

Effect of specific IgG on IgE-induced systemic anaphylaxis in the rabbit*

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Summary. Immunization of rabbits as neonates and periodically thereafter has been shown to induce the long-term preferential production of specific IgE antibodies. Specific IgG antibodies are not detected in the majority (greater than 70%) of rabbits when classical immunological detection techniques are used, including heterologous PCA in guinea-pig skin. Nevertheless, in this study we demonstrate that all rabbits neonatally immunized to the antigen horseradish peroxidase (HRP) do produce low levels of specific IgG antibody detectable by an ELISA technique. Serum levels of anti-HRP IgG were found to be log normally distributed, with a geometric mean for the heterologous PCA-negative sera of $31.6 \times / \div 2.69 \mu\text{g/ml}$. Serum anti-HRP IgE levels ($\log_2$ homologous PCA titres) are bimodally distributed. Specific IgG and IgE levels in individual rabbits have a significant direct relationship. Six heterologous PCA-negative and seven heterologous PCA-positive rabbits were challenged intravenously with HRP. All of the respiratory and circulatory alterations typical of IgE anaphylaxis occurred in every challenged rabbit. Regression analysis of percentage changes in the physiological

variables *vs* log specific IgE level indicated that none of the changes was either directly or inversely related to the specific IgG levels. Also the mean changes of the heterologous PCA-positive *vs* negative rabbits did not differ significantly. Thus, we could find no evidence for either a blocking or enhancing effect of the specific IgG antibodies (range 10–529 $\mu\text{g/ml}$ serum) on the IgE-induced anaphylactic reaction.

INTRODUCTION

Two immunological pathways which lead to systemic anaphylaxis in the rabbit have been described. One route, initially characterized by Jackson (1935), involves the intravenous challenge of rabbits producing high levels of serum antibody (induced by repeated intraperitoneal or intravenous immunization of adult rabbits). Severe reactions require precipitating antibody levels in the mg/ml range. Administration of precipitated antigen-antibody complexes to non-immune rabbits can mimic this type of anaphylaxis, referred to as 'aggregate anaphylaxis' (McKinnon *et al.*, 1957). These workers linked the pathophysiology to the sequestration of insoluble complexes in the pulmonary and other vascular beds. Schachter (1953) demonstrated that histamine was released following antigen challenge in rabbits sensitized in a similar way to those of Jackson's study. However, whether homocytotropic antibodies are also produced in this type of immunization schedule is not known.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; i.m., intramuscularly; i.p., intraperitoneally; PBS-Tween 20, 0.12 M phosphate in 0.15 M saline containing 0.05% Tween-20 and 0.5% gelatin; PCA, passive cutaneous anaphylaxis; s.c. subcutaneously.

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The second form of anaphylaxis is thought to be triggered by antigen combining with cell-bound IgE antibodies. Anaphylaxis was shown by Spitzer *et al.* (1973) to occur in rabbits which had detectable serum levels of specific IgE and IgG antibodies, but not in those with only IgG of comparable (and relatively low) levels. Intravenous antigen challenge also induces systemic anaphylaxis in rabbits neonatally immunized which have demonstrable IgE but not specific antibody of other classes when tested by the Farr antigen-binding capacity, passive haemagglutination, Ouchterlony analysis and heterologous passive cutaneous anaphylaxis (PCA) (Pinckard, Halonen & Meng, 1972; Halonen *et al.*, 1976).

Clinical signs of the two forms of anaphylaxis are similar (McKinnon *et al.*, 1957; Halonen, Pinckard & Meng, 1973) and both types can be lethal. In rabbits sensitized in a similar way to those in the Jackson study, Karczewski & Widdicombe (1969) found that the physiological alterations of anaphylaxis include an increase in pulmonary resistance, a decrease in dynamic compliance, an increase in right atrial pressure, bradycardia and systemic hypotension. All of these alterations also occur in IgE anaphylaxis (Halonen *et al.*, 1976), but a comparison of the immunological trigger(s) cannot be made in these two models since the levels of specific IgG and/or IgE antibodies in the Karczewski & Widdicombe study were not assessed. To add to the immunological complexity, Henson & Cochrane (1968) and Stux & Ovary (1976) have reported that adult rabbits immunized with high dose antigen in complete Freund's adjuvant also produce homologous skin-sensitizing IgG antibodies (in addition to IgE antibodies), thus providing another potential immunological participant in the induction of anaphylaxis in this species.

