The Cutaneous Basophil Response to Particulate Antigens[1] (36978)

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The presence of basophilic leukocytes in inflammatory reactions associated with hypersensitivity states has been known for some time (1–3). More recently, however, it was noted that a clear predominance of these cells (up to 65%) occurs in inflammatory exudates early after immunization with soluble protein antigens alone, or in incomplete Freund's adjuvant. These reactions had been termed "Jones-Mote" hypersensitivity (4) or according to Richerson, Dvorak and Leskowitz "cutaneous basophil hypersensitivity" (CBH) (5) as opposed to the classical delayed or tuberculin hypersensitivity. In the latter, while basophils may also occur, as proven by skin biopsies or "skin window" exudates (6), they appear in much smaller numbers (usually not more than 10%) (7–10). The evaluation of the numbers of basophils in these reactions is based mainly on the morphologic recognition of this highly characteristic cell although a correlation between the incidence of basophils and titers of skin histamine has been demonstrated (11).

The presence of high numbers of basophils in other conditions such as contact allergy and allograft rejection has also been pointed out (12, 13) and a separate subdivision for CBH within the category of cell mediated reactions has been suggested. Unfortunately, the role of the basophilic leukocyte in these cellular reactions has not yet been elucidated and a great deal of speculation exists as to the significance of its contents and its possible function.

The various reactions studied for basophils, have in the past, usually been elicited by immunization and challenge with soluble antigens. To establish the spatial and functional relationship between this cell and the antigen, we have attempted to use a particulate antigen that could be easily recognized in the histologic material and distinguished from the cells participating in the inflammatory reaction. In one experiment, a soluble antigen was covalently coupled to sheep red blood cells (SRBC) and in another SRBC itself acted both as antigen and marker.

Materials and Methods. Two experiments were undertaken.

Experiment A. Antigen for immunization. A specific precipitate was prepared by adding a solution of 0.5 mg of crystalline hen egg albumin (EA) (Pentex, Inc., Kankakee, IL) in saline to 3 ml of a potent rabbit antiserum which was then incubated at 37° for 0.5 hr, refrigerated at 4° over night and washed three times with cold saline. The supernatant was tested for excess antibody by addition of more EA.

Antigens for skin testing. Sheep red blood cells (SRBC) were used as a particulate marker. To confer additional resistance and protection against accelerated digestion and lysis, they were treated with formaldehyde (f-SRBC) according to the method described by Butler (14). The EA was covalently conjugated to f-SRBC using 1-ethyl 3-(3-dimethylaminopropyl) carbodiimide HCl (ECDI) (Ott Chemical Co., Muskegon, MI) as reagent (15). These EA coupled cells (f-SRBC-EA) remained stable in saline suspension for long periods.

Immunization. Twelve female white guinea pigs (300–400 g) were immunized with the specific precipitate incorporated in an emulsion with incomplete Freund's adjuvant (IFA). A total of 0.1 ml of emulsion containing 6 μg EA was distributed in the animal's four foot pads.

Skin testing. One week after immunization, the animals were shaved, depilated and skin

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tested with 0.1 ml of the following antigens: (a) a 10% suspension of f-SRBC-EA, (b) a 1% suspension of f-SRBC-EA, (c) a 10% suspension of f-SRBC, (d) a 1% suspension of f-SRBC, (e) 50 μg EA and (f) 10 μg EA.

Histology. Animals were sacrificed at 3, 6, 18, 24, 48, 72 and 96 hr after the skin tests and skin sites were removed, fixed in Karnovsky II fixative (16), postfixed with OsO_4, dehydrated, embedded in Epon, cut in 1 μm sections and stained with Giemsa.

Experiment B. Immunization. Six female white guinea pigs (300–400 g) were immunized via the foot pads with either 0.1 ml of an emulsion of a 1% suspension of SRBC in incomplete Freund's adjuvant or 0.4 ml of a 10% suspension of SRBC in saline.

Skin testing. After 7 days, all animals plus 2 unimmunized controls were shaved, depilated and skin tested with 0.1 ml of (a) 10% suspension of SRBC, (b) 1% suspension SRBC, (c) 10% suspension of f-SRBC and (d) 1% suspension of f-SRBC, all in saline.

