# CUTANEOUS BASOPHIL (JONES-MOTE) HYPERSENSITIVITY AFTER "TOLEROGENIC" DOSES OF INTRAVENOUS OVALBUMIN IN THE GUINEA PIG*, ‡

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The term cutaneous basophil hypersensitivity (CBH)[1] has recently been used to describe the type of delayed hypersensitivity reaction produced transiently early in the course of immunization with soluble antigen (1–3). Immunogenic and antigenic requirements distinguish it from classic delayed hypersensitivity (classic DH) and antibody-mediated reactions (2), and the histology of the skin reaction is uniquely characterized by heavy infiltration with basophils (3). CBH has recently been reported in contact dermatitis[2] and allograft rejection (4). Studies of tolerogenesis in adult guinea pigs by Asherson and Stone (5) revealed reduction in classic DH by preimmunization injection of soluble or alum-precipitated antigen without a comparable effect on antibody production; this phenomenon was called "immune deviation." Dvorak et al. (6) were able to induce specific immunologic unresponsiveness to human serum albumin and bovine gamma globulin in adult guinea pigs with intravenous injections of antigen in saline within 3 wk of subsequent immunization with the same antigen in complete Freund's adjuvant (CFA); classic DH and hemolytic antibody were suppressed more completely and for a longer time than was passive cutaneous anaphylaxis (PCA) antibody.

In the investigations reported here, the effects of intravenous administration of antigen on CBH are compared with tolerogenesis toward classic DH

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[1] *Abbreviations used in this paper:* CBH, cutaneous basophil hypersensitivity; CFA, complete Freund's adjuvant; classic DH, classic delayed hypersensitivity; HSA, human serum albumin; IFA, incomplete Freund's adjuvant; MIF, migration-inhibitory factor; OA, ovalbumin; PCA, passive cutaneous anaphylaxis; PPD, purified protein derivative of tuberculin.

[2] Dvorak, H. F., B. A. Simpson, R. C. Bast, and S. Leskowitz. 1971. Cutaneous basophil hypersensitivity. III. Participation of the basophil in hypersensitivity to antigen-antibody complexes, delayed hypersensitivity and contact allergy. Submitted for publication.

and antibody production. Results show that relatively large doses of intravenous ovalbumin in guinea pigs stimulate immunogenesis for CBH and PCA antibody while at the same time inducing tolerance for classic DH and hemolytic antibody.

## *Materials and Methods*

*Animals.*—Random-bred white guinea pigs (weighing 300–400 g) were used in these experiments for active immunization and skin testing, and for measuring PCA antibody.

*Antigen.*—Ovalbumin (5x crystallized, Pentex Biochemical, Kankakee, Ill.) diluted in saline was used throughout. PPD (purified protein derivative of tuberculin) was purchased from Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada, and diluted appropriately in saline.

*Tolerogenesis Protocol.*—The initial schedule for comparison of tolerogenic requirements in CBH, classic DH, gamma$_1$ and gamma$_2$ antibody production is shown below:

| Day 0 | Day 7 | Day 14 | Day 15 | Day 28 |
|---|---|---|---|---|
| Intravenous ovalbumin (OA) (graded doses) | Skin test (100 $\mu$g OA) | Skin test (100 $\mu$g OA) | Immunize (10 $\mu$g OA in CFA) | Bleed for antibody<br>Skin tests (10 $\mu$g OA 10 $\mu$g PPD) |

Various doses of ovalbumin (OA) were injected intravenously into the hind foot vein on day 0. 1 wk later, 100 $\mu$g of OA was injected intradermally in a volume of 0.1 ml, which experience had shown immunizes the guinea pig for CBH (see Results and Table IV). At 2 wk, the animal was again skin tested and the test read at 4 and 24 hr. Subsequent immunization was carried out by injection of 10 $\mu$g of OA emulsified in CFA in a total volume of 0.1 ml divided equally among the four footpads. CFA was made with 8.5 parts light mineral oil and 5 parts Arlacel A (Atlas Chemical Industries, Inc., Wilmington, Del.) to which was added 5 mg/ml of killed mycobacteria. 2 wk after immunization the animals were bled for antibody studies and skin testing was repeated using 10 $\mu$g of OA to judge tolerance for classic DH, and 10 $\mu$g of PPD to evaluate possible nonspecific suppression. Reactions were read at 4 and 24 hr; the diameter of erythema, degree of induration, and presence or absence of necrosis were recorded. Later studies of sensitization by intravenous antigen alone and of dose and route requirements for CBH sensitization are detailed under Results.

