

# Eosinophil leukocyte responses and hypersensitivity reactions in the *Bordetella pertussis*-treated mouse

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*Studies were concerned with an evaluation of hypersensitivity as one factor in the emergence of eosinophilia from antigen-antibody complex formation. Investigative approaches combined two experimental procedures in susceptible CFW strain mice: (1) Bordetella pertussis treatment to effect models of histamine-serotonin sensitivity and anaphylactic responsiveness and (2) sensitization and intraperitoneal challenge with egg albumin (EA) to induce local eosinophil responses in cell populations sampled during the resultant inflammatory process. Groups of 10 animals each were studied for these developments in varied modifications of the basic procedure. These experimental factors included single priming and multiple immunization injections, 4, 10, and 20 day sensitization schedules, and peritoneal fluid recovery at 6 hour and at 4 day periods after intraperitoneal antigenic challenge. In eight paired series there were no significant differences in quantitated eosinophilia between the control and B. pertussis-treated animals. Eosinophil cell responses could not be related to: the nonspecific leukocytosis of inflammation or lymphocyte-related leukocytosis of B. pertussis; techniques favorable for effecting histamine responsive states, anaphylactic manifestations, or adjuvant immunizing effects of the vaccine; levels of 2-mercaptoethanol (2-ME)-sensitive hemagglutinating antibody; or the presence of precipitins to EA. A role for reaginic antibody reactivity in experimental eosinophilia is suggested but requires further study.*

**T**he demonstration of eosinophil cell accumulations within involved tissue sites and secretions of allergic man occurs with sufficient frequency to document this association as a clinical pattern. In turn, a finding of eosinophilia is often accepted as a valid lead in the diagnostic study of suspected hypersensitivity states. Where eosinophil leukocytosis accompanies parasitic infections, it is considered likely that it emerged as a manifestation of hypersensitivity during a tissue-invasive stage of the helminth. For here, as in the atopic diseases, the development of eosinophilia is associated with demonstrated intracutaneous whealing responses to specific antigenic challenge (e.g., in trichinosis, schistosomiasis, echinococcosis, ascariasis) and the potential for systemic allergic reac-

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tivity (e.g., with the formation of echinococcus hydatid cyst fluid). Yet it is not clear whether the appearance of eosinophils in increased numbers may simply be a nonspecific sequel of immune reactions or whether an associated hypersensitivity response may be one critical requirement for the eosinotactic mechanism. Investigations based upon experimental animal models do not give sufficient evidence for definite delineation of this point. Our studies and the reports of other investigators<sup>1-3</sup> lend themselves only to an interpretation of eosinophil cell responses as a function of antigen-antibody union, e.g., formation of antigen-antibody complexes<sup>4-6</sup> or physicochemically related molecular aggregates<sup>5, 7-8</sup> and/or their disposition,<sup>9-11</sup> or to the effects of mononuclear cells sensitized by antigen during the immune process.<sup>12, 13</sup> Some studies<sup>14, 15</sup> have even indicated that not every antigen-antibody reaction may be accompanied by significant eosinophilia. On the other hand, there have been reported observations<sup>16-19</sup> suggesting a relationship between eosinophil leukocytes and chemical mediators that may be associated with allergic inflammatory processes.

In seeking an adaptable experimental animal for further study of this question, consideration of the *Bordetella pertussis*-treated mouse is of special interest and relevance. Though the mouse represents a species relatively refractory to histamine and anaphylactic shock, potential susceptibility for the induction of these pathophysiologic phenomena within some specific strain members has been found.<sup>20, 21</sup> The many contributions to this subject have been extensively reviewed by Munoz and Bergman.<sup>22-24</sup> Thus, defined conditions of *B. pertussis* treatment and sensitization in the CFW Swiss-Webster strain allowed for comparative evaluation of eosinophil cell responses to antigenic challenge per se and in models of histamine sensitivity and anaphylactic responsiveness.

## MATERIALS AND METHODS

### Experimental animals

CFW strain, Swiss-Webster, female, albino mice, 4 to 7 weeks of age (Carworth Farms, New City, N. Y.) were used in groups of 10 for each of the experimental procedures described.