Even if the circulating IgG antibodies are not of the skin-sensitizing type and are not present in sufficiently high levels to lead to aggregate anaphylaxis, it is still conceivable that antibodies of the IgG isotype could modulate IgE-induced anaphylaxis in either a positive or negative direction via complement activation, stimulation of other cells or simply by competing for antigen. Thus, the first objective of the present study was to develop a quantitative enzyme-linked immunosorbent assay (ELISA) in order to examine more rigorously the neonatally immunized, IgE-producing rabbits with respect to whether IgG antibodies were present at levels below the threshold of the assays previously used. Secondly, we sought to determine whether variation in the level of specific IgG had a

detectable effect on the physiological alterations of anaphylaxis induced in the IgE-producing rabbits.

MATERIALS AND METHODS

Animals and immunizations

Randomly bred California rabbits of both sexes were sensitized so as to produce IgE antibodies specific for horseradish peroxidase (HRP: Type II, Sigma Chemical Co., St Louis, MO) by an immunization schedule previously reported (Pinckard *et al.*, 1972; Halonen *et al.*, 1976) which is begun within 24 h of birth. Four adult New Zealand White rabbits were immunized to produce high titres of IgG antibodies to HRP, and sera were collected to make a reference pool for calibration of the anti-HRP IgG ELISA. HRP (2 mg/ml in saline) was mixed with 1 ml of complete Freund's adjuvant (Gibco Laboratories, Grand Island, NY) and was injected in two s.c. and two i.m. sites. Two booster injections in incomplete Freund's adjuvant were given monthly, and rabbits were bled weekly during Weeks 8–12. All sera which tested positive for precipitation (0.1 ml serum mixed with 0.1 ml of 120 µg/ml HRP) were pooled. Non-immunized 2–3 kg California rabbits were used as recipients for homologous PCA assays. All rabbits were purchased from Olson's Blue Ribbon Ranch, Tucson, AZ, and neonatally immunized rabbits were maintained there until 84 days of age.

Hartley guinea-pigs of both sexes (350–600 g), purchased from Southwest Research Animals, Tucson, AZ, were used to test for HRP-specific serum IgG antibodies by heterologous PCA.

Homologous and heterologous PCA

Circulating levels of anti-HRP IgE were semi-quantitatively assessed by homologous PCA and anti-HRP IgG antibodies by heterologous PCA as previously described (Halonen *et al.*, 1976). For purposes of clarity, we should emphasize that rabbit IgG is capable of sensitizing guinea-pig skin, but rabbit IgE is not (e.g. Stux & Ovary, 1976).

Modified antigen-binding capacity

This procedure was performed on the ELISA reference serum pool (see below) to provide a weight estimate of the anti-HRP antibody in the pool, and thus allow us to quantify the ELISA assay. The procedure was that described by Mulligan, Osler & Rodriguez (1966), except that we used goat anti-rabbit

IgG, whole molecule-specific (Pel Freez Biologicals, Rogers, AR), to precipitate the HRP-anti-HRP complexes, since 50% ammonium sulphate precipitates uncomplexed HRP. Minden, Farr & Trembath (1975) established the comparability of these two precipitating techniques. We also used 100- μ l rather than 1-ml volumes, and the diluent was borate buffer containing 1% and 10% normal rabbit serum for antigen and antiserum, respectively (rather than 20%). Increasing amounts of ^{125} I-labelled HRP were added to aliquots of the serum pool diluted 1/810, and the antigen bound for each antigen concentration was calculated according to the method of Farr (1958). A plot of HRP bound vs amount of HRP added yielded a typical saturable binding curve. From the double reciprocal plot of these values, the y intercept of the least squares regression line represented the reciprocal of the antigen bound at infinite antigen concentration. Assuming that antibody binds two molecules of antigen and has a molecular weight of 160,000, the antibody concentration of the reference serum pool was 3.9 and 4.0 mg/ml anti-HRP antibody in two separate assays.

