SUPPRESSION OF T CELL-MEDIATED CUTANEOUS BASOPHIL HYPERSENSITIVITY BY SERUM FROM GUINEA PIGS IMMUNIZED WITH MYCOBACTERIAL ADJUVANT*

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Delayed-type hypersensitivity reactions are mediated by specifically sensitized T cells that recruit auxiliary effector cells via release of lymphokines (1). Classical tuberculin-type delayed hypersensitivity (DH) reactions are induced when an antigen is administered in an adjuvant consisting of a water-in-oil emulsion containing mycobacteria (complete Freund's adjuvant [CFA]) (2). However, reactions with a delayed time-course can also be elicited in guinea pigs immunized with protein antigens administered in saline or water-in-oil emulsion not containing mycobacteria. These reactions differ from DH by their lack of induration and their early disappearance after immunization and testing (3). These evanescent delayed reactions that occur in animals immunized without mycobacterial adjuvants contain masses of basophils and are called cutaneous basophil hypersensitivity (CBH), a term that allows differentiation from DH reactions that contain few basophils (4). CBH reactions are "carrier specific" and are mediated by sensitized T cells (5-8), but immune serum can also mediate cutaneous basophil responses (9, 10). These latter reactions are hapten specific and can be transferred with small amounts of guinea pig IgG1 antibody (11). Thus, both T and B cell-derived factors participate in the generation of delayed basophil-rich cutaneous hypersensitivity responses.

The fact that T cells can mediate both DH and CBH suggests that the two reactions are due to separate T cell subsets or that a common T cell is responsible for both reactions but that the final resulting components of the response (such as basophil content) are subject to regulatory factors. This latter possibility is suggested by the fact that guinea pigs immunized with CFA and skin tested at 3-4 wk have classical DH reactions with few basophils present, but recipients of T cells from these animals have delayed skin test reactions containing large basophil infiltrates (7). Thus, animals immunized for basophil-poor classical tuberculin reactions have T cells that can mediate CBH. The CBH activity of these cells appears to be suppressed, and it has been proposed that transfer removes them from a suppressive influence, thus allowing

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** Abbreviations used in this paper: CBH, cutaneous basophil hypersensitivity; CFA, complete Freund's adjuvant; DEAE, diethylaminoethyl; DH, tuberculin-type delayed hypersensitivity; HBSS, Hank's buffered salt solution; Ox-CLH, oxazolone-keyhole limpet hemocyanin; Ox-SRBC, oxazolone-sheep erythrocytes; PBS, phosphate-buffered saline; PEC, peritoneal exudate cells; Pic-HSA, picryl-human serum albumin.
them to express CBH. The nature of such a suppressive influence is not known. The current study reports that when serum and peritoneal exudate cells (PEC) from guinea pigs immunized with CFA are co-transferred intravenously to normal recipients, the cell-mediated transfer of CBH is suppressed. The responsible serum factor is nonantigen specific, has a molecular weight of ~70,000, and acts preferentially on cells from donors that express basophil-poor DH.

Materials and Methods

**Animals.** Hartley strain guinea pigs were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. Female animals weighing from 250–300 g were used.

**Immunization.** Guineas pigs were immunized by footpad injection (0.1 ml × 4) using hapten-protein conjugate antigens (100 μg/animal) emulsified with CFA (H37 Ra; Difco Laboratories, Detroit, MI) with an added 3 mg/ml of ground Mycobacterium tuberculosis. The CFA emulsions contained either oxazolone coupled to keyhole limpet hemocyanin (Ox-KLH) or Picryl-human serum albumin (Pic-HSA) obtained and prepared as described previously (7, 10, 11).

**Skin Testing.** Intradermal skin testing was performed in the shaved flank by injecting soluble protein antigens in 0.1 ml phosphate-buffered saline (PBS; 10 mM potassium phosphate-buffered 0.15 M NaCl, pH 7.4). Macroscopic reactions were read at 4, 24, and 48 h by estimating the diameter and extent of erythema and induration. Animals were skin tested with the antigen to which the donor had been immunized (Ox-KLH and PPD or Pic-HSA and PPD).