*Antibody Studies.*—Blood was collected by cardiac puncture, allowed to clot at room temperature, and the serum separated by centrifugation and stored frozen until examined. Passive cutaneous anaphylaxis was used to measure 7S gamma$_1$ antibody (7). Serum dilutions in volume of 0.1 ml were injected intradermally in the back of duplicate guinea pigs at multiple sites (up to 16). 4 hr later, a mixture of 2% Evan's blue dye and 1 mg of OA in a total volume of 1 ml was injected intravenously; reactions were measured after 30 min. Titers of 7S gamma$_2$ and macroglobulin (hemolytic) antibody were measured by hemolysis of antigen-coated tanned sheep red cells by the method of Kabat and Mayer (8) as modified by Bloch et al. (9). A concentration of 2 mg/ml of antigen was used to coat the tanned sheep cells. The highest dilution giving complete hemolysis was taken as the end point.

*Histology.*—Histological sections were kindly prepared in the laboratory of John C. Hoak, M.D., Hematology Section, Department of Internal Medicine, University of Iowa. Tissues were fixed in gluteraldehyde, postfixed in osmium tetroxide, were dehydrated in graded alcohol solutions and propylene, and were embedded in Epon Araldite. For light microscopy, 1 $\mu$ sections were cut and stained for 1 hr in Giemsa diluted 1:10 in 2% sodium borate solution. For electron microscopy, sections were cut on a Reichert ultramicrotome (C. Reichert,

Wien, Austria) and examined with a Philips EM 300 electron microscope (Philips Electronic Instruments, Mount Vernon, N.Y.).

## RESULTS

*Tolerogenesis.*—The tolerogenesis protocol outlined above proved suitable to demonstrate differences in tolerogenic requirements for classic DH, PCA antibody, and hemolytic antibody. Results are given in Table I. After intra-

TABLE I

*Results of Tolerogenesis Protocol*

| Time | Dose of i.v. OA at day 0 | Delayed skin reaction to | | | Antibody response | |
|---|---|---|---|---|---|---|
| | | 100 μg OA | 10 μg OA | 10 μg PPD | PCA (Average titer) | Hemolytic (Average titer) |
| *wk* | *mg* | | | | | |
| 1 | none | 0− (6/6)* | | | | |
| | 1 | 13− (4/4) | | | | |
| | 10 | 15− (4/4) | | | | |
| | 100 | 4− (2/6) | | | | |
| 2 | none | 18− (6/6) | | | | |
| | 1 | 0− (0/4) | | | | |
| | 10 | early rxn‡ (3/4) | | | | |
| | 100 | early rxn (4/6) | | | | |
| 4 | none | | 18+++ (6/6) | 19+++ (6/6) | 600 (6/6) | 2000 (6/6) |
| | 1 | | 10+ (4/4) | 18+++ (4/4) | 520 (4/4) | 112 (4/4) |
| | 10 | | 12+ (4/4) | 19+++ (4/4) | 400 (4/4) | |
| | 100 | | 6− (3/5) | 18+++ (5/5) | 150 (5/5) | 45 (2/5) |

* Skin reactions are recorded as average diameter of erythema in millimeters followed by the average degree of induration graded from − to +++. Figures in parenthesis denote No. of guinea pigs reacting per number tested.

‡ rxn, reaction.

venous injection of large amounts of soluble OA, subsequent immunization with OA in CFA resulted at 4 wk (2 wk postimmunization) in mild suppression of PCA titers, markedly diminished hemolytic antibody titers, and specific suppression of classic DH. This is in agreement with the results of Dvorak et al. (6) using other antigens.

Of great interest, however, was the observation that the delayed skin test 7 days after the tolerogenic intravenous OA injection was frequently positive and had the morphologic characteristics of CBH. At 14 days, skin testing resulted in an earlier reaction, probably antibody-mediated, reaching maximum intensity at 4–6 hr and appearing faded at 24 hr as discussed elsewhere (3).

The control group showed good CBH reactivity at 2 wk, 1 wk after the initial skin test (see Table IV). The phenomenon of immunization for CBH by intravenous antigen led to further studies on the effects of route and dosage of OA on the immune response.