ELISA reagents

ELISA assays were performed in 96-well flat-bottomed EIA Microtitration plates (Linbro, Flow Laboratories, McLean, VA). Antigen (HRP) was diluted in 0.027 M carbonate buffer, pH 9.6, containing 0.02% sodium azide (prepared within 24 hr of use). The diluent for the test and reference sera and the conjugate contained 0.12 M phosphate (pH 7.2), 0.85% sodium chloride and 0.5% gelatin. Plates were washed three times between reagent incubations with the phosphate-buffered saline diluent containing 0.05% Tween-20 (PBS-Tween) dispensed and aspirated with a Nunc-Immuno Wash 8 (Gibco). Three second antibody conjugates were initially compared: alkaline phosphatase-conjugated, affinity purified, goat anti-rabbit IgG, Fc-specific (Pel Freez Biologicals); alkaline phosphatase-conjugated, affinity isolated, goat anti-rabbit IgG, whole molecule-specific (Sigma), and Protein A conjugated to alkaline phosphatase (Seragen, Boston, MA). The substrate used throughout was 1 mg/ml disodium *p*-nitrophenyl phosphate (Sigma) dissolved in 1.0 M ethanolamine, pH 9.8, containing 5.9 mM Mg^{2+} . This buffer was freshly prepared every month.

ELISA procedure

The procedure is based on that of Engvall & Perlmann

(1972). Wells were coated with 100 μ l of 100 μ g/ml HRP (optimal concentration determined by checker-board titration). The plates were incubated for 1 hr at room temperature (RT) and overnight at 4°, washed and then stored at 4° for up to 60 days. Quadruplicate 100- μ l aliquots of serial 10-fold dilutions of sera were added to the antigen-coated wells and incubated for 2 h at RT. Each plate contained dilutions of a normal rabbit serum pool as negative control. Dilutions of the reference pool were included in every batch. Every plate was set up in duplicate. After incubation, plates were washed with PBS-Tween at 4°. Conjugated antibody (diluted as recommended) was added to the plates (100 μ l) and incubated at 4° overnight. Plates were washed and allowed to dry for 30 min. Substrate (100 μ l at RT) was added to each well and incubated at RT for 30 min. The plates were read at 405 nm on a Dynatech ELISA Reader (Alexandria, VA), the use of which was kindly provided by Dr Paul Finley, Department of Pathology, Arizona Health Sciences Center, AZ.

While establishing optimal assay conditions, we tested the activity of three different second antibody preparations, each conjugated to alkaline phosphatase. The concentration-absorbance curve for our reference anti-HRP pool (see below and Fig. 1) was shifted to the right by 50-fold when conjugated protein A was used as a second 'antibody' compared to goat anti-rabbit IgG Fc. Increasing the concentration of

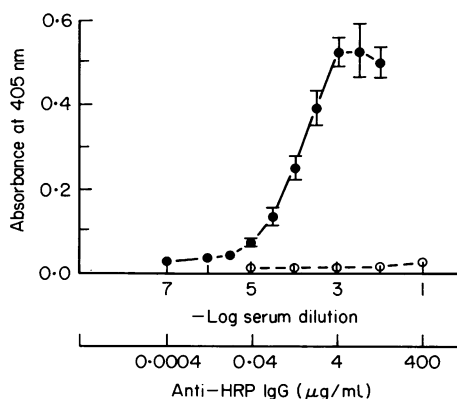


Figure 1. Log concentration-absorbance curves for ELISA reference pool (●) and normal rabbit serum (○). Points represent mean of 15 separate assays, error bar = SEM. Lower abscissa provides quantitative calibration based on a concentration of 4.0 mg/ml anti-HRP antibody in the pool (see Materials and Methods). The threshold of detectable anti-HRP IgG (arbitrarily defined as three times the absorbance of normal rabbit serum) is 40 ng/ml.

conjugated Protein A did not produce a significant change. Adsorption of a serum sample containing anti-HRP IgG with protein A-Sepharose (Pharmacia, Piscataway, NJ) removed all detectable activity when assayed with either the Fc-specific or Protein A conjugate. Thus, both conjugates detected anti-HRP IgG, but the protein A conjugate was less efficient. Goat anti-rabbit IgG (whole molecule) yielded essentially the same curve (in both slope and maximum) as that obtained with the Fc specific preparation, except that a reduction in absorbance (*vs* a plateau with the Fc specific antibody) was observed at high concentrations (dilutions less than 10^{-3}) of the reference serum. The alkaline phosphatase-conjugated goat anti-rabbit IgG Fc was used routinely for the testing of all sera in this study.