**Quantitative Histology.** After 48 h, animals were killed by ether anesthesia, and flank skins were removed and placed in baths of freshly prepared Helley’s fixative (Zenker-Formal; Fisher Scientific Co., Pittsburgh, PA) at room temperature. 24 h later, tissue blocks (2 mm × 10 mm) were excised from the center of skin reactions, washed with frequent changes of tap water, and stored in 70% ethanol. Subsequently, they were embedded in paraffin and 4-μm thick sections were cut and Giemsa stained at optimum conditions for identification of basophils (10). Differential cell counts were made in five adjacent central 180 μm Diam oil objective (X 1,000) fields in the uppermost dermis (10).

**Harvesting Cells and Serum.** PEC were obtained by the intraperitoneal injection of donors with 30 ml of sterile light mineral oil (Markol 52; Exxon Corp., Linden, NJ). This was usually done on day 25 post-immunization, except in those experiments where cells were used early after immunization (day 7) when it was done on day 4. 3 d later, cells were harvested with Hanks’ buffered salt solution (HBSS), drained from the peritoneal cavity into 250 ml sterile siliconized centrifuged bottles, and washed three times with HBSS and then resuspended in RPMI media 1640 with added penicillin (100 U/ml) and streptomycin (100 μg/ml) and 10% fetal calf serum (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) at 4°C.

**Blood.** Blood was obtained from the same animals by intracardiac needle puncture under ether anesthesia before cell harvesting. Serum was separated and used without storage.

**Intravenous Transfers.** Serum transfer was performed by intravenous injection using the dorsal foot veins. A volume of 5 ml was administered to each recipient. Cell transfer was also performed intravenously. This was done after serum injection in cases where both were administered. Approximately 175–200 × 10⁶ PEC was given to each recipient, with a donor:recipient ratio of 1:1. The transferred cells were at least 85% viable using the Eosin dye exclusion technique.

**Experimental Protocol (Fig. 1).** Donors were sensitized with antigens in CFA. On day 28 they were either skin tested or were killed for harvest of serum and PEC for intravenous transfer. Normal recipients were skin tested 1–2 h later. After 48 h, macroscopic skin reactions were read, and animals were killed for fixation and processing of delayed skin reaction sites for quantitation of the cellular infiltrate.

**Affinity Chromatography.** Ox-KLH (40 mg) was conjugated to 25 g of Sepharose 2B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) that had been activated previously with cyanogen bromide (12). A 32-ml column of Ox-KLH Sepharose was calibrated, and 16 ml of Ox-KLH plus CFA serum was applied, followed by PBS containing 0.02% sodium azide (Fisher Scientific Co.). The rate of flow was 5 ml/h. The serum that passed through the column...
Hartley female guinea pigs 

Peritoneal injection of mineral oil

Skin test (PPD, Ox-KLH)

Harvest and transfer

Skin test recipients (PPD, Ox-KLH)

Measure macroscopic erythema (at 24 and 48 hours) and microscopic basophils (48 hours)

PEC (1:1) Serum 5 ml + PEC + serum

became diluted to 25 ml and was concentrated back to 16 ml using negative pressure. Then the 
surface was dialyzed overnight against sterile PBS and was then transferred.

Ion-Exchange Chromatography. Serum globulins were precipitated using 18 g% sodium sulphate 
(Na₂SO₄). The precipitate was dissolved with PBS and dialyzed for 3 d against 0.01 M 
potassium phosphate buffer, pH 8.0 (starting buffer). The solution was centrifuged at 10,000 g 
for 10 min to remove debris, and the supernatant was applied by gravity to a 10- × 2.5-cm 
column containing diethylaminoethyl (DEAE) cellulose (Whatman, Maidstone, Kent, U. K.) 
that had been equilibrated previously with the starting buffer. Fractions were collected every 5 min at a flow rate of 30 ml/h. IgG₂ eluted with the starting buffer and then IgG₁ eluted with 
a second buffer (0.04 M phosphate buffer, pH 6.3). The third buffer (0.5 M NaCl) eluted the 
other proteins (11). Nominal protein concentrations in all samples were calculated on the basis 
that each 1 mg/ml had an absorbance at 280 nm of 1.3 (the extinction coefficient of IgG). 
Correction factors were used to account for dilution or concentration of the fraction pools when 
compared with the original serum. All samples were dialyzed overnight against sterile PBS and 
were then transferred.