*Immune Response after Intravenous Antigen.*—Studies were designed to evaluate the immune response after varying doses of intravenous ovalbumin without subsequent immunization. Table II summarizes the results of one such experiment. Groups of guinea pigs were injected with log doses of OA, skin tested at 7 days, and bled weekly. The control group was also skin tested at 1 wk and bled weekly. CBH was present 7 days after intravenous injection of OA, most consistently after a dose of 1 mg. PCA antibody was not detectable in any of the groups tested on day 7 (lowest dilution tested was 1:10), but was regularly present 14 days after intravenous OA dosages of 1, 10, or 100 mg.

TABLE II

*Immune Response after Intravenous Ovalbumin*

| Dose given | Delayed skin rxn at 7 days to 100 μg OA | PCA Ab* | | | | Hemolytic Ab Titer on pooled sera | |
|---|---|---|---|---|---|---|---|
| | | 2 wk | | 3 wk | | | |
| | | 1:10 | 1:100 | 1:10 | 1:100 | 2 wk | 3 wk |
| 100 mg OA i.v. | 8+ (3/6)‡ | 13 | 3 | 10.5 | 6 | <1:2 | <1:10 |
| 10 mg OA i.v. | 14+ (4/6) | 6 | 0 | 14 | 12 | <1:2 | <1:10 |
| 1 mg OA i.v. | 17+ (6/6) | 8 | 0 | 15 | 12 | <1:2 | <1:10 |
| Control | 0+ (0/6) | 0 | 0 | 11 | 0 | <1:2 | <1:10 |

* PCA at stated dilutions was performed on pooled sera for each group and is recorded as average size of lesion (mm) in three passive recipients. Ab, antibody.

‡ See Table I for grading of skin reactions.

No hemolytic antibody was detectable at any time after administration of intravenous OA alone. The control group was negative throughout except for the presence of PCA antibody 2 wk after skin testing with 100 μg of OA. A positive CBH reaction can be regularly elicited 7 days after such skin testing (see Tables I and IV).

A second similar experiment was carried out to eliminate the effect of skin testing as a possible booster for antibody formation, and to examine for CBH earlier than the 7th day in animals receiving 100 mg of OA intravenously. The results are summarized in Table III. After intravenous injection of 100 mg of OA, none of three animals developed CBH after skin testing on the 4th day, whereas six of seven animals showed CBH when skin tested on day 5. Skin testing made little difference in PCA titers. Animals receiving 100 mg of OA intravenously developed slightly higher PCA titers 14 days later than did animals receiving 1 mg of OA intravenously. No hemolytic antibody was detectable.

*Effect of Route and Antigen Dose on Sensitization for CBH.*—Previous studies have concluded that minute doses of antigen are effective for the production of CBH (10). The above findings with the intravenous route and skin test immunization raised the question of whether dosage requirements for subsequent CBH varied with the route of administration of the antigen. Results of studies addressed to this question are summarized in Table IV. Clear differences occur among the three routes of immunization in respect to optimum dosage for CBH when tested 7 days later. When OA is incorporated in incomplete Freund's adjuvant (IFA) and injected into footpads, the optimum dose is about 1 μg. When soluble antigen is injected intravenously, however, a 1000-fold higher optimum dose of DBH is noted, i.e., about 1 mg. When animals are sensitized by injecting soluble OA as a skin test, the optimum

TABLE III

*Immune Response after Intravenous Ovalbumin (Continued)*

| Dose of OA i.v. at day 0 | Delayed skin rxn to 100 μg OA | | PCA titer at day 14 (average titer) | Hemolysin Titer day 14 |
|---|---|---|---|---|
| | Day 4 | Day 5 | | |
| *mg* | | | | |
| 100 | 0− (0/3)* | ND | 32 (3/3)‡ | <1:2 (0/3) |
| 100 | ND§ | 14− (6/7) | 20 (3/3) | <1:2 (0/3) |
| 100 | ND | ND | 36 (5/6) | <1:2 (0/6) |
| 1 | ND | ND | 13 (5/6) | <1:2 (0/6) |

* See Table I for grading of skin lesions.

‡ PCA in these experiments is recorded as average titer of individual sera; figures in parenthesis denote animals reacting/number tested.