Calibration of ELISA

The concentration-absorbance curve for our reference pool prepared from sera of rabbits hyperimmunized to HRP is illustrated in Fig. 1. Modified antigen-binding capacity assay yielded a value of 4.0 mg/ml anti-HRP antibody for this pool. Based on this value, the second (lower) abscissa in Fig. 1 quantifies the ELISA and demonstrates that the threshold of sensitivity for the assay (arbitrarily defined as three times the absorbance of normal rabbit serum) is approximately 40 ng/ml anti-HRP IgG. Each test sera from the neonatally immunized rabbits was quantified by determining the serum dilution giving an absorbance equivalent to 25% of the maximum absorbance for the reference pool, and then multiplying the reciprocal dilution by the anti-HRP IgG concentration of the reference pool at that 25% maximum absorbance point. This point of 25% maximum absorbance was arbitrarily selected for internal consistency. Binding curves for test sera often had more shallow slopes than that for the reference pool. Since variation in slope probably reflects variation in antibody affinity, the quantitative determination of antibody levels in this study must be regarded as approximate.

Physiology studies

By methods previously reported (Halonen *et al.*, 1980), we monitored changes in the following physiological variables before and after intravenous antigen (200 μ g HRP) challenge of immunized rabbits: aortic and right ventricular pressure, heart rate, breathing frequency, airflow, tidal volume, transpulmonary pressure, dynamic lung compliance and total pulmonary resistance. The point of maximum and minimum

change was determined for each variable, and the percentage change from prechallenge baseline calculated. Circulatory measurements were made by averaging the beats over one breathing cycle (minimum of six beats), and respiratory measurements were averages of six consecutive breaths.

Statistical analyses

All methods are described by Richmond (1964). Geometric mean-specific IgG levels in 3- and 4-month-old rabbits were compared by the Student's *t*-test (unpaired data), as were group means for maximal changes in the physiological alterations (with $P < 0.05$ considered significant). Frequencies of positive heterologous PCA sera from the two age groups were compared by Chi Square with Yates' correction factor. Regression analyses were performed by the method of least squares, plotting the percentage change in each physiological variable *vs* serum level of specific antibody.

RESULTS

Specific IgG levels in neonatally immunized rabbits

The distribution of anti-HRP IgG levels, quantified by ELISA, in 27 sera from neonatally immunized rabbits aged 81–99 days is displayed in Fig. 2. The values were found to be log normally distributed with a geometric mean of $42.7 \times / \div 3.16 \mu\text{g/ml}$ (range 6–424 $\mu\text{g/ml}$). Six of the 27 sera were positive for heterologous PCA. The

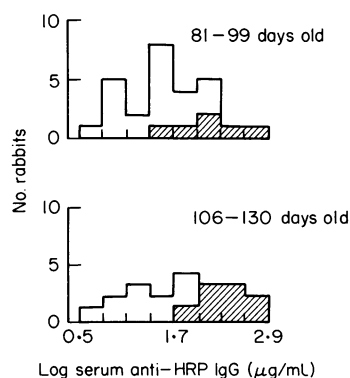


Figure 2. Distribution of specific serum IgG concentrations for two age groups of neonatally immunized rabbits. Sera were assayed for anti-HRP IgG by ELISA. Open bars show sera that has no detectable specific IgG by heterologous PCA; hatched bars represent heterologous PCA-positive sera.

21 heterologous PCA-negative sera had a geometric mean of $31.6 \times / \div 2.69 \mu\text{g/ml}$ (range 6–129 $\mu\text{g/ml}$).

Three months is the age at which we have routinely used these rabbits for our previous studies of anaphylaxis (e.g. Pinckard *et al.*, 1972; Halonen *et al.*, 1976). In order to determine the relative stability of the induced IgG response, we similarly assayed anti-HRP IgG levels in the sera of 20 rabbits aged 106–130 days (Fig. 2). The geometric mean level was $61.7 \times / \div 3.80 \mu\text{g/ml}$ (range 6–529 $\mu\text{g/ml}$). Nine of the 20 were positive for heterologous PCA. Although values for this group appear somewhat higher, neither the mean level specific IgG nor the frequency of sera positive for heterologous PCA differed significantly in the two age groups.