Gel Filtration Chromatography. A column 5.0 × 100 cm was filled with Sephadex G-200 
(Pharmacia Fine Chemicals, Div. of Pharmacia Inc.). After calibration, 19 ml of fresh immune 
serum was applied by gravity, followed by PBS containing 0.2% azide and 5 mM EDTA at 
4°C. The column was run over 4 d by upward flow at a pressure of 10 cm and a rate of 28 ml/ 
h, with fractions collected every 15 min. The absorbance at 280 nm was determined for each 
fraction and three peaks were pooled and processed as stated above and used immediately.

Titration of Hemagglutinating Antibodies. Coupling of oxazolone to sheep erythrocytes (SRBC) 
and hemagglutination was performed as described previously (13). Duplicate serial twofold 
dilutions of each sample were made in PBS containing 2% heat-inactivated normal rabbit 
serum, absorbed previously with Ox-SRBC (13). Then 25 μl of 0.25% vol/vol Ox-SRBC in the 
same buffer were added to each 25-μl dilution in conical-bottomed microtiter trays. The trays 
were incubated for 1 h at room temperature and then overnight at 4°C. The titer was read as 
the last well showing definite agglutination. For anti-KLH titers, glutaraldehyde was used to 
couple KILH to SRBC (14).

Passive Cutaneous Anaphylaxis Titration of Antibody. Antisera (0.1 ml) in various dilutions were 
injected intradermally into 250-g normal guinea pigs. 3-4 h later, 1 mg antigen in 1 ml of 1% 
Evans blue dye was injected intravenously. The sites were read 20 min later for the diameter of 
extravasated dye.
Statistical Analysis. An unpaired t test was used to compute the significance ($P < 0.05$) of differences between groups.

Results

Cell Transfer of CBH from Guinea Pigs Immunized for DH (Fig. 2). PEC from donors immunized with Ox-KLH plus CFA 28 d previously was administered intravenously to recipients and transferred the ability to elicit delayed responses to the immunizing antigen Ox-KLH (Fig. 2a). Macroscopically, the reaction size in the recipients was significantly smaller ($P < 0.001$) than those elicited in the donors. As reported previously (7), skin reactions elicited with Ox-KLH in the recipients were basophil rich (Fig. 2a), unlike the DH responses of the donors that were basophil poor ($P < 0.001$). Fig. 2b shows that PPD skin testing yielded results comparable to those seen with the immunizing hapten protein conjugate. The reactions elicited in cell transfer recipients were smaller than those of the actively immunized ($P < 0.001$) and contained significantly increased numbers of basophils ($P < 0.001$). These results suggest that (a) cells are able to transfer delayed responses but are insufficient to induce DH and instead induce reactions resembling CBH, and (b) actively immunized animals seem to possess the ability to inhibit cell-mediated CBH.

Immune Serum Modulates Macroscopic and Microscopic Aspects of Delayed Skin Test Responses

![Graphs showing macroscopic and microscopic responses](image-url)

**Fig. 2.** Macroscopic erythema and microscopic basophils in skin test responses of actively sensitized guinea pigs or recipients of immune PEC. Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC were harvested at 28 d. Data were pooled from six experiments. Actively immunized and PEC recipient animals were skin tested with 100 μg Ox-KLH (Fig. 2a) and with 25 μg PPD (Fig. 2b). □, erythema; □, basophils.
in Recipients of Immune Cells (Fig. 3 and Table I). When immune PEC and immune serum that were harvested 28 d after immunization were transferred intravenously, skin testing with the immunizing conjugate Ox-KLH elicited delayed reactions that were macroscopically larger than those seen in animals that received cells alone (Fig. 3a). Such augmented responses were evident at 24 and 48 h (only 48-h responses are shown). Early (4 h) indurated and hemorrhagic lesions (i.e., Arthus-like) were not seen in recipients. Immune serum alone transferred the ability to elicit skin test reactions to the immunizing conjugate that were smaller than those seen with cells. PPD responses were less, though significantly ($P < 0.001$) augmented by concomitant administration of PEC and serum, whereas immune serum alone did not have the ability to transfer reactivity to PPD skin testing (Fig. 3b).