### Antigen

Egg albumin (EA) (Pentex, Inc., Kankakee, Ill.), 5 $\times$  crystallized, in a dose of 1 mg. was given into the tail vein as a single intravenous injection or was adsorbed on aluminum hydroxide gel and given in a series of 5 subcutaneous injections: 1 mg. on Days 1 and 2 and 2 mg. on Days 3, 6, and 13 in a volume of 0.3 ml. per dose. Intraperitoneal challenging injections consisted of 1 mg. of EA per 0.3 ml. of Al(OH)<sub>3</sub> gel per dose.

### *Bordetella pertussis* vaccine

Organisms, 6  $\times$  10<sup>8</sup>, in 0.5 ml. volume from a killed suspension of *B. pertussis* (supplied by Eli Lilly Co., Indianapolis, Ind.) were given intravenously to the indicated groups at the time of single injection of EA or 4 or 10 days prior to intraperitoneal challenge with EA in those immunized by multiple subcutaneous injections.

### Peritoneal fluid cell study

At 6 hours or at 4 days following the intraperitoneal challenging injection of EA, animals were killed and peritoneal cavities irrigated by injection of 5 ml. of TC 199 culture medium

through an abdominal incision. The abdominal walls were quickly laid open and the fluid contents recovered by thorough aspiration. The recovered fluid was centrifuged at 1,500 r.p.m. for 15 min. at 4° C. and the measured packed cells then resuspended in unit volumes of TC 199 culture medium. Determinations on samples were made for total cells, leukocytes, and eosinophils and for differential cell study of glass slide smears stained with May-Gruenwald-Giemsa.

### Immunization, sensitization, and challenge

The design of procedures for the experimental series was based upon the application of a different set of treatment conditions for each group of 10 mice. Variations in combinations of immunization techniques, associated *B. pertussis* administration, timing of antigenic challenge, and the subsequent time periods allowed for sampling of peritoneal fluids. Effects of the following factors were thus studied: 1. immunization by a single intravenous EA injection: A. with or without associated *B. pertussis* administration; B. intraperitoneal injection of corresponding antigen (EA) 4 or 10 days later; C. peritoneal fluid recovery at 6 hours or 4 days following intraperitoneal challenge. 2. multiple subcutaneous injections of EA followed by specific antigen intraperitoneal injections on Day 20: A. *B. pertussis* administration either omitted or given either 10 days or 4 days prior to intraperitoneal challenge; B. recovery of peritoneal fluid at either 6 hours or 4 days following intraperitoneal challenge.

One group of mice received a single intravenous immunizing injection of EA and *B. pertussis* and was then challenged intraperitoneally on Day 20 for the purpose of evaluating this sensitization time period which was employed in the multiple subcutaneous injected series.

One series of mice received multiple subcutaneous injections of aluminum phosphate-adsorbed ultra-refined tetanus toxoid (Wyeth Laboratories, Philadelphia, Pa.)—0.1 ml. on Days 1 and 2, 0.2 ml. on Days 3, 6, and 13, and challenged intraperitoneally with 0.05 ml. on Day 20—in order to compare the effects of this antigen employed by Speirs and associates<sup>12, 13</sup> with EA utilized in this study.

The series numbers for these groups according to the reagents given and procedures employed are identified and listed in Table I.

For control purposes additional series were included for peritoneal study in CFW mice: (1) untreated and injected intraperitoneally with TC199 culture medium; (2) untreated and injected intraperitoneally with 1 mg. of a purified protein, EA, or bovine gamma globulin (BGG) adsorbed on Al(OH)<sub>3</sub> gel; (3) single intravenous injection of *B. pertussis* and injected intraperitoneally with the purified protein preparation; (4) sensitized by intravenous injection of EA and challenged intraperitoneally with an unrelated purified antigen, e.g., bovine gamma globulin.

### Antibody determinations

At the time of sacrifice, blood was collected by axillary incision and pooled in lots to 5 animals per pool. Serum was prepared for study of: (1) hemagglutination of EA-coated tanned sheep erythrocytes by a micromodification technique<sup>25</sup> and repeated with serum samples treated with 0.2M 2-ME; (2) precipitin formation to EA (range of 10 to 40 mg. per milliliter) within agar-filled 1.7 mm. diameter glass tubes according to the double diffusion method of Preer.<sup>26</sup>

### RESULTS

Members of all 16 groups of CFW mice challenged intraperitoneally at different time periods following either single or multiple priming injections of EA demonstrated significant eosinophilia of peritoneal cell populations. The figures for mean total leukocyte and eosinophil determinations and serum antibody levels are summarized in Table I.