Figure 2 also portrays the (ELISA-assessed) threshold level of anti-HRP IgG detected in the heterologous PCA assay. The serum with the lowest specific IgG concentration positive for heterologous PCA was 38 $\mu\text{g/ml}$ (17 heterologous PCA negative sera had lower values). Eight out of 23 sera (35%), with values between 38 and 200 $\mu\text{g/ml}$, were positive for the heterologous PCA assay. The seven sera with specific IgG greater than 200 $\mu\text{g/ml}$ were all heterologous PCA-positive. This threshold for the heterologous PCA assay is substantially higher than the 0.2 $\mu\text{g/ml}$ value originally reported by Ovary (1964). In order to determine if this difference could be attributed to competition for skin fixing sites by non-HRP-specific IgG present in the serum, we compared the heterologous PCA titre obtained with saline *vs* normal rabbit serum dilutions of our ELISA reference pool (concentration 4 mg/ml anti-HRP IgG). The heterologous PCA titre for the saline-diluted pool was 1600 (equivalent to a threshold sensitivity of 2.5 $\mu\text{g/ml}$ anti-HRP IgG), whereas the titre of the serum-diluted pool was 20 (equivalent to a threshold of 200 $\mu\text{g/ml}$). Thus, the threshold concentration of specific IgG for the heterologous PCA assay varied with the amount of competing non-HRP-specific IgG present, as has been previously reported by Biozzi, Halpern & Binaghi (1959).

Specific IgE levels in neonatally immunized rabbits

Specific IgE levels were determined by homologous PCA titre in the sera of the 81–99-day-old rabbits and were found to distribute bimodally, with 22% of the rabbit sera in one peak at titres of 0–1 and a second peak (30%) at a titre of 16 (Fig. 3). The 106–130-day-old rabbits showed a similar bimodal distribution, with the second peak at a titre of 32. Regression

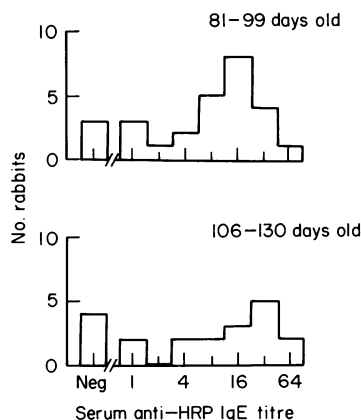


Figure 3. Serum anti-HRP IgE levels in neonatally immunized rabbits. The distribution of homologous PCA titres in the 27 3-month-old rabbits is bimodal, with one peak between 0 (undetectable)–1 and a second peak at 16. The 20 4-month-old rabbits showed a similar bimodal distribution, with the second peak at 32 rather than 16.

analysis revealed a significant direct relationship between specific IgG and IgE levels in individual sera (Fig. 4).

Effect of specific IgG antibody on IgE-induced systemic anaphylaxis

We first compared the seven rabbits with heterologous PCA-negative sera (Group 1) to six heterologous PCA-positive rabbits (Group 2) with respect to the group mean respiratory and circulatory alterations which accompanied anaphylaxis. Log mean ELISA values were $29.5 \times / \div 2.51$ and $295 \times / \div 1.66 \mu\text{g/ml}$ anti-HRP IgG for Groups 1 and 2, respectively. All six

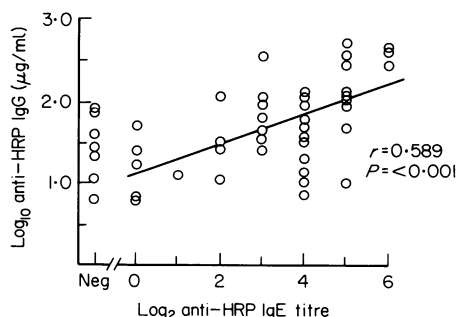


Figure 4. Regression analysis of specific IgG *vs* specific IgE in neonatally immunized rabbits. IgE titres were determined by homologous PCA, and IgG levels were quantified by ELISA in the sera of 47 rabbits.

