mediating the 48-hour passive cutaneous anaphylaxis (PCA) reaction is IgE, and that a non-reaginic antibody mediating the 2-hour passive cutaneous anaphylaxis (PCA) reaction is IgGa (Stechschulte, Austen and Bloch, 1967; Stechschulte, Orange and Austen, 1970; Orange, Stechschulte and Austen, 1970). The present study was undertaken to explore the predilection for reaginic antibody synthesis in several inbred strains of rats. Following immunization with various concentrations of purified ovalbumin in aluminum hydroxide gel, it was found that the several inbred strains of rats studied could be segregated into high and low producers of reaginic antibody. In the high responding BN strain, reaginic antibody was persistent, elicited with low concentration of antigen, and could be boosted; while in the ACI strain of rats reaginic antibody could not be detected by the methods employed.

MATERIALS AND METHODS

Rats

Six-week-old male and female BN, F344 and ACI inbred, as well as outbred Wistar rats were obtained from Microbiological Associates, Bethesda, Maryland. In addition, colonies have been established for these inbred rats in our laboratory.

Antigen

Ovalbumin (OA), five times recrystallized, was obtained from K & K Laboratories, Plainview, New York. The ovalbumin was incorporated in aluminum hydroxide ($Al(OH)_3$) gel as previously reported (Levine and Vaz, 1970).

Immunization schedules

In the initial protocol, the inbred and outbred rats were immunized with a single intraperitoneal injection of 0·1 mg of OA in $Al(OH)_3$ gel at 7 weeks of age, and bled periodically for 80 days. In a second intraperitoneal immunization protocol, the inbred and outbred rats were initially immunized at 7 weeks of age with 0·1 mg of OA in aluminum gel and boosted at 9 weeks and again at 16 weeks with the same concentration of antigen. These animals were bled periodically for 20 weeks. In the third protocol, which assessed the effect of several different concentrations of antigen, groups of BN rats were immunized with either 0·1, 0·01 or 0·001 mg of OA in $Al(OH)_3$ gel intraperitoneally. These animals were bled periodically for 80 days. All bleedings were by cardiac puncture during ether anaesthesia. After allowing the blood to clot at 25° for 1–2 hours, the serum was separated by centrifugation and stored at −20° until studied.

Passive cutaneous anaphylaxis (PCA) assay

(A) The 48-hour PCA reaction to assess reaginic antibody was employed according to the methods of Stechschulte *et al.* (1967). A challenge dose of 5 mg of ovalbumin (OA) incorporated in Evan's Blue dye was administered intravenously to outbred unimmunized male Wistar rats which served as passive recipients. The antibody titres presented in the results are the means of the greatest serum dilutions which produced a lesion with at least 5-mm diameter of blueing.

(B) The 2-hour PCA reaction to assess non-reaginic antibody production was performed with sera heated at 56° for 1 hour (Stechschulte *et al.*, 1967). Antibody titres were quantified and are recorded in the results as described above in (A).

Passive haemagglutinating antibody (HA)

HA antibodies specific for OA were performed according to the methods of Stavitsky (1954) as modified by Levine *et al.* (Levine, Wyman, Broderick and Ipsen, 1960).

Binding avidity

Serum from BN rats obtained 18 weeks after immunization at 0, 9 and 16 weeks with 0·1 mg of OA in $Al(OH)_3$ gel was assessed for reaginic antibody binding avidity by comparison with serum obtained 9 days after a single immunization with the same dose of OA. 0·05 ml of peak serum titre was injected intradermally into various skin sites of three outbred Wistar males which served as passive recipients. After 48 hours of incubation each recipient animal was injected via the tail vein with either 200 mg, 2 mg or 0·2 mg of OA.