Concomitant transfer of immune serum with immune cells altered the histological nature of the elicited skin test reactions. Individually, cells and serum allowed the elicitation of respectively strong and weak cutaneous basophil responses. Together, their administration resulted in suppression of the strong basophil infiltrate that occurred with cells alone. The suppression of basophil infiltration was evident to skin testing with the immunizing conjugate Ox-KLH (Fig. 3a) and PPD (Fig. 3b). The reduced basophil response is all the more surprising when viewed in the light of the

**Fig. 3.** Immune serum modulates skin test responses in recipients of immune PEC. Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC (1 donor:1 recipient) and immune serum (5 ml) were harvested at 28 d and transferred intravenously. Data were pooled from six experiments. Actively immunized and recipient animals were skin tested with 100 μg Ox-KLH (Fig. 3a) and with 25 μg PPD (Fig. 3b). Statistically significant differences represent comparisons to the group that received cells alone. □, erythema; ○, basophils.
Table I

| Transferred | Number of animals | Skin test dose | Microscopic cells per 5,000× fields ± SE |
|-------------|-------------------|----------------|----------------------------------------|
|             |                   |                | Macroscopic erythema | Basophils | Eosinophils | Neutrophils | Mononuclear |
| Cells (PEC) | 6                 | 30             | 9 ± 1                   | 102 ± 24  | 11 ± 4 | 3 ± 1 | 74 ± 11 |
|            | 18                | 100            | 14 ± 1                  | 134 ± 12  | 7 ± 1 | 12 ± 5 | 54 ± 7  |
|            | 6                 | 300            | 16 ± 2.0                | 139 ± 30  | 13 ± 3 | 5 ± 3 | 67 ± 20 |
| Serum      | 6                 | 30             | 3 ± 1                   | 17 ± 8    | 7 ± 1 | 12 ± 4 | 34 ± 3  |
|            | 6                 | 100            | 9 ± 4                   | 29 ± 6    | 19 ± 6 | 4 ± 2 | 37 ± 7  |
|            | 6                 | 300            | 13 ± 1                  | 24 ± 7    | 17 ± 3 | 11 ± 3 | 66 ± 7  |
| Cells + serum | 6          | 30             | 16 ± 2‡                 | 49 ± 10‡  | 13 ± 4 | 10 ± 3 | 55 ± 17 |
|            | 18                | 100            | 27 ± 2‡                 | 54 ± 10‡  | 18 ± 4 | 30 ± 12‡ | 79 ± 4‡ |
|            | 6                 | 300            | 32 ± 4‡                 | 33 ± 10‡  | 18 ± 4 | 87 ± 20‡ | 191 ± 11‡ |
| Nil        | 6                 | 30             | 0                       | 6 ± 3     | 6 ± 3 | 1 ± 1 | 43 ± 3  |
|            | 6                 | 100            | 2 ± 1                   | 2 ± 1     | 6 ± 3 | 8 ± 6 | 34 ± 3  |
|            | 6                 | 300            | 2 ± 2                   | 5 ± 3     | 4 ± 2 | 10 ± 6 | 72 ± 14 |

* Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC and immune serum harvested at 28 d were transferred intravenously at a dose of 1 donor to 1 recipient for the PEC and 5 ml for the serum. 1–2 h after transfer, recipient animals were skin tested with 0.1 ml PBS containing 30 μg, 100 μg, and 300 μg of Ox-KLH.

‡ Statistically significant (P < 0.05) when compared with the group that received cells alone.

The finding that immune serum augmented the macroscopic size of reactions elicited with the immunizing conjugate in recipients of immune PEC was demonstrated at a wide range of skin test doses, 30 μg, 100 μg, and 300 μg, as was suppression of basophil infiltration (Table I). Thus, immune serum modulation of the macroscopic and microscopic aspects of cell-dependent reactions was not dependent on any particular skin test dose.

Concomitant transfer of immune cells (1:1 ratio) and fourfold dilutions of serum revealed that significant macroscopic augmentation and microscopic basophil suppression was not present at a volume of 1.25 ml or less. Normal serum (5 ml) did not possess either activity (data not shown).