§ ND = not done.

dosage is clearly less than by the intravenous route and more than that with emulsion into footpads.

*Histology of Delayed Skin Lesions Produced by Intravenous and Skin Test Immunization.*—An important consideration is whether the delayed skin reaction produced by intravenous or intradermal injection of soluble antigen has the histological characteristics of CBH produced by antigen emulsified in IFA and injected into footpads (1, 3). Grossly, the skin reactions are identical regardless of immunization route; at 24 hr there is an area of well-circumscribed erythema, frequently rather faint in intensity, unaccompanied by induration, and appearing faded in 48 hr. Histological studies of the delayed skin reactions after immunization with soluble antigen intravenously or intradermally revealed a picture identical to that previously described of CBH (1, 3). Light microscopy revealed substantial accumulations of basophils accompanied by other mononuclear cells; electron microscopy confirmed the presence of groups of basophils (Figs. 1 and 2). Thus, the histological picture substantiates the identity of these reactions as CBH.

## DISCUSSION

The results described here indicate that intravenous injection of relatively large doses of ovalbumin in guinea pigs, sufficient to produce subsequent tolerance with respect to classic delayed hypersensitivity and hemolytic antibody, actually stimulates the development of CBH and PCA antibody. In addition, a wide variation in dosage optimum for CBH has been demonstrated to depend on route of immunization.

Immune deviation and partial or split tolerance have been reported in adult guinea pigs given soluble or alum-precipitated antigen before immunization with the same antigen emulsified in CFA (5, 6). The question addressed in the present studies was whether tolerance for CBH could be produced relatively

TABLE IV

*Effect of Route on Dosage Requirements for Optimum CBH*

| Dose of OA for immunization | Delayed skin reactivity at 7 days to 100 μg OA after immunization by route shown | | |
|---|---|---|---|
| | Footpads in IFA | i.d. skin test | Intravenous |
| *mg* | | | |
| 0.00001 | 0− (0/6)* | | 0− (0/4) |
| 0.0001 | 14− (14/16) | 5− (1/4) | 0− (0/4) |
| 0.001 | 18− (16/16) | 11− (3/4) | 7− (2/4) |
| 0.01 | 8− (6/10) | 12− (6/8) | 7− (2/4) |
| 0.1 | 4− (1/6) | 14− (8/10) | 4− (1/4) |
| 1.0 | 3− (1/6) | 13− (4/4) | 15− (10/10) |
| 10.0 | 0− (0/6) | 0− (0/4) | 13− (10/14) |
| 100.0 | | | 5− (5/16) |

* See Table I for grading of skin reactions.

easily, in which case it would in this respect resemble classic DH and $gamma_2$ (hemolytic) antibody, or whether tolerogenesis for CBH is difficult, in which respect it would then resemble $gamma_1$ (PCA) antibody production. The tolerogenesis protocol confirmed earlier reports of partial tolerance, with classic DH and hemolytic antibody production being suppressed by intravenous antigen before immunization with antigen emulsified in CFA, while PCA antibody production was less affected. An unexpected finding with the tolerogenesis protocol was that the intravenous tolerogenic dose of antigen sensitized the animal for CBH. After intravenous ovalbumin, CBH was elicited at 5–7 days, at a time when no detectable antibody was present in the serum. By 12–14 days, PCA but not hemolytic antibody was demonstrable. Thus, intravenous OA in doses sufficient to be tolerogenic for classic DH and hemolytic antibody actually produced both CBH and subsequent PCA antibody.

Experiments of others concerning antigenic requirements for Jones-Mote hypersensitivity concluded that very small amounts of antigen were necessary