RESULTS

Nine days after a single immunization with 0·1 mg of OA-$Al(OH)_3$ gel, BN rats produced reaginic antibody which had a maximum titre of 1:120 and these reagins persisted in detectable concentrations for at least 70 days. In contrast, inbred ACI rats failed to generate detectable reaginic antibody subsequent to immunization with 0·1 mg of

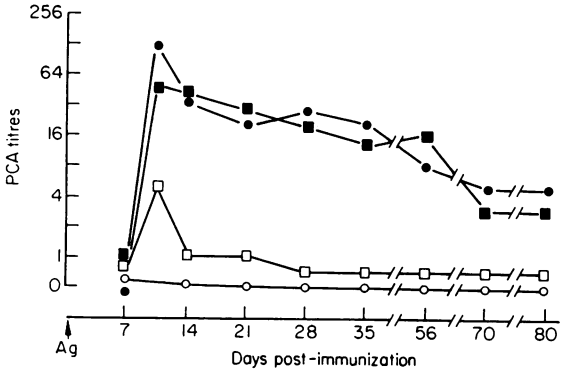

FIG. 1. Mean reaginic antibody titres in (●) twelve BN rats, (■) six Wistar rats, (□) six F344 rats, and (○) six ACI rats after a single intraperitoneal injection of 0·1 mg OA in $Al(OH)_3$ gel, as measured by the 48-hour PCA reaction and expressed as the reciprocal of the titre. Ag=antigen administration.

OA in $Al(OH)_3$ gel. The F344 rats were poor reaginic antibody producers, having maximum mean titres of no greater than 1:5, and having no reagins detectable after 28 days. The outbred Wistar rats produced reaginic antibody of comparable titre and duration, to that observed for the inbred BN rats (Fig. 1).

In addition to immunization with 0·1 mg of OA in $Al(OH)_3$ gel, BN rats were also injected intraperitoneally with 0·01 or 0·001 mg of OA in $Al(OH)_3$ gel (Fig. 2). Initially, lower mean peak serum reagin titres were found after intraperitoneal injection of 0·01 or 0·001 mg of antigen in $Al(OH)_3$ gel, as compared to 0·1 mg of antigen. However, after 14

days the responses to the three different concentrations were quite similar and there were no statistical differences ($P>0{\cdot}5$, Student's t-test) in the reaginic antibody responses regardless of the immunizing dose of antigen employed.

After an initial intraperitoneal immunization of 0·1 mg of OA in $Al(OH)_3$ gel, mean serum reagin titres of inbred BN and outbred Wistar rats peaked at 9 and 21 days and then

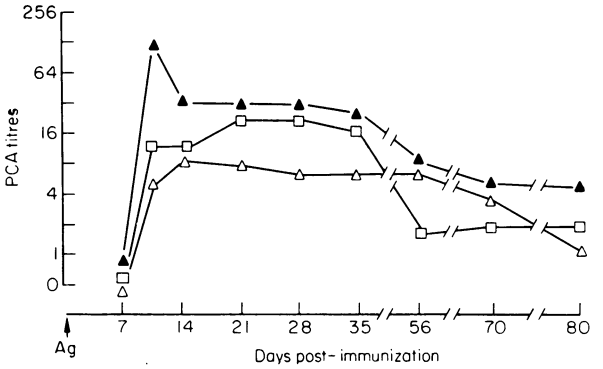

FIG. 2. Mean reaginic antibody titres after a single intraperitoneal injection of 0·1 mg of OA in $Al(OH)_3$ gel in six BN rats (▲), as compared to six BN rats immunized with 0·01 mg of OA in $Al(OH)_3$ gel (□), and six BN rats immunized with 0·001 mg of OA in $Al(OH)_3$ gel (△), as measured by the 48-hour PCA reaction and expressed as the reciprocal of the titre. Ag=antigen administration.

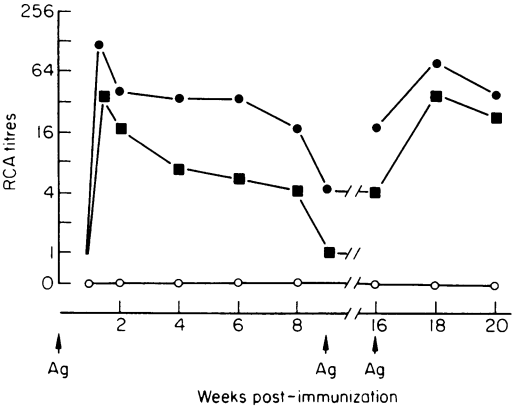

FIG. 3. Mean reaginic antibody titres in six BN (●), six Wistar (■), and six ACI inbred rats (○) after an initial intraperitoneal injection of 0·1 mg of OA in $Al(OH)_3$ gel, followed by two additional injections of 0·1 mg of OA in $Al(OH)_3$ gel, as measured by the 48-hour PCA reaction and expressed as the reciprocal of the titre. Ag=antigen administration.