Histology. After 24 hr they were sacrificed, the skin sites were removed and processed as above.

Results. Experiment A. Grossly, there was mild erythema and induration at the sites injected with 50 and 10 μg soluble EA and the 10% suspension of f-SRBC-EA in all animals. The reaction could not be detected in the early hours, was maximal between 18 and 24 hr, weaker at 48 and had disappeared after 72 hr. No reactions were seen at sites injected with f-SRBC or 1% f-SRBC-EA.

Histologically, the lesions produced by EA and f-SRBC-EA were composed of a mixed cell exudate, where macrophages, basophils, lymphocytes, occasional neutrophils, mast cells and rare eosinophils could be recognized. The total cell population was sparse in the early hours (lymphocytes and macrophages seemed to be the first cells to appear), at 24 hr it had markedly increased and was maintained thereafter. A small, but approximately constant number of mast cells was present from the beginning and these cells were easily distinguished from the basophilic leukocytes by their size, nucleus and type of granules.

The f-SRBC were easily identified in the cell exudate and maintained a surprising structural integrity. They were located primarily in the deep aspect of the dermis and became gradually incorporated into macrophages (Fig. 1). In many instances up to 5–7 intact red cells could be seen in the cytoplasm of the macrophages. The f-SRBC fragmented progressively with time and the fragments could be observed free or in macrophage cytoplasms. Fragmentation and phagocytosis seemed to start and be more intense superficially, whereas the f-SRBC in the deeper layers remained intact throughout the entire experiment.

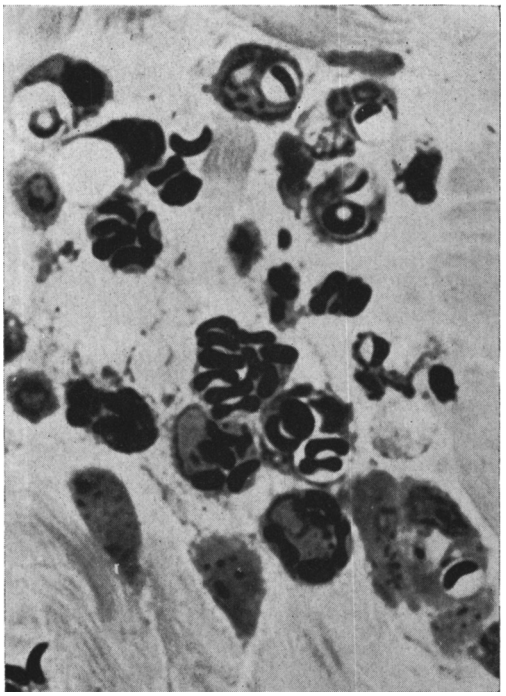

FIG. 1. Cutaneous basophil hypersensitivity reaction in guinea pig at 24 hr showing particulate antigen (f-SRBC-EA) in deep dermis, practically all within the cytoplasm of macrophages (1000×).

There was no difficulty in distinguishing the f-SRBC from the guinea pigs own red cells due to the extravascular location and deeper staining of the former.

Only occasional basophilic leukocytes could be found 3 or 6 hr after skin test, at 24 hours their concentration reached a peak which decreased slowly thereafter. As shown in Fig. 2, the basophils appeared in the superficial dermis within blood vessels or in the vessel walls, near macrophages or lympho-

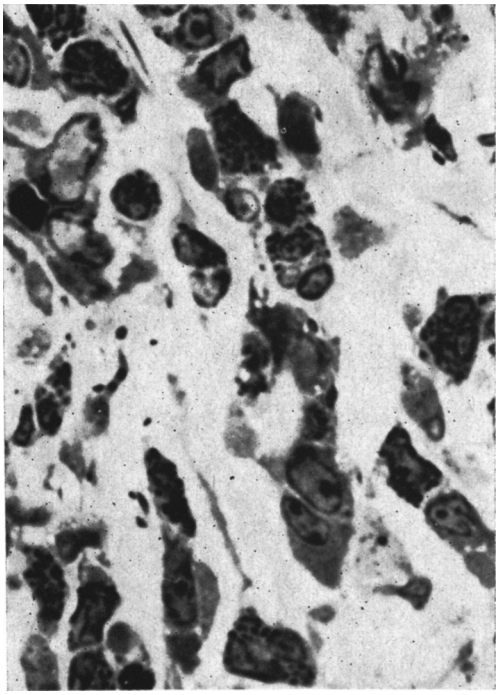

FIG. 2. Cutaneous basophil hypersensitivity reaction in guinea pig at 24 hr showing many basophils present in superficial dermis. None of the particulate antigen eliciting this response is visible in this field (1000×).