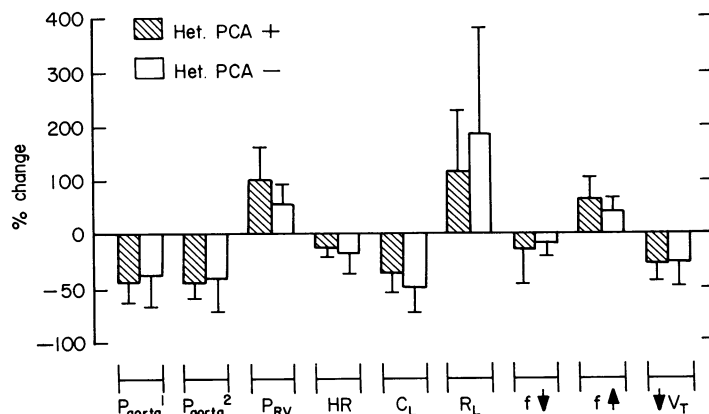


Figure 5. Effect of specific IgG on physiological alterations of IgE anaphylaxis. Six rabbits heterologous PCA-positive for anti-HRP IgG and seven heterologous PCA-negative rabbits (all of which had detectable specific IgE antibodies levels) were challenged intravenously with a sublethal 200 μ g dose of HRP. Aortic and right ventricular pressures (P_{aorta} and P_{RV}), heart rate (HR), dynamic compliance (C_L), total pulmonary resistance (R_L), breathing frequency (f), and tidal volume (V_T) were monitored. Values are expressed as maximum percentage change from prechallenge baseline values (mean $\pm$ SD). Since aortic pressure showed two phases of hypotension, one peaking at less than 5 min (P_{aorta} 1) and the other after 5 min (P_{aorta} 2), each is treated as a separate change. Breathing frequency initially increased (in concert with a decrease in tidal volume) and later decreased (representing brief apnoea in some rabbits). None of the respiratory or circulatory alterations in heterologous PCA-positive rabbits differed significantly from those which occurred in heterologous PCA-negative rabbits. (Analysis is based on the Student's t -test for differences between means, unpaired data.)

heterologous PCA-positive rabbits were also positive when screened at a dilution of 1/32. Anti-HRP IgE levels (homologous PCA titres) ranged from 1 to 32 in Group 1 and from 4 to 64 in Group 2. Intravenous antigen challenge induced respiratory and circulatory alterations typical of anaphylaxis in every rabbit. Mean maximum percentage change values induced by the 200 μ g HRP challenge were lower than those previously obtained with 5 mg HRP challenge (Halonen *et al.*, 1980) (except for the brief increase in breathing frequency and decrease in tidal volume), indicating that the values were on the ascending portions of the antigen concentration-response curves for the monitored variables. None of the mean maximum percentage changes were significantly different for the two groups (Fig. 5). Linear regression analyses of percentage change in each variable *vs* log anti-HRP IgG concentration for the 13 rabbits also supported the lack of a role for IgG modulating the anaphylactic response over this range of IgG concentrations (10–529 μ g/ml). None of these analyses yielded a significant correlation coefficient. Plots for pulmonary resistance and dynamic compliance *vs* anti-HRP IgG concentration are shown as examples of these analyses in Fig. 6. Similar regression analyses

of percentage change in the physiological variables *vs* serum anti-HRP IgE titre also yielded insignificant correlation coefficients.

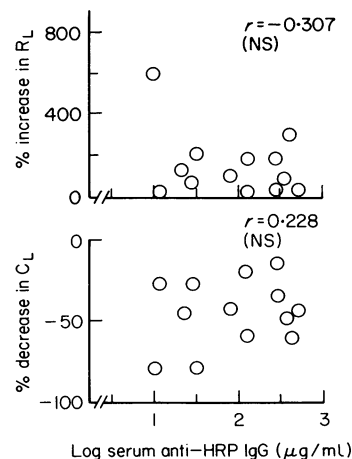


Figure 6. Examples of regression analyses for the anaphylactic physiological alterations *vs* specific IgG. No significant correlation is detectable between the percentage increase in pulmonary resistance or the percentage decrease in dynamic compliance (occurring after intravenous challenge with HRP) and the specific IgG level in the 13 challenged rabbits.

DISCUSSION

We have previously reported (Pinckard *et al.*, 1972), and others have confirmed (Shampain *et al.* 1982), that intraperitoneal immunization of rabbits, if begun in the neonatal period, leads to the long-term preferential synthesis of specific IgE antibodies. However, specific antibodies of other isotypes were not detectable by Farr antigen-binding capacity, heterologous PCA, passive haemagglutination or precipitin analyses in greater than 70% of the neonatally immunized rabbits (Pinckard *et al.*, 1972; Halonen *et al.*, 1976). Nevertheless, in this study we have demonstrated that IgG antibodies are produced in the neonatally immunized rabbits at levels below the sensitivities of the previously employed assays. Thus, these rabbits are hyporesponsive but not completely unresponsive to antigen with respect to production of the IgG isotype.