declined at 9 weeks to 1:5 and 1:1 respectively. At 9 weeks, and again at 16 weeks 0·1 mg of OA in $Al(OH)_3$ gel was administered to these animals. Measurement of serum reagins, subsequent to this additional administration of antigen, disclosed elevated levels of mean reaginic antibody titres at 18 weeks of 1:100 in BN, and 1:50 in outbred Wistar rats. Those inbred ACI rats which were immunized at 0, 9, and 16 weeks with OA in $Al(OH)_3$ gel failed to generate detectable reagins by 20 weeks after the administration of OA. The persistence of reagin synthesis could be demonstrated for at least 20 weeks in both the inbred BN and outbred Wistar animals (Fig. 3).

There were no statistical differences ($P>0{\cdot}5$, Student's t-test) between the reagin antibody titres observed in the male and female animals of the inbred BN, F344, and outbred Wistar strains after immunization with 0·1 mg of OA in $Al(OH)_3$ gel (Fig. 4).

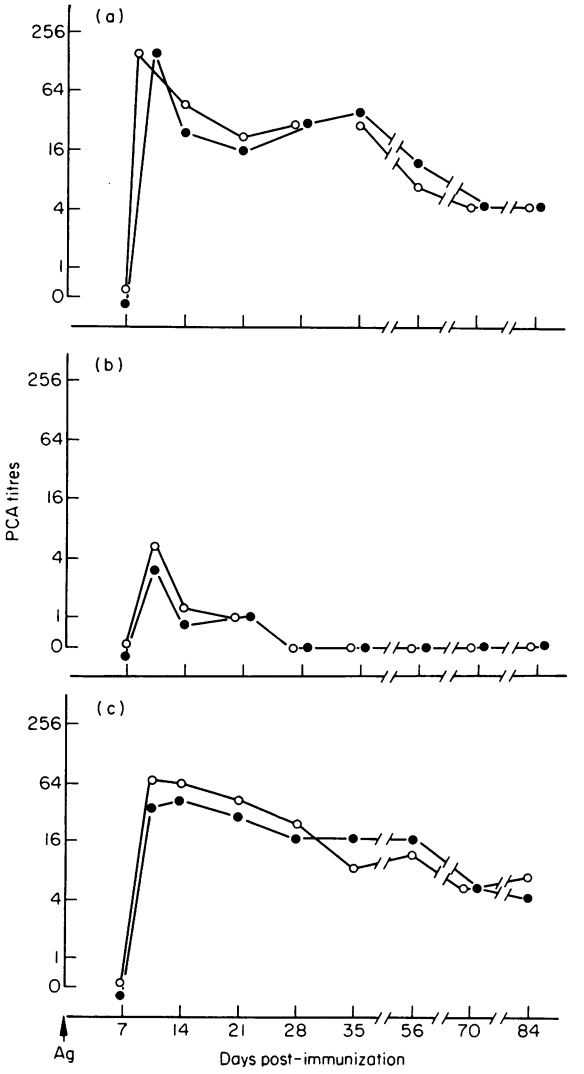

FIG. 4. Mean reaginic antibody titres in males (●) and females (○) after a single intraperitoneal injection of 0·1 mg of OA in $Al(OH)_3$ gel, as measured by the 48-hour PCA reaction and expressed as the reciprocal of the titre in (a) BN, (b) F344 and (c) Wistar rats. Ag=antigen administration.

Not only did the male and female animals produce peak reagin antibody on approximately the same day but the antibody persisted for a similar duration in both sexes.

The 2-hour PCA titres for both BN and outbred Wistar rats following immunization with 0·1 mg of OA in Al(OH) gel are shown in Fig. 5. The inbred BN and outbred Wistar rats responded similarly with maximum mean antibody titres of 1/100 demonstrable by

9 days after immunization. The 2-hour PCA reaction to the same antigen differed in the ACI strain, where a mean maximum titre of 1/20 was not observed until 21 days after immunization. The 2-hour PCA antibody activity then persisted at approximately the same level in all three groups of rats for at least 70 days. In contrast, mean haemagglutinating antibody concentrations were comparable in the BN, ACI and F344 inbred strains, as well as the outbred Wistar rats. All strains of rats, including the poor reagin producing