Macroscopic Augmentation Is Antigen Specific, and Microscopic Suppression of Basophil Infiltrates Is Nonspecific (Fig. 4 and Table II). To ascertain whether the ability of
Fig. 4. Specificity of immune serum that modulates skin test responses in recipients of immune PEC. Separate groups of donors were immunized with either Ox-KLH or Pic-HSA (100 μg each) emulsified with CFA. PEC and immune serum were harvested at 28 d. Animals received immune cells alone, immune cells plus homologous immune serum, or immune cells plus heterologous immune serum. Animals were skin tested with 100 μg Ox-KLH (Fig. 4a) or with 100 μg Pic-HSA (Fig. 4b). △, erythema; ▲, basophils.

TABLE II
Antigen-Affinity Chromatography of Immune Serum That Modulates Skin Test Responses in Recipients of Immune Cells*

| Immune components transferred | Recipient’s skin test responses |
|------------------------------|--------------------------------|
|                              | Recipient's skin test responses |
|                              | Macroscopic erythema | Microscopic basophils per 5,000 fields | mm ± SE |
| Cells alone                   | 12.3 ± 1.2          | 87.7 ± 4.5 |
| Cells + serum                 | 21.0 ± 1.5‡         | 53.3 ± 3.5‡ |
| Cells + Ox-KLH Sepharose      | 13.7 ± 0.3          | 46.3 ± 11.3‡ |
| column-passed serum           |                   |        |

* Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC and immune serum were harvested at 28 d. Serum was passed through an Ox-KLH Sepharose affinity column. Recipient animals were skin tested with 100 μg Ox-KLH. Each group consisted of three animals.
‡ Statistically significant (P < 0.05) when compared with cells alone group.
immune serum to macroscopically augment reaction size and suppress basophil infiltration was antigen specific, two groups of animals were immunized, each with a different hapten-protein conjugate in CFA. Ox-KLH and Pic-HSA were used as the immunizing antigens. Crossover studies were done, whereby immune cells of one specificity were transferred either alone with homologous immune serum or with heterologous immune serum.

Fig. 4a shows that macroscopic augmentation of elicited reactions was antigen specific. Pic-HSA plus CFA immune serum did not significantly enlarge Ox-KLH responses elicited after transfer of cells with Ox-KLH specificity, whereas Ox-KLH plus CFA immune serum did. In contrast, both homologous and heterologous immune CFA serum had the ability, when administered with Ox-KLH cells, of suppressing basophil infiltration. Fig. 4b shows that the heterologous Pic-HSA plus CFA serum that nonspecifically suppressed the ability of immune cells with Ox-KLH specificity to transfer the elicitability of basophil-rich delayed reactions could suppress basophil infiltration in a homologous system when administeed with cells of Pic-HSA specificity. Also, Ox-KLH plus CFA-immune serum transferred with immune cells of Pic-HSA specificity suppressed the infiltration of basophils into Pic-HSA skin test lesions (Fig. 4b). Macroscopically, homologous Pic-HSA-immune cells plus immune serum did augment reaction size, but this augmentation did not reach statistical significance. In contrast, Ox-KLH plus CFA serum had a much reduced effect upon the macroscopic size of Pic-HSA skin tests when given in combination with Pic-HSA-immune cells.

These data suggest that the ability of immune CFA serum to macroscopically augment the elicited skin reaction size in recipients of immune cells is antigen specific, as it is only significant in a homologous system. However, immune CFA serum can suppress the infiltration of basophils into such reactions in an antigen-nonspecific manner, as evidenced in two different immunization protocols.

An antigen affinity column was used to further investigate the dual activity of immune CFA serum. 16 ml of 28-d Ox-KLH plus CFA-immune serum was passed through an Ox-KLH-Sepharose column. All anti-oxazolone and anti-KLH hemagglutinating antibody was removed (data not shown). The passed serum was concentrated and administered in 5-ml equivalent volumes. Table II shows that immune serum that macroscopically augmented reactions elicited in immune cell recipients lost this activity after passage through an antigen affinity column. In contrast, the ability of the immune serum to suppress basophil infiltration into such reactions was not altered by passage through an antigen affinity column. This further suggests that these two serum activities are separable and distinct, as one (macroscopic augmentation) binds to an antigen column and one (basophil suppression) does not.