Animals that did not survive intraperitoneal anaphylactic challenge for

**Table 1.** Leukocyte and eosinophil cell contents of peritoneal fluids recovered after intraperitoneal antigenic challenge subsequent to single injections of EA and Bordetella pertussis vaccine and serum antibody determinations in CFW strain mice

| Series* | Treatment                          |                 | Peritoneal fluid |       |                          |                 | Serum antibody§ |                 |
|---------|------------------------------------|-----------------|------------------|-------|--------------------------|-----------------|-----------------|-----------------|
|         | B. pertus-sist                     | Challenge (day) | Sampling period  |       | Cells ( $\times 10^5$ )† |                 | Hemag-glutinin  | Pre-<br>cipitin |
|         |                                    |                 | 6 Hour           | 4 Day | Leukocytes               | Eosinophils     |                 |                 |
| S1      |                                    | 4               | X                |       | 23.6 $\pm$ 12.3          | 1.2 $\pm$ 2     | 0               | 0               |
| S2      | X                                  | 4               | X                |       | 14.9 $\pm$ 11.9          | 0.7 $\pm$ 0.8   | 0               | 0               |
| S3      |                                    | 10              | X                |       | 28.2 $\pm$ 13.2          | 3.5 $\pm$ 2.3   | 0               | 0               |
| S4      | X                                  | 10              | X                |       | 35.4 $\pm$ 25.8          | 0.8 $\pm$ 1.8   | 0               | 0               |
| S5      |                                    | 4               |                  | X     | 18.6 $\pm$ 7.1           | 3.4 $\pm$ 2.9   | 0               | 0               |
| S6      | X                                  | 4               |                  | X     | 39.8 $\pm$ 24.4          | 2.3 $\pm$ 2.7   | 0               | 0               |
| S7      |                                    | 10              |                  | X     | 63.3 $\pm$ 28.3          | 9.8 $\pm$ 7.5   | 64<br>(8)       | 0               |
| S8      | X                                  | 10              |                  | X     | 48.6 $\pm$ 27.1          | 7.5 $\pm$ 8.1   | 8<br>(0)        | 0               |
| S9      | X                                  | 20              |                  | X     | 56.5 $\pm$ 37.9          | 2.7 $\pm$ 2.1   | 2,048<br>(128)  | +               |
|         | Day given<br>prior to<br>challenge |                 |                  |       |                          |                 |                 |                 |
| M1      |                                    | 20              | X                |       | 16.9 $\pm$ 13.6          | 3.9 $\pm$ 7.3   | 4<br>(0)        | 0               |
| M2      | 4                                  | 20              | X                |       | 12.2 $\pm$ 6.1           | 1.4 $\pm$ 0.9   | 256<br>(16)     | 0               |
| M3      | 10                                 | 20              | X                |       | 93.2 $\pm$ 75.1          | 0.8 $\pm$ 0.8   | 1,024<br>(128)  | +               |
| M4      |                                    | 20              |                  | X     | 67.3 $\pm$ 40.1          | 28.6 $\pm$ 23.7 |                 |                 |
| M5      |                                    | 20              |                  | X     | 42.1 $\pm$ 32.7          | 13.8 $\pm$ 9.6  | 1,024<br>(128)  | +               |
| M6      | 4                                  | 20              |                  | X     | 41.7 $\pm$ 23.6          | 13.6 $\pm$ 9.7  | 2,048<br>(128)  | +               |
| M7      | 10                                 | 20              |                  | X     | 23.9 $\pm$ 9.5           | 0.4 $\pm$ 0.5   | 2,048<br>(64)   | +               |

Control series of mice receiving nonspecific or unrelated protein antigenic intraperitoneal challenge demonstrated: total leukocytes 200 to 2,700, 0 to 1 per cent eosinophils, 5 to 43 per cent polymorphonuclear leukocytes, 52 to 94 per cent mononuclear cells.