cytes, or alone. On rare occasions, they were observed within the epidermis. Basophilic granules could often be dispersed extracellularly and also incorporated in the cytoplasm of macrophages. The number and distribution of the basophils was essentially similar in skin sites tested with EA or f-SRBC-EA. Only slight increase in basophil count and total cellularity were seen with the higher concentrations of antigens. However, when only f-SRBC were used, the striking difference was that the basophilic leukocytes were almost totally absent. Interestingly, although the largest number of f-SRBC-EA were present in the deep dermis, the highest concentrations of basophils were located in the superficial dermis. In consequence, there was no direct topographical relationship between the antigen and the basophil; and only randomly and infrequently could both cells be observed one next to the other. Neither intact nor fragmented f-SRBC were ever seen within the basophils.

Experiment B. Grossly there was significant erythema and induration at the site of all skin tests. Reactions were somewhat greater in the animals immunized with 0.4 ml of a 10% suspension of SRBC in IFA. Smaller reactions were, in general, obtained with 1% than with 10% suspensions. Reactions to f-SRBC were only slightly less than to SRBC. Nonimmunized control animals gave no visible reaction with any antigens.

Histologically, the picture was in all instances comparable to skin sites of Expt A biopsied at similar time intervals. The exudates were composed of the same proportions of cells, but at 24 hr SRBC had undergone a pronounced fragmentation and destruction and the number of identifiable cells and particles had markedly decreased as opposed to the f-SRBC, which again maintained their shape and integrity and could be easily identified and localized. Fragments of SRBC could also been seen free or in macrophages in greater amounts and with a more random distribution within the dermis than with f-SRBC.

A basophil response of comparable magnitude to that of Expt A was observed in the immunized animals, whereas, in the controls these cells were almost totally absent. Maximal numbers of basophils were found in animals immunized with SRBC in saline and skin tested with SRBC. A slightly smaller number were present in sites tested with f-SRBC. The values obtained with the animals immunized with SRBC in IFA were somewhat smaller although the relative proportions of basophils in the skin tests with SRBC and f-SRBC were maintained.

Similar features were observed in this experiment as to the localization of the basophils with respect to the antigen. Basophils were again concentrated in the superficial dermis, whereas, the SRBC and especially the f-SRBC, were mainly in the deep dermis. Again, there was no topographical relationship between the antigen and the basophils.

Discussion. The work reported here confirms previous findings that the basophilic leukocyte appears in significant numbers in animals sensitized with soluble or particulate