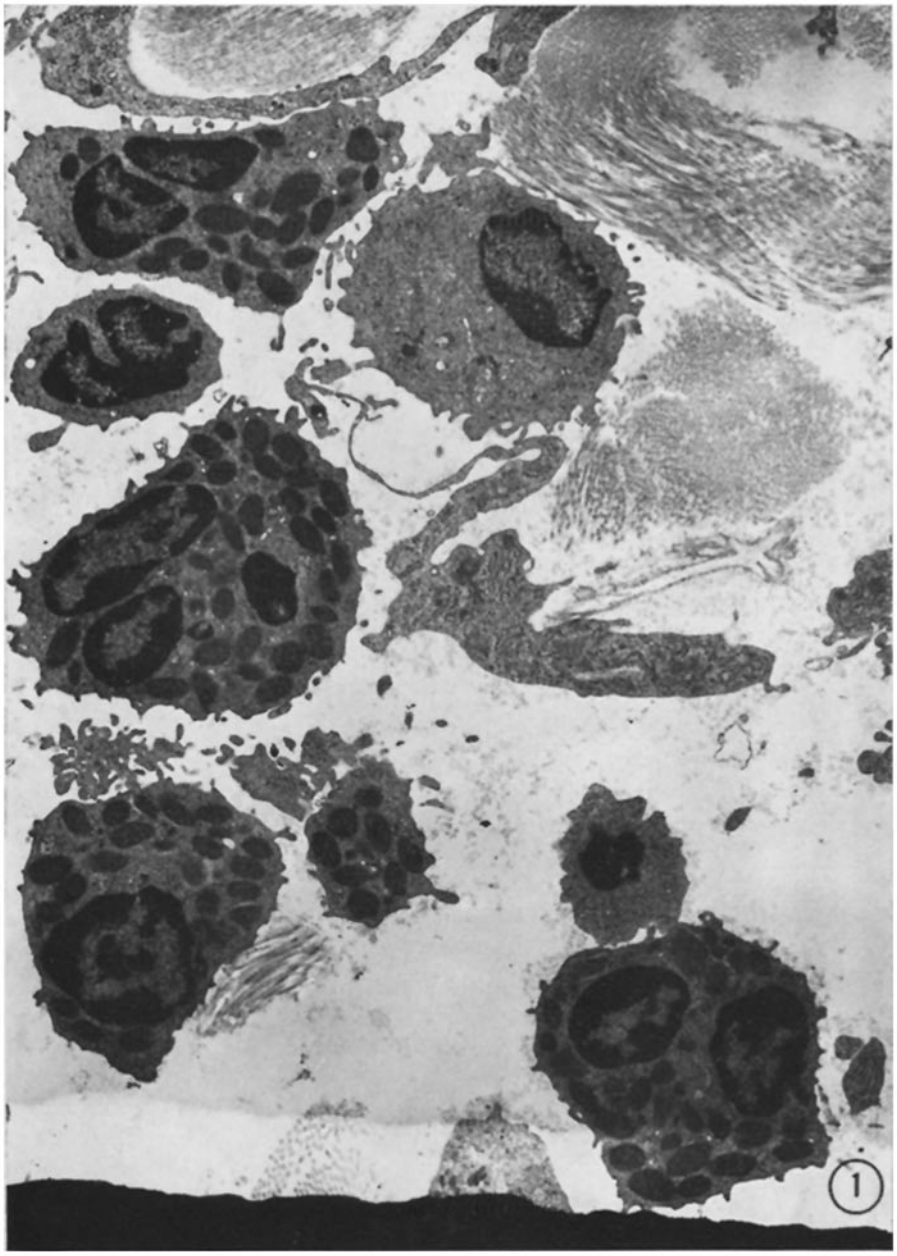


FIG. 1. Electron photomicrograph of delayed skin reaction at 24 hr after skin test with 100 $\mu$g of ovalbumin (OA) in a guinea pig sensitized 5 days earlier with 100 mg of OA intravenously. Note cluster of basophils, a common scene throughout the sections. × 5600.