The antibodies detected by homologous PCA in this neonatal immunization model were previously characterized as belonging to the IgE immunoglobulin class based on (i) elution from DEAE at 0.1 M NaCl, pH 8.0, (ii) loss of activity following reduction and alkylation, (iii) variable (partial to complete) lability to heating for 4 hr at 56°, and (iv) molecular weight estimation of 240,000 by molecular sieve chromatography (Pinckard *et al.*, 1972). They differ from homologous skin-sensitizing IgG antibodies in all of these respects (Stux & Ovary, 1976).

Anaphylaxis occurs in the neonatally immunized rabbits when challenged intravenously with antigen at 3 months of age (e.g. Pinckard *et al.*, 1972). This anaphylaxis has been attributed to IgE antibodies bound to mast cells and/or basophils based on several lines of evidence. Benveniste, Henson & Cochrane (1972) demonstrated that, even in adult immunized rabbits, antigen-induced basophil degranulation and mediator release from washed blood cells were IgE-dependent events: anti-IgE, but not anti-IgG, could similarly induce degranulation and mediator release from these cells. *In vivo*, following intravenous antigen challenge of neonatally immunized rabbits, circulating basophils disappeared within 2 min of antigen challenge, and histamine and platelet activating factor were released into the plasma (Pinckard, Farr & Hanahan 1979; Halonen & Kaliner, 1984). In adult immunized rabbits, Spitzer *et al.* (1973) reported that, among rabbits with similar levels of anti-HRP IgG antibodies, anaphylaxis occurred only in those which had demonstrable specific IgE antibodies. Thus, although ultimate demonstration that IgE is the

necessary and sufficient trigger of anaphylaxis awaits the transfer of purified IgE to normal rabbits, a preponderance of indirect evidence strongly implicates IgE as the trigger of anaphylaxis in IgE-producing rabbits. The findings of this study add to this evidence by demonstrating that no significant role could be detected for specific IgG either negatively or positively modulating the circulatory or respiratory alterations which occur during anaphylaxis in rabbits with specific IgG levels varying over a 50-fold range. These results contrast with those of Shampain *et al.* (1982) who found that the presence of specific IgG tended to inhibit the lung mechanical alterations induced by aerosol-administered antigen in IgE-producing rabbits, despite higher IgE levels in the IgG-positive *vs* IgG-negative rabbits (assessed by heterologous PCA). Although heterologous PCA titres with different antigens may not be directly comparable, the rabbits in our heterologous PCA-positive group had titres at least as great as did those in the study of Shampain *et al.* (1982). Thus, the inhibitory effect of IgG in their study may indicate that actively synthesized IgG in the rabbit is a more effective blocking antibody for antigen delivered by aerosol than for antigen administered intravenously.

It is also evident that IgE titres do not correlate with the severity of respiratory and/or circulatory alterations. As the log IgE titre is directly correlated with the log IgG concentration in these rabbits, we cannot completely rule out the possibility of concurrent opposing effects of increasing concentrations of these isotypes (enhancement by one and inhibition by the other) on the responses. It seems more likely, however, that even a low level of circulating IgE reflects adequate sensitization of cells, and that the levels of IgG in this study were not capable of eliminating antigen prior to its reaching the sensitized cells. This interpretation is supported by the *in vitro* studies of May *et al.* (1972) and Schumacher & Jeffery (1979), demonstrating little to no inhibition of mediator release from allergic human donor leucocytes by the presence of autologous or heterologous sera, despite the presence of antigen-binding activity in the sera. Although others have reported inhibition of mediator release by autologous sera (e.g. Lichtenstein & Osler, 1966), their studies involved incubation of serum and antigen *prior* to the addition of cells—a protocol which does not simulate the intravascular antigen challenge.

In this study, we have characterized more fully the rabbit neonatal immunization model as a model for studying IgE reactions *in vivo* without the complicat-

ing presence of other participating homologous skin-sensitizing antibodies and only low levels of IgG antibodies that do not sensitize homologous skin. No effect of these IgG antibodies on the physiological alterations of anaphylaxis was detectable. Some recent evidence from studies in guinea-pigs implies that the antigen dose required and the mediators released may be different in reactions triggered by IgE vs IgG₁ homocytotropic antibodies (Andersson, 1980; Regal, 1984; Udem, Buckner & Graziano, 1984). Further *in vitro* and *in vivo* studies with models that have one or the other of these antibodies may well provide a much clearer understanding of the pathways by which immediate hypersensitivity reactions occur.

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