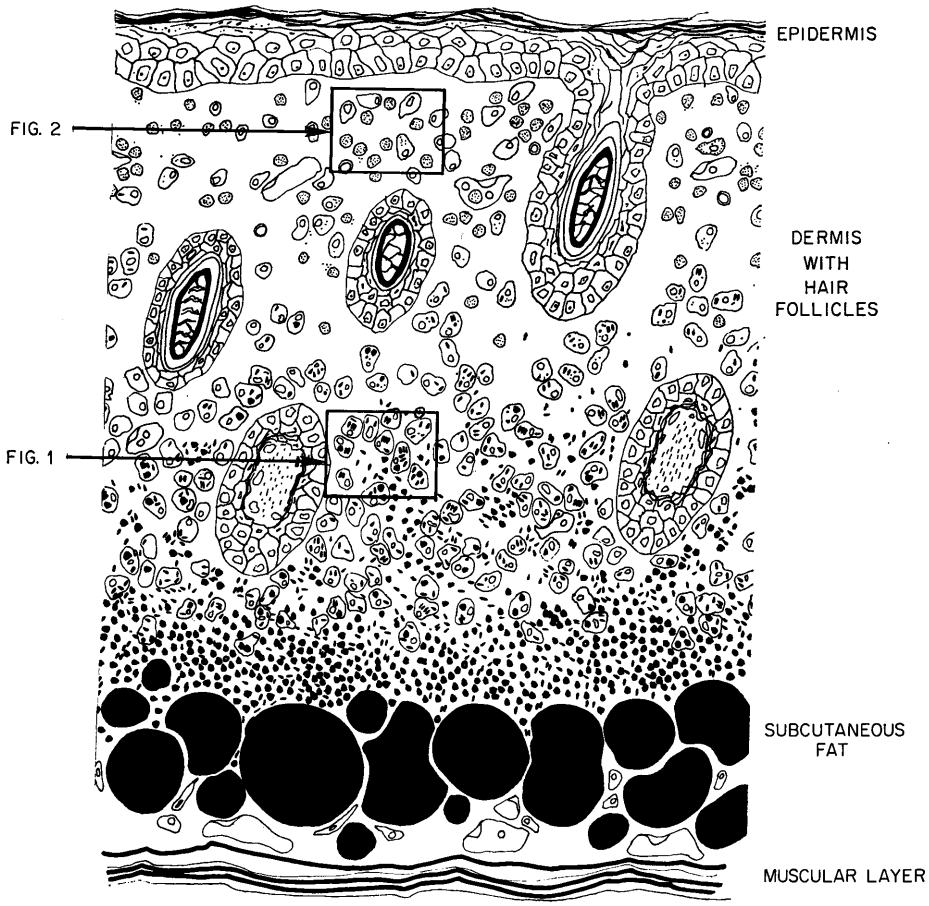

FIG. 3. Schematic drawing of guinea pig skin illustrating its different components. Rectangles outline regions having typical appearance seen in Figs. 1 and 2.

antigens whether or not they are incorporated in IFA (17). The point of departure was using particulate antigens for eliciting reactions and providing visible markers for the presence and location of such antigen. From the experiments using f-SRBC-EA in animals sensitized to EA (Expt A), it could be concluded that no close topographical relationship existed between the entering basophil and the enciting antigen, the former appearing primarily in the upper dermis and the latter in the deep dermis (see Fig. 3).

Since the possibility existed that EA could come off the f-SRBC-EA conjugate, diffuse to the upper dermis and generate a basophil response there, the studies were repeated using SRBC itself as antigen (Expt B). Essentially similar findings were obtained as to number and location of the basophils.

It can, therefore, be concluded that the antigen does not exert a direct chemotactic attraction for the basophilic leukocyte nor does it seem that this cell is one engaged in phagocytosis since none were ever seen to engulf antigen-coated SRBC. However, since it was conceivable that the eliciting antigen-coated SRBC had migrated to the deep dermis before the arrival of the basophil, an experiment was attempted in which the red cells were injected into a preformed 24 hr old CBH skin site. Twenty-four hours later, biopsy of skin sites still revealed no phagocytosis by the basophils of injected red cells.

To explain these observations, we suggest

the production of a mediator from some cell present early in the inflammatory reaction that would exert a chemotactic effect on basophils. As with classic delayed hypersensitivity the first reaction might be between antigen and a circulating, sensitized lymphocyte. Stimulation by antigen of lymph node cells from animals exhibiting CBH reactivity has already been reported *in vitro* (18), and we have successfully produced local CBH reactions by intradermal injection of live lymph node cells plus antigen into unimmunized recipients.

The reason why the largest concentration of basophils appears always in the superficial dermis rather than near the antigen in the deeper dermis remains to be answered, but it seems more likely that this depends on a particular local environment or specific site of egress from blood vessels rather than on the basophil itself.

The role of the invading basophil also remains in question since a phagocytic function similar to that of granulocytes or macrophages could not be supported by our observations. One possibility is that they play a significant role in the inflammatory process through uptake or release of various substances stored in their prominent granules (19).

Summary. Cutaneous basophil hypersensitivity was elicited with particulate antigens consisting of egg albumin coupled to formalinized sheep red blood cells and sheep red blood cells themselves. By observation of these visible markers, it could be seen that basophils appeared predominantly in the superficial dermis while the antigen was engulfed by macrophages in the deeper dermis.

The absence of contact between basophils and particulate antigen suggests that they are not primarily phagocytic in function and appear in response to chemotactic substances released from other cells.

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