Fractionation of Immune Serum That Modulates Skin Test Responses in Recipients of Immune Cells (Tables III and IV). An 18 g% Na2SO4 globulin fraction of immune serum (Ox-KLH plus CFA) was applied to a DEAE column and eluted as three peaks with stepwise changes in buffer. Hemagglutination titers, PCA titers, and macroscopic augmenting and basophil-suppressing activity were assessed for each fraction. The initial globulin fraction retained the ability to both macroscopically augment reactions and suppress the infiltration of basophils when administered with immune PEC to recipient animals that were skin tested with Ox-KLH. The IgG2-containing fraction retained the ability of the whole serum to augment macroscopic reaction size under
TABLE III
DEAE Ion Exchange Chromatography of Immune Serum That Modulates Skin Test Responses in Recipients of Immune Cells*

| Immune components transferred | Recipient's skin test responses |
|------------------------------|--------------------------------|
|                              | Macroscopic erythema | Microscopic basophils per 5,000× fields ± SE |
|                              | mm ± SE               |                                             |
| Cells alone                  | 13.0 ± 0.9            | 146.8 ± 12.0                                |
| Cells + serum                | 27.3 ± 1.3$\ddagger$  | 47.0 ± 16.5$\ddagger$                       |
| Cells + IgG$_2$ fraction     | 18.2 ± 1.3$\ddagger$  | 137.2 ± 13.2                                |

* Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC and immune serum were harvested at 28 d. Serum was fractionated on a DEAE cellulose column. Data is shown only for the IgG$_2$ fraction. Recipient animals were skin tested with 100 μg Ox-KLH. Results were pooled from two experiments with six animals in each group. $\ddagger$ Statistically significant when compared with the cells alone group.

TABLE IV
Sephadex G200 Gel Filtration Chromatography of Immune Serum That Modulates Skin Test Responses in Recipients of Immune Cells*

| Immune components transferred | Recipient's skin test responses |
|------------------------------|--------------------------------|
|                              | Macroscopic erythema | Microscopic basophils per 5,000× fields ± SE |
|                              | mm ± SE               |                                             |
| Cells alone                  | 11.2 ± 0.5            | 112.8 ± 6.3                                 |
| Cells + excluded fraction    | 10.0 ± 1.2            | 112.0 ± 24.6                                |
| Cells + IgG-sized fraction   | 18.7 ± 1.8$\ddagger$  | 127.0 ± 10.7                                |
| Cells + albumin-sized fraction | 19.0 ± 2.52$\ddagger$ | 31.0 ± 7.1$\ddagger$                        |

* Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC and immune serum were harvested at 28 d. Serum was fractionated on a Sephadex G-200 column. Recipient animals were skin tested with 100 μg Ox-KLH. Each group consisted of three animals. $\ddagger$ Statistically significant when compared with the cells alone group.

similar circumstances, but this fraction did not have the basophil-suppressing activity (Table III). Neither the IgG$_1$ serum fraction nor the third fraction from the DEAE column had macroscopic augmenting activity or detectable basophil-suppressing activity; this latter activity might have been lost during fractionation. The IgG$_2$ fraction had most of the hemagglutinating anti-oxazolone and anti-KLH antibody activity of whole serum, and the IgG$_1$ fraction had virtually all of the PCA activity of both specificities (data not shown).

A Sephadex G-200 column (90 × 5 cm) was used to assess the size of serum factors that had the ability to suppress basophil infiltrates. 19 ml of immune serum was applied to the column, and three major protein peaks were collected. Concentrated pools of fractions representing these three peaks were transferred concomitantly with immune cells. Table IV shows that neither the excluded fraction nor the IgG-sized fraction had the ability to suppress basophil influx to the local reaction site. The
Comparison of 7-d vs. 28-d Immune Serum Activity in Modulation of Skin Test Responses in Recipients of 7-d Immune Cells

| Immune components transferred | Recipient's skin test responses |
|------------------------------|--------------------------------|
|                              | Macroscopic erythema | Microscopic basophils per 5,000× fields ± SE |
| Cells (7 d)                  | 13 ± 2.3             | 134 ± 33.2          |
| Cells (7 d) + serum (7 d)    | 11 ± 3.0             | 156 ± 51.6          |
| Cells (7 d) + serum (28 d)   | 17 ± 1.2             | 115 ± 12.5          |