\*Female, 4 to 7 weeks of age, groups of 10 each; challenged intraperitoneally with 1 mg. of EA/Al(OH)<sub>3</sub> gel. S series—received single intravenous priming injection of 1 mg. of EA. M series—immunized by multiple subcutaneous injections of EA/Al(OH)<sub>3</sub> (see text), except M4.

† $6 \times 10^9$  Killed organisms given: (1) simultaneously with EA as marked (X) in the S series and (2) on day indicated in M series.

‡Total leukocytes and eosinophils recovered by peritoneal lavage (Mean  $\pm$  S.D.).

§Antibody determinations report identical results obtained on 2 serum pools per series, up to 5 animals per pool. Hemagglutination figures are reciprocals of titers. Figures in parentheses are titers after treatment of sera with 2-ME.

||Series M4: Animals received aluminum phosphate-adsorbed tetanus toxoid by multiple subcutaneous immunizing injections and intraperitoneal challenge (see text) in place of EA.

**Table II.** Significance of differences in peritoneal fluid cell contents of total leukocytes and of eosinophils between series of CFW strain mice related to: (1) the respective methods for their sensitization and intraperitoneal challenges with egg albumin, (2) the variable administration of Bordetella pertussis vaccine, and (3) serum antibody determinations

| Experimental procedures*<br>(paired series†)                      | Significance‡ of |              | Serum antibody§ |             |
|-------------------------------------------------------------------|------------------|--------------|-----------------|-------------|
|                                                                   | Leukocytosis     | Eosinophilia | Hemagglutinins  | Precipitins |
| <i>Recovery period after anti-<br/>gen challenge</i>              |                  |              |                 |             |
| 6 Hours vs. 4 Days                                                |                  |              |                 |             |
| S1 S5                                                             | 0                | +            | 0:0             | 0:0         |
| S2 S6                                                             | +                | +            | 0:0             | 0:0         |
| S3 S7                                                             | +                | +            | 0:64            | 0:+         |
| S4 S8                                                             | 0                | +            | 0:8             | 0:0         |
| M1 M5                                                             | +                | +            | 4:1024          | 0:+         |
| M2 M6                                                             | +                | +            | 256:2048        | 0:+         |
| M3 M7                                                             | 0                | 0            | 1024:2048       | +:+         |
| <i>Challenge time after single<br/>immunization</i>               |                  |              |                 |             |
| 4 Days vs. 10 Days                                                |                  |              |                 |             |
| S1 S3                                                             | 0                | +            | 0:0             | 0:0         |
| S5 S7                                                             | +                | +            | 0:64            | 0:0         |
| <i>B. pertussis administration<br/>prior to antigen challenge</i> |                  |              |                 |             |
| None vs. 4 Days                                                   |                  |              |                 |             |
| S1 S2                                                             | 0                | 0            | 0:0             | 0:0         |
| S5 S6                                                             | +                | 0            | 0:0             | 0:0         |
| M1 M2                                                             | 0                | 0            | 4:256           | 0:0         |
| M5 M6                                                             | 0                | 0            | 1024:2048       | +:+         |
| <i>B. pertussis administration<br/>prior to antigen challenge</i> |                  |              |                 |             |
| None vs. 10 Days                                                  |                  |              |                 |             |
| S3 S4                                                             | 0                | 0            | 0:0             | 0:0         |
| S7 S8                                                             | 0                | 0            | 64:8            | 0:0         |
| M1 M3                                                             | +                | 0            | 4:1024          | 0:+         |
| M5 M7                                                             | 0                | 0            | 1024:2048       | +:+         |
| 4 Days vs. 10 Days                                                |                  |              |                 |             |
| S2 S4                                                             | +                | 0            | 0:0             | 0:0         |
| S6 S8                                                             | 0                | 0            | 0:8             | 0:0         |
| M2 M3                                                             | +                | 0            | 256:1024        | 0:+         |
| M6 M7                                                             | +                | +            | 2048:2048       | +:+         |
| 4 Days vs. 20 Days                                                |                  |              |                 |             |
| S6 S9                                                             | 0                | 0            | 0:2048          | 0:+         |
| 10 Days vs. 20 Days                                               |                  |              |                 |             |
| S8 S9                                                             | 0                | 0            | 8:2048          | 0:+         |

\*Female, 4 to 7 weeks of age, groups of 10 each, challenged intraperitoneally with 1 mg. of EA/Al(OH)<sub>3</sub> gel. S series—received single intravenous priming injection of 1 mg. of EA. M series—immunized by multiple subcutaneous injections of EA/Al(OH)<sub>3</sub>.