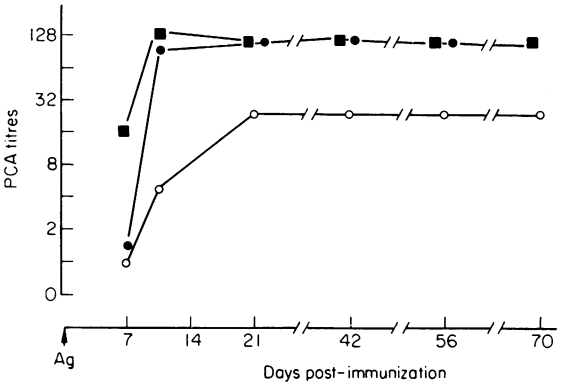

FIG. 5. Mean IgGa-like antibody titres in BN (●), Wistar (■) and ACI (○) rats after a single intraperitoneal injection of 0·1 mg of OA in $Al(OH)_3$ gel, as measured by the 2-hour PCA reaction and expressed as the reciprocal of the titre. Ag=antigen administration.

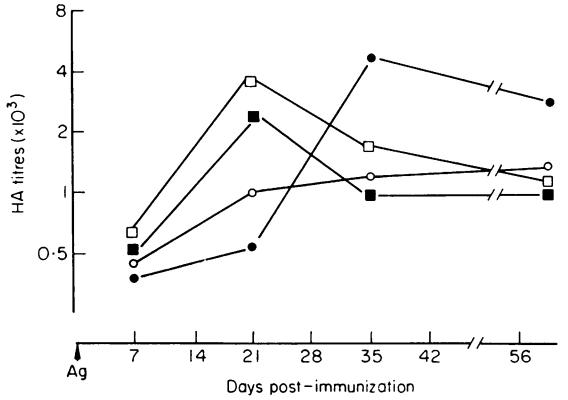

FIG. 6. Mean haemagglutinating (HA) antibody titres in BN (●), F334 (□), Wistar (■) and ACI (○) rats after a single intraperitoneal injection of OA in $Al(OH)_3$ gel, as measured by passive haemagglutination and expressed as the reciprocal of the titre. Ag=antigen administration.

ACI strain synthesized serum haemagglutinating antibodies in titres of 1:1000 or greater within 35 days after a single immunization with 0·1 mg of OA in $Al(OH)_3$ gel (Fig. 6).

Reaginic antibody binding avidity estimated for inbred BN rats is summarized in Table 1. A 1000-fold reduction in the amount of OA challenge dose administered to recipient Wistar rats passively sensitized with serum (mean titre 1:120) obtained from BN rats 9 days after immunization completely abolished the 48-hour PCA reaction. In distinction,

the 48-hour PCA reaction in animals sensitized with serum (mean titre 1:100) obtained from BN rats which received an initial immunization, as well as two subsequent injections of OA, was unchanged.

TABLE 1

BINDING AVIDITY OF REAGINIC ANTIBODY TO OVALBUMIN (OA) IN BN RATS

Immunogen dose 0·1 mg of OA	Antisera obtained (day)	Amount of OA used to elicit 48-hour PCA reaction		
		200 mg	2 mg	0·2 mg
0·1	9	3–4+*	1+	0
0·3 (0·1 × 3)	112	3–4+	3+	3+

* Intensity of 48-hour PCA reaction in millimetres of blueing: 1+ = 5 mm; 2+ = 6–10 mm; 3+ = 11–15 mm; 4+ = 15 mm.

DISCUSSION

The present experiments have demonstrated that strain differences for reaginic antibody production exist in the rat. Inbred BN rats synthesized reaginic antibody similarly to outbred Wistar rats, whereas inbred ACI and F344 rats were deficient in their reaginic antibody responses after immunization with the identical antigen. A single intraperitoneal injection of OA incorporated in $Al(OH)_3$ gel as an adjuvant elicited reaginic antibodies in the BN rats with titres similar to those observed by Clausen in outbred rats which received saline extracts of *Bordetella pertussis* organisms as an adjuvant; however, reagins elicited in BN rats with $Al(OH)_3$ gel as an adjuvant persisted for longer periods of time (70 days) in comparison to 14–21 days when *Bordetella* extracts were utilized as the adjuvant (Clausen, Munoz and Bergman, 1969; Clausen, Munoz and Bergman, 1970). Bloch and co-workers have described a potentiated reaginic antibody response specific for OA when outbred rats were immunized with OA in $Al(OH)_3$ gel and subsequently infected 14 days later with *Nippostrongylus brasiliensis* (Bloch, Ohman, Waltin and Cygan, 1973). They documented the maximum reaginic antibody response 14 days after parasitic administration, which rapidly decreased and reached minimal levels 25 days after infection (Bloch *et al.*, 1973). Additional intraperitoneal injections of OA in $Al(OH)_3$ gel into the Wistar and BN rats in the present study boosted and sustained elevated levels of reaginic antibody, similar to that reported in the mouse with the use of $Al(OH)_3$ gel adjuvant (Levine and Vaz, 1970), but not previously described in outbred rats which received Freund's adjuvant, *Bordetella* extracts, or parasites (Mota, 1964; Clausen, Munoz and Bergman, 1969; Clausen *et al.*, 1970; Bloch *et al.*, 1973).