* Donors were immunized with 100 μg Ox-KLH emulsified with CFA. PEC and immune serum were harvested at either 7 d or 28 d. Early (7 d) cells were administered with either early (7 d) serum or late (28 d) serum. Recipient animals were skin tested with 100 μg Ox-KLH. There were three animals in each group. Later (28 d) serum had the property of significantly suppressing basophil infiltration when given with late (28 d) cells.

Discussion

Delayed cutaneous reactions that are elicited by specific antigen in a sensitized host are complex processes consisting of several components. When guinea pigs are immunized with a protein antigen emulsified with complete Freund's adjuvant, classical tuberculin-type DH reactions are elicited by PPD or by the immunizing protein antigen. Macroscopically, DH reactions have a characteristic delayed onset (6–8 h after testing), a broad peak over 24–48 h, and are characterized by strong erythema and induration. Microscopically, DH reactions are rich in monocyte/macrophages that are recruited from the bone marrow via the blood. Basophils comprise <5% of the infiltrate of these classical DH reactions. In contrast, basophils
comprise 25–60% of the infiltrate in delayed, weakly erythematous, and nonindurated CBH responses that are elicited in guinea pigs immunized with proteins administered without mycobacterial adjuvant (4, 7, 15).

The T cell dependence of DH responses is apparent from the hapten-carrier specificity of reactions elicited in actively sensitized animals and by the fact that elicitability of delayed reactions of similar fine specificity requirements is transferable from these animals to normal recipients by live sensitized T cells and not by soluble serum components (7, 16). However, careful examination of reactions elicited in recipients of immune T cells reveals that these responses differ significantly from those of the donors. In particular, recipient responses are weaker in intensity of erythema and induration, and microscopically they contain significant infiltrates of basophils (7). Therefore, recipient reactions resemble CBH responses rather than DH responses that are elicited in the donors. Thus, the landmark cell transfer experiments of Landsteiner and Chase in 1942 were in fact probably T cell transfers of CBH from donors with DH. These T cell transfers of CBH from donors with DH are not due to major histocompatibility complex restrictions or an allogeneic effect, as they occur between strain 13 inbred guinea pigs (7) as well as between outbred Hartley guinea pigs.

The current study has compared reactions elicited in recipients of cells alone to those of animals receiving both immune cells and immune serum. Our findings indicate that components of immune serum act in concert with immune T cells to produce elicitation of reactions in recipients that more fully resemble those of the donors than do reactions in recipients of cells alone. At least two serum components are responsible for this modulation of T cell-dependent delayed reactions. One is an antigen-specific IgG2 antibody that leads to macroscopic augmentation of the cell-mediated response. The other is a nonantigen-specific albumin-sized serum component that leads to suppression of the T cell-dependent microscopic basophil infiltrates.

The macroscopic augmentation was antigen specific, as it was not present with serum from donors immunized with another antigen in CFA and was removed by an antigen affinity column. Asherson and Loewi (17) also reported a synergistic action of immune cells and immune serum in the passive transfer of delayed hypersensitivity. However, partial augmentation of cell transfer could be demonstrated after the induction of an irrelevant antigen-antibody reaction at the skin test site (18, 19). The current experiments support the concept that an antigen-antibody reaction is responsible for macroscopic augmentation by demonstrating that IgG2 antibody has this property. This antibody isotype has the ability to fix complement, and such a mechanism or antigen retention at the skin test site might participate in the augmented response. However, the exact mechanism of augmentation by antibody is not clear, as there is differing evidence available for different antigens and immunization protocols (18, 20, 21). Only a small but significant increase in macroscopic reactions was demonstrable when skin testing with PPD, whereas a more marked augmentation was present when testing with the hapten-protein conjugate. A poor IgG antibody response to PPD at 4 wk after immunization might explain this difference.