†Members of each paired series differed in only one factor of otherwise common experimental procedure, i.e., the indicated variable listed. Summaries of treatment for each numbered series are given in Table I.

‡By t test ( $p < 0.05$ ).

§Reciprocals of hemagglutination titers. Figures for antibody determinations given in left and right sides of column correspond to the respective column position for the series member in each pair.

the 6 hour or 4 day period required for peritoneal cell sampling were not included in these reported series. The chance of overt anaphylactic shock resulting from a challenging injection of antigen was minimized by giving the EA on  $\text{Al}(\text{OH})_3$  gel to delay absorption from the peritoneal cavity. In procedures in which the administration of *B. pertussis* and 20 day sensitization periods resulted in anaphylactic manifestations on challenge, giving EA/ $\text{Al}(\text{OH})_3$  in divided doses at short intervals effectively bypassed this development.

In this as in other models,<sup>4-6, 27</sup> individual animals varied quantitatively in the total leukocyte and eosinophil responses. However, defined ranges and a uniformity of pattern for each group allowed for comparative study of the data. For this evaluation, series were arranged in pairs and their respective results compared. Each member of a paired series differed in only one factor of otherwise common experimental procedure, and that variable in turn served as specific subject for study. The basis for the pairing of experimental groups is identified with the respective procedures listed in the first column of Table II. Differences between groups in their mean cell counts for total leukocytes and for eosinophils were compared for significance by the *t* test. Results of these with values for  $p < 0.05$  are given in Table II.

#### **Recovery period of peritoneal cells after intraperitoneal antigenic challenge**

Higher eosinophil counts were found at 4 days than at 6 hours in 6 of 7 paired groups. Within series having identical time periods for cell recovery, multiple antigen injections effected higher eosinophilia than a single priming dose when these animals were sampled 4 days after challenge.

#### **Effects of *B. pertussis* treatment**

Greater eosinophilia was demonstrated in animals antigenically challenged intraperitoneally at 10 days than at 4 days after a single EA injection alone ( $S3 > S1$ ,  $S7 > S5$ ). However, this was not found when similar procedures were accompanied by *B. pertussis* treatment ( $S2-S4$ ,  $S6-S8$ ,  $S9$ ). The additional injection of *B. pertussis* 4 days or 10 days prior to intraperitoneal specific antigenic challenge did not result in any significant differences in the degree of resultant peritoneal eosinophilia as compared to groups given EA alone either by single intravenous or by multiple subcutaneous injections.

#### **Relationships between peritoneal cell populations**

High total leukocyte counts reflected increases in the numbers of mononuclear cells and to a lesser extent polymorphonuclear leukocytes. While in some instances elevated total leukocyte and eosinophil counts were found in the same animal and increased eosinophil levels contributed to the leukocytosis, no consistent relationship could be found. Eosinophil counts could not be correlated with fluctuations of either predominating lymphocyte or polymorphonuclear leukocyte percentages by differential cell study.

#### **Antibody determinations**

Only low titers of 2-ME-sensitive hemagglutinating antibody to EA could be identified in the sera of some groups of mice initially injected intravenously,

challenged intraperitoneally on Day 10, and sampled 4 days later. Where the interval to challenge had been lengthened to 20 days (S9), the increased hemagglutinin titers and the appearance of secondary antibody phase precipitins paralleled the findings in groups receiving multiple subcutaneous injections over equal time periods. While higher eosinophil counts were found in some series with such serum antibodies (e.g., M5, M6), this was not the case for other groups (e.g., S6, M7).