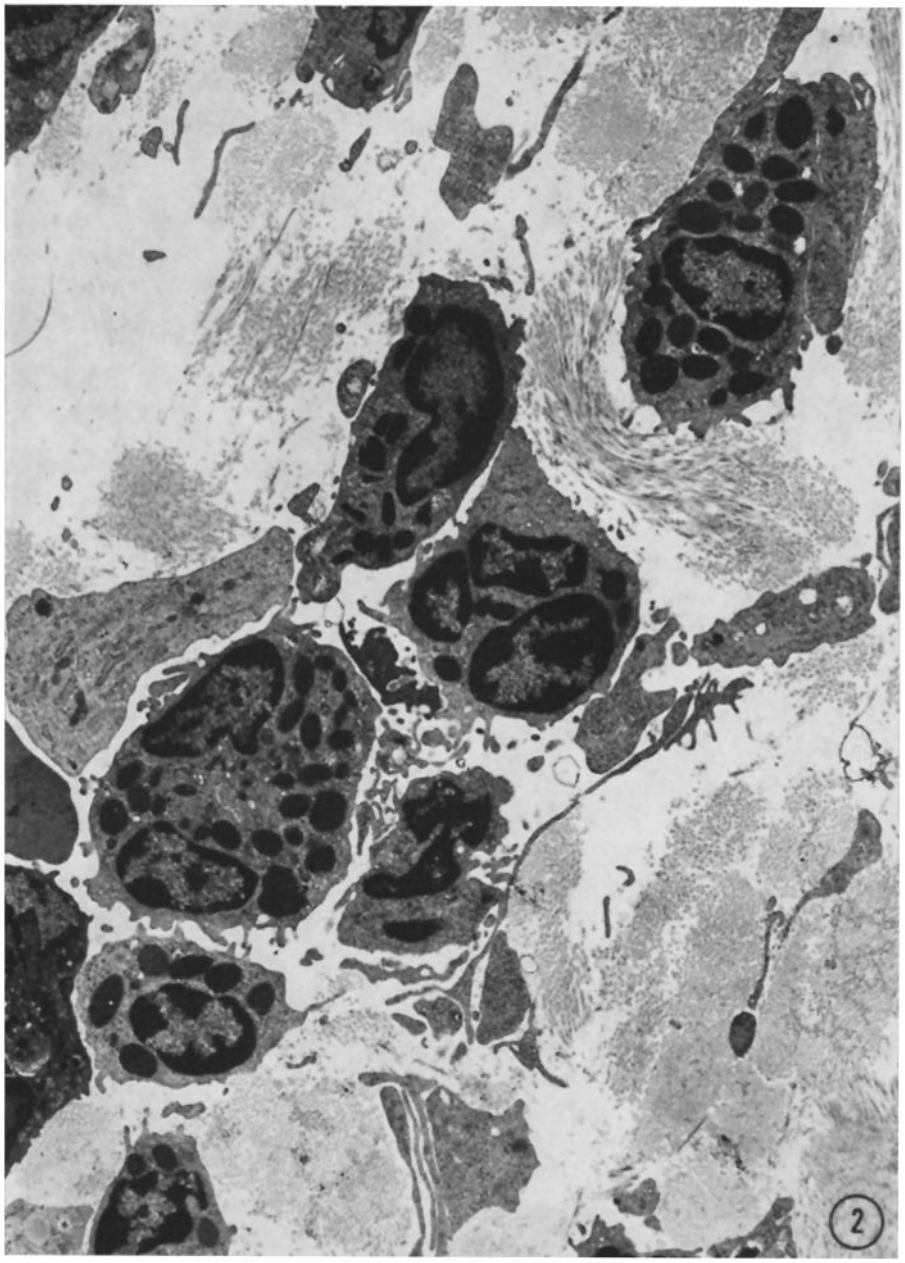


FIG. 2. Electron photomicrograph of delayed skin lesion taken 24 hr after skin test with 100 $\mu$g of ovalbumin (OA) in guinea pig sensitized 7 days earlier with 10 $\mu$g of OA intradermally on flank. Note accumulation of basophils, and there is one eosinophil (with ovoid granules containing central bar at higher magnification.) $\times$ 5600.

when injected in the soluble form (10). Our own findings confirm that low doses (i.e. 1 $\mu$g) are optimal for CBH when ovalbumin is incorporated in IFA; on the other hand, a 1000-fold larger dose is optimal for CBH sensitization when injected intravenously; and soluble OA injected intradermally as a skin test has intermediate dosage requirements. These differences may reflect a subtle balance between tolerogenesis and immunogenesis, or may perhaps be due to an antigen localization requirement for CBH stimulation.

Very recently, Bast et al. (11, 12) have studied in vitro responses of lymphoid cells taken from animals made tolerant to human serum albumin (HSA); the "tolerizing" injection of HSA was given intravenously immediately before immunization with HSA in CFA or IFA, and this routine resulted in a diminished CBH response 1 wk but not 2 wk later. The effect of intravenous tolerizing antigen alone was not studied. The results of thymidine incorporation indicated an evolution of cellular immunity with selection of lymphoid cells responding to decreasing concentrations of antigen as time passed. Cells responsive at low antigen concentration evolved later, were easily rendered tolerant by intravenous antigen, and were correlated with classic delayed hypersensitivity skin reactions. Cells responsive at high antigen concentration evolved earlier, were resistant to tolerogenic antigen, and correlated in vivo with CBH (11). The appearance of lymphocytes reacting to low concentrations of HSA was associated with a progressive increase in the degree of induration of delayed skin reactions, i.e., classic delayed hypersensitivity rather than CBH.