Previous studies in the mouse have demonstrated that single 0·001 mg antigen immunization produced lower titres of reaginic antibody in comparison to repeated low dose immunization (0·0001 mg), in which a six-fold selective increase of reagin titres were found (Levine and Vaz, 1970). In the present experiment, when the immunizing dose of OA was lowered from 0·1 mg to either 0·01 or 0·001 mg, subsequent mean reagin titres were comparable or slightly lower than those obtained when immunization was carried out with a relatively high dose of antigen. This suggests that in the BN strain of rats, the

threshold for recognition of OA, at least when incorporated in $Al(OH)_3$ gel and administered intraperitoneally, is quite low; yet, maximal reagin titres do not vary greatly with changes in the strength of the dose employed for immunization. Sera obtained from the BN strain following repeated intraperitoneal administration of the antigen demonstrated that the reagins present in boosted sera had a greater estimated avidity for OA than did the reagins present in sera after a single immunization of OA.

Previous studies of rat reaginic antibody synthesis after immunization with various antigens have not evaluated the role of sex in the immune response (Orr *et al.*, 1972; Stechschulte *et al.*, 1967; Orange *et al.*, 1970; Bloch *et al.*, 1973). In contrast to inbred strains of mice in which female animals produced greater amounts of reaginic antibody than their male counterparts after immunization with hapten–protein conjugates (Levine and Vaz, 1970; Vaz *et al.*, 1971a, b; Levine and Chang, 1971;), both male and female BN rats produced similar amounts of reagins after immunization with OA in $Al(OH)_3$ gel. This suggests that in the BN rat reaginic antibody production is controlled by autosomal genetic factors, in distinction to genes associated with, or located on the female X chromosome. Gill and co-workers have documented that within certain rat strains, at least two independently segregating genes are responsible for the full expression of IgG antibody synthesis following immunization with synthetic polypeptides (Gill *et al.*, 1970; Gill and Kunz, 1971; Gill *et al.*, 1971), but similar findings have not yet been observed for reaginic antibody.

The development of non-reaginic, as well as reaginic antibody specific for OA concurrent with each bleeding obtained after a single immunization with 0·1 mg OA in $Al(OH)_3$ gel in the BN rats and Wistar rats demonstrates the heterogeneity of the immune response. These data are in agreement with previous reports of both inbred strains of mice and rats when hapten–protein conjugates were employed as the antigen (Vaz *et al.*, 1971b; Gill *et al.*, 1970; Stechschulte *et al.*, 1970). In spite of an inability to synthesize detectable reaginic antibody, inbred ACI rats produced comparable levels of non-reaginic antibody to that found in inbred BN, F344, and outbred Wistar rats following a single intraperitoneal injection of OA in $Al(OH)_3$ gel as measured by passive haemagglutination. The production of IgGa antibodies, as measured by the 2-hour PCA reaction in the ACI inbred strain of rats was detectable but not of the same magnitude as that found in either inbred BN or outbred Wistar rats.

Repeated inbreeding of the BN and ACI stock in our animal facility has established that both of these inbred strains breed true in respect to both reaginic and non-reaginic antibody synthesis following intraperitoneal immunization with OA in $Al(OH)_3$ gel. Thus, another animal model is now available to the investigator to study the genetic predilection for reaginic antibody synthesis that is also observed in man. Further speculation with regard to the genetic mechanisms operative in the synthesis of reaginic antibody in the inbred strains of rats reported here must await further experimentation.

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