## DISCUSSION

The design of this investigation was based upon application of procedures described by Munoz and Bergman<sup>22-24</sup> and by Malkiel and Hargis.<sup>28</sup> Through these an experimental animal model for controlled histamine and serotonin sensitivity, anaphylactic susceptibility, and corresponding nonresponsive states could be effected. Preliminary studies verified the adaptability of these *B. pertussis*-treated CFW strain mouse models to experimental techniques for evoking eosinophil responses. Through combinations of these procedures we then sought to study the question of possible relationships between eosinotactic immune mechanisms and delineated hypersensitivity states. In order to establish prerequisite conditions for antigen administration, challenge, and cell sampling at affected sites, several variations in experimental approach were evaluated. Some of our resultant observations of interest and relevance to allergic inflammatory cell processes are reported here.

The bulk of knowledge of experimentally induced eosinophilia in the mouse has been derived from the contributions of Speirs and associates.<sup>12-14, 29-32</sup> We therefore employed their basic experimental model<sup>29</sup> to evaluate possible critical factors on which our own studies were to be based. Our data established: (1) that, in mice of the required strain, age, and sex,<sup>22-24</sup> antigen-antibody-related eosinophil cell accumulations could be induced and recovered from the peritoneal cavity; (2) that EA, with antigenic properties favorable for anaphylaxis in the *B. pertussis*-treated model,<sup>28</sup> could serve equally well as tetanus toxoid<sup>29</sup> as an eosinotactic antigen (series M4 and M5); and (3) that, while high eosinophil counts were found in mice challenged intraperitoneally after a 20 day course of multiple subcutaneous and final intraperitoneal injection (M5), the peritoneal fluid of those primed only by a single intravenous injection of EA 10 days previous to challenge also contained significant cell populations (S7). The procedure described by Speirs and co-workers<sup>12-14</sup> utilizing tetanus toxoid as the immunizing and challenging antigenic agent evoked larger eosinophil responses (M4 > M5). However, we chose to substitute ovalbumin throughout these experiments because of its documented ability to effect anaphylactic responsiveness in CFW mice<sup>22-24, 28</sup> and because the use of this purified protein allowed us to bypass unknown qualities that might be inherent in a preparation of multiple antigenic components.

Speirs and associates<sup>14, 29, 31</sup> have interpreted results of their mouse experiments as indicating two distinct phases in eosinophil responses to antigenic influences. The first, a transient phase that occurs within the first 24 hours, may be a reflection of eosinophil participation in the early cellular inflammatory stimulus of initially formed antigen-antibody complexes<sup>4-6</sup> and/or nonspecific

large molecular aggregates.<sup>5, 7-8</sup> The second is a prolonged phase that peaks on the fourth day, lasts for 5 to 7 days, and owes its specificity in immunized animals to the chemotactic attraction of eosinophils to sensitized mononuclear cells reacting to the reinjected antigen. Our corroboration here in identifying significant eosinophil accumulations in mouse peritoneal fluid at 4 days is at variance with the results of our earlier studies with other species. In working with passive sensitization techniques and the injection of preformed antigen-antibody complexes and protein molecular aggregates, we noted the peak of induced eosinophil infiltrations into rabbit pulmonary vascular tissue within 24 hours<sup>33</sup> and into regional lymph nodes of rabbit<sup>3</sup> and guinea pig<sup>5-6</sup> models at 6 to 8 hours. In attempting to reconcile this divergence, several possibilities are suggested, although exact reasons are not known.

First to be questioned are whether there may be relevant differences in: (1) animal species, (2) natural conditions within tissues of different structure and function (i.e., lymph node and peritoneal surfaces) that in turn may influence the pathogenesis and evolution of local eosinophilia and related lesions, and (3) pathophysiologic pathways of inflammation and cell responses to different mechanisms of sensitization (i.e., active and passive immunization in contrast to the effects of immune complexes per se). Another important factor for consideration may be the physicochemical state of the antigenic challenge exerting an influence on the duration of effects. To evoke a 4 day cellular response, EA had to be given adsorbed to aluminum hydroxide gel presumably to ensure retention within the peritoneal cavity. A further observation of Athanassiades and Speirs<sup>32</sup> is especially noteworthy in that the continued presence of retained antigen led to an even longer-lasting and persisting phase of peritoneal eosinophilia with granuloma formation.