Our own experiments could thus be interpreted as demonstrating a population of antigen-responsive cells which clone with high-dose antigen (even when administered intravenously), are resistant to tolerogenesis, and participate in the CBH delayed skin reaction. The three routes of immunization employed may have different localizing propensities for delivery of antigen to antigen-responsive cells, and thus vary in dosage requirements.

Borel and David (13) have reported that peritoneal exudate cells from partially tolerant guinea pigs fail to elaborate migration-inhibitory factor (MIF) when incubated with the tolerance-inducing antigen. Bast and Dvorak (14) also found MIF elaboration limited to classic DH, whereas blast transformation occurred in lymphocyte cultures from animals sensitized either for CBH or classic DH, with differing time and dosage relationships (12). Our data support the correlation of indurated delayed skin reactions (i.e. classic delayed hypersensitivity) with the presence of lymphocytes capable of stimulation with low antigen concentrations and easily rendered tolerant; and, conversely, the correlation of CBH reactions with the appearance of lymphocytes stimulated by high-dose antigen and resistant to tolerogenesis. In addition to the evidence cited above on MIF production, studies with carrageenan suggest that a basic difference between classic DH and CBH is the role of the macrophage, which participates only in the indurated lesion of classic DH (2).

Presumably, then, a high-affinity lymphocyte easily rendered tolerant is responsible for classic DH and elaboration of MIF.

The significance for clinical medicine of the specific cellular and antibody responses to high-dose intravenous tolerizing antigen is uncertain. The importance of lymphoid cells responding to high-dose antigen in allograft rejection has not been determined, although Dvorak (4) has found that skin allograft rejection in the guinea pig is associated with many of the morphologic features of CBH. The production of complete tolerance by the intravenous administration of soluble transplantation antigens may therefore not be a practical possibility. The unfolding heterogeneity of the cellular immune response increasingly complicates control of this type of hypersensitivity.

The immunogenic-tolerogenic stimilarities between CBH and PCA (gamma$_1$) antibody suggests an intrinsic relationship between these two types of immune response. Of interest is that in the human, as originally described by Jones and Mote (15), delayed hypersensitivity precedes wheal-and-flare antibody responses and reappears as the latter wanes; similar observations have been made in the guinea pig by Sell and Weigle (16). Of relevance also may be the report that delayed skin reactions may be "unmasked" if the wheal-and-flare skin reaction is inhibited by antihistamines in grass pollen-sensitive patients (17). Further work is required in the guinea pig to completely characterize the relationship between CBH and PCA antibody, particularly in view of the recent reports of a guinea pig IgE immunoglobulin class (18, 19). Intriguing, however, is the apparent importance of the basophil in both CBH and type I (anaphylactic) hypersensitivity reactions (20). It seems more than coincidence that CBH calls the basophil to the site of action and PCA antibody utilizes the same cell to carry out its work.

## SUMMARY

Cutaneous basophil hypersensitivity (CBH) was studied for tolerogenic requirements. Graded doses of intravenous ovalbumin (OA) were given to guinea pigs which were subsequently immunized appropriately to produce CBH, classic delayed hypersensitivity (classic DH), and/or antibodies of both passive cutaneous anaphylaxis (PCA) and hemolytic types.

Results showed that doses of intravenous antigen sufficient to induce subsequent tolerance for classic DH and hemolytic antibody actually stimulate CBH reactivity and PCA antibody production.

Other studies of dose-route relationships for CBH production demonstrated that optimal immunogenic dosage requirements for CBH varied widely with route of antigen employed. OA in incomplete Freund's adjuvant (IFA) injected into footpads had low dosage requirements, intravenous OA had high dose requirements, and intradermal soluble OA dosage requirements were intermediate.

The observation that blatant immunogenic responses occur during the early period of tolerance induction amplifies the significant heterogeneity of the cellular immune response and may be of importance in understanding tolerogenesis.

Similar immunogenic-tolerogenic requirements and the prime role played by the basophil suggest a developmental or functional relationship between CBH and PCA antibody response.

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