Immune serum from animals immunized with CFA was found to have the very important property of suppressing the basophil-rich histology present after transfer of immune cells alone. Interestingly, this property was not found in the IgG2 serum fraction and thus could be differentiated from the macroscopic augmenting ability.
The activity of suppressing basophils was not antigen specific, as it could be elicited when immune cells were transferred in combination with serum from donors immunized with another antigen in CFA, and basophil-suppressing activity was still present after specific immune serum was passed through an antigen affinity column. Gel filtration chromatography revealed that the basophil-suppressing activity resided in the albumin-sized fraction, though possibly it was caused by an even smaller molecule that was albumin bound. It is tempting to speculate that the activity was caused by a cell product and to compare it with analogous regulatory factors in CFA-induced serum that selectively suppress IgE antibody responses (22). In the mouse, Kishimoto (23) has demonstrated that such a factor is a T cell product with a 55,000–60,000 mol wt. Thus far, our attempts at producing the basophil-suppressing factor in vitro have not been successful (Mitchell and Askenase, unpublished observations).

Basophil-rich delayed-onset reactions are elicited at early intervals after immunization with soluble protein antigens emulsified with CFA, but, at later intervals, basophil-poor and mononuclear cell-rich DH reactions are elicited (4, 15). Comparison of reactions elicited after transfer only of early cells (7-d postimmunization) with those elicited after transfer of early cells plus early serum revealed that macroscopic augmentation and basophil suppression did not occur. Later serum (28 d), which was known to possess basophil-suppressing activity when administered with late (28 d) cells, failed to suppress cell-dependent basophil infiltrates when transferred with early (7 d) cells. These results suggest a distinct difference between early and late immune cells after immunization with CFA. Early cells are resistant to CBH-suppressive activity of late serum, and it is at this stage that early cells are most effective in recruiting basophils. It has been shown (24) that cells from animals immunized with CFA in which hospital infiltrates are suppressed are potent producers of lymphokines that are chemotactic for basophils. The serum activity we described might either prevent the production of, or render inoperative, such chemotactic stimuli.

In conclusion, our results suggest that elicitibility of DH reactions is not achieved by transfer of T cells alone, although these responses are T cell dependent. Additional antigen-specific and antigen-nonspecific factors in immune serum are required to elicit delayed responses in recipients that more faithfully reproduce those elicited in the donors. The biological utility of this phenomenon is not clear. However, delayed basophil infiltrates are required for immune rejection of some large multicellular parasites, whereas monocyte/macrophage-rich responses are more appropriate to immune resistance to facultative intracellular microorganisms, such as mycobacteria (1, 25, 26). Therefore, the ability of specific and nonspecific factors in immune serum to modulate the basophil vs. monocyte/macrophage component of T cell-dependent tissue responses might have important biological consequences.

Summary

Guinea pigs immunized with protein antigens emulsified with complete Freund's adjuvant (CFA) and skin tested at 3–4 wk have classical tuberculin-type delayed hypersensitivity (DH) reactions with few basophils present. However, recipients of T cells from these animals have delayed responses containing large basophil infiltrates

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2 Brown, S. J., S. J. Galli, G. Gliech, and P. W. Askenase. 1982. Ablation of immunity to *Amblyomma americanum* by anti-basophil serum: cooperation between basophils and eosinophils in expression of immunity to ectoparasites (ticks) in guinea pigs. *J. Immunol.* In press.
and thus resemble basophil-rich cutaneous basophil hypersensitivity (CBH) responses that are elicited in animals immunized without CFA. This suggests that animals immunized with CFA have T cells with basophil-recruiting capacity but that this activity is suppressed.

Using a transfer system, we found that immune serum from donors immunized with CFA had the ability to suppress the basophil-recruiting capacity of immune T cells. When immune serum and peritoneal exudate cells from guinea pigs immunized with CFA were co-transferred intravenously to normal recipients, the cell-mediated transfer of basophil-rich responses was suppressed. The responsible serum factor was antigen nonspecific, had an ~70,000 mol wt, and acted preferentially on cells from donors that express basophil-poor DH responses.

Thus, tuberculin-type delayed hypersensitivity and CBH might be mediated by a common T cell, but the resulting basophil component of the delayed response depends on the modulation of T cell recruitment of basophils by factors in CFA-immune serum.

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