Associated lymphocytosis effects of *B. pertussis*<sup>34</sup> treatment apparently did not influence eosinophil cell responses discernible at the challenged site. Nor could any consistent relationships be identified between local eosinophilia and the response patterns of other cell types in the study of peritoneal populations. Thus the appearance of the eosinophil in this situation apparently does not depend upon its participation as a nonspecific component of cellular infiltrate within the inflammatory milieu.

In all eight variations of sensitization and challenge procedures there were no significant differences found in quantitated eosinophilia between those given *B. pertussis* to effect associated states of histamine-serotonin sensitivity or susceptibility to anaphylactic shock and the nontreated groups. It is therefore of interest to take note of suspected mechanisms for *B. pertussis* influences in susceptible mouse strains. The work of Munoz and Bergman<sup>22-24</sup> has pointed to an increase in permeability of capillaries and perhaps tissues in general accompanying induced histamine-responsive states. Associated factors of reduced histaminase activity<sup>35, 36</sup> and of increased production of histidine decarboxylase<sup>37</sup> in treated animals have been reported. Apart from this, Fischel and co-workers<sup>38</sup> have suggested that a functional imbalance in reactivity of  $\alpha$ - and  $\beta$ -adrenergic receptors in related neural pathways results from *B. pertussis* treatment simulat-

ing a state of  $\beta$ -adrenergic blockade (BAB). Results of our studies were not definitive in this regard. However, it is relevant to note that the eosinophilia was not greater with *B. pertussis* treatment, since elimination of eosinopenic influences in homeostatic control<sup>39</sup> would be anticipated to occur if BAB states had been induced.

It is to be expected that highest serum antibody levels would be found under experimental conditions (1) associated with *B. pertussis* treatment in view of its adjuvant effects,<sup>28, 40</sup> and (2) in which procedures were followed for multiple antigenic stimulation (M5) or the time period for sampling beyond 10 days after single EA injection was extended (S9). In addition, reduction in circulating antibody by complexing with reinjected corresponding antigen soon thereafter could be expected. Thus, levels from serum samples collected 6 hours later cannot be compared to those at 4 days when anamnestic responses are underway.<sup>41</sup> In this evaluation the lack of correlation between quantitation of antibody and eosinophil cell responses can apply only to antibody characterized as hemagglutinin (largely 2-ME sensitive, equated with 19S character), as precipitin (2-ME-resistant, 7S), or by activity in enhancing anaphylactic shock. The latter was especially prominent in experimental procedures followed for developing series S9, M3, M7. Turner and co-workers<sup>30</sup> actually found in mouse models an inverse correlation between circulating antitoxin (presumably IgG) at challenge and subsequent eosinophil responses. In the guinea pig, Litt<sup>42</sup> has identified experimentally produced eosinophilia favored by  $\gamma_1$  antibody (PCA) over that of  $\gamma_2$  (precipitin).

The fact that states of histamine sensitivity and anaphylactic responsiveness did not enhance eosinophil cell responses in this mouse model appears relevant to our previous demonstrations of the eosinotactic properties of immune complexes of varied character<sup>5, 6</sup> and with both 7S and 19S antibody moieties.<sup>43</sup> However, our preliminary observations from other work in progress suggest that eosinophil prominence may be related to processes involving reaginic antibody. If so, this in turn might explain the eosinophilia emerging from antigen-antibody reactions as early as 4 days and increasing by Day 10 after a single antigen injection, a time period that could be correlated with reagin production in the mouse.<sup>44, 45</sup> By the same token, an adjuvant effect of *B. pertussis* for enhanced production of IgM and IgG classes could mask results that might reveal the significance of reaginic antibody appearing transiently within this critical time period. Since eosinophilia commonly accompanies parasitic worm infections, it is of related interest to note the presence of corresponding reagins in affected mice<sup>46</sup> and in man given *Ascaris* extract<sup>47</sup> and his immediate skin test reactivity in clinical trichinosis, schistosomiasis, filariasis, and involvement with *echinococcus* and *Fasciola hepatica*.<sup>48</sup> Eosinophilia, so characteristic of atopic man whose reaginic antibodies are now identified with IgE,<sup>49</sup> has also been found associated with skin-sensitizing antibody during the course of penicillin drug reactions.<sup>50</sup> Since our preliminary observations require further study, we are continuing these investigative approaches in developing experimental models designed to delineate the relative eosinotactic influence of this antibody class.

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