Induction of Delayed-Type Hypersensitivity to Staphylococcal Antigens in Guinea Pigs

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Delayed-type hypersensitivity against staphylococcal antigens was induced in guinea pigs using Freund’s incomplete as well as Freund’s complete adjuvant. In animals sensitized with Freund’s complete adjuvant the induction time and the duration of delayed-type hypersensitivity were longer and the skin reactions were stronger compared to animals sensitized with Freund’s incomplete adjuvant. The difference between animals sensitized with Freund’s incomplete or complete adjuvant may reflect the difference between tuberculin- and Jones-Mote type hypersensitivity.

Certain types of eczematous lesions contain more bacterial cells than does healthy skin. Up to 94% of all patients with eczema grow Staphylococcus aureus (S. aureus), sometimes even as pure strain, in their lesions [1,2]. As early as 1892 Unna [3] proposed a microbial etiology for eczema. Scholtz and Raab [4] produced eczema-like lesions in man with a broth incubated 24 hr with S. aureus. Robert [5] studied skin sensitization with a variety of culture filtrates of epidermal bacteria and observed the strongest reactions with S. aureus. Storck [1] proposed that these were allergic rather than toxic reactions. He sensitized guinea pigs with S. aureus and culture filtrates (concentrated tenfold by vacuum distillation) by repeatedly rubbing the antigen into the depilated flank using a short-haired brush [6]. Similar experiments were performed by Schröpl, Müller, and Röckl [7] using S. aureus cultured on blood agar plates, covered with cellophane films to prevent contamination with components of the medium. Delayed-type hypersensitivity was induced by Taubler [8] by injecting 10⁸ viable staphylococci subcutaneously into mice 8 times in weekly intervals. This delayed-type hypersensitivity could be transferred to normal recipients by lymphoid cells and was demonstrated in vitro by spleen cells migration inhibition [9]. The authors used a freeze-dried homogenate as the test antigen. Peter [10] sensitized guinea pigs intracutaneously by injecting 4 mg freeze-dried staphylococcal cells in Freund’s complete adjuvant into the hind footpads and by skin-painting or scarring with freeze-dried or viable staphylococci in sodium chloride or sodium dodecylsulfate. He obtained the strongest reactions with cell wall material 3-6 weeks after epicutaneous sensitization and with the cytoplasmic fraction 26 weeks after intracutaneous sensitization. Kowalski and Berman [11] sensitized Hartley strain guinea pigs with 3 injections of 1 mg formalin-killed staphylococcal cells into all footpads and the nuchal skin. They obtained delayed-type hypersensitivity reactions with cell walls and teichoic acid-peptidoglycan complexes. Sensitization of guinea pigs and rabbits with the cell wall component Protein A also leads to a delayed-type hyper-

sensitivity which can be transferred by lymphoid cells [13]. According to Storck and Schwarz-Speck [14] differences exist in the grade and quality of epicutaneous and intracutaneous test reactions, depending on the mode of sensitization and the nature of the antigen. Easmon and Glynn [15] induced delayed-type hypersensitivity against staphylococcal antigens in mice after 4 subcutaneous injections of viable staphylococci on cotton dust.

Our future aim is to find the principal staphylococcal antigens which are responsible for the induction of delayed-type hypersensitivity. Since delayed-type hypersensitivity is an immunological phenomenon, its induction does not depend on living cells. We therefore used a staphylococcal homogenate, avoiding the production of undefined antigens by living bacteria in the skin. In addition, in a homogenate, extracellular as well as intracellular antigens are exposed to the immune system. In the experiment presented in this report we intended to find the best conditions leading to a delayed-type hypersensitivity to staphylococcal antigens in guinea pigs.

MATERIALS AND METHODS

Randomly bred albino guinea pigs, living in a closed colony, were used in all experiments.

The Staphylococcus aureus strain used throughout the study was isolated from a patient with microbial eczema in 1962 (strain 502). The strain is coagulase and DNase positive and contains no Protein A, as tested with 2-dimensional immunodiffusion. The strain was checked monthly in our bacteriological laboratory. The cells were grown on synthetic broth (Difco, 0352-02-21) [16], supplemented with 0.1% glucose in a 4-liter Fermentor (New Brunswick Scientific Co., Inc., Somerset, New Jersey, USA) at 37°C and 400 rpm. Growth was controlled by continuous registration of the turbidity at 546 nm.

For preparation of staphylococcal antigen, stationary phase cells were harvested and washed 3 times for 30 min in a refrigerated centrifuge (Sorvall, Du Pont Company, Wilmington). The washed sediment was resuspended in phosphate buffered saline to an approx. 50% (v/v) suspension and homogenized in a Braun MSK homogenizer (Braun, Melsungen, BRD) with Ballotini glass beads (0.17-0.18 mm) [17] for 20 min at 5 min intervals using liquid CO₂ as coolant. The resulting homogenate was heated for 1 hr at 60°C to kill any remaining intact cells [18].

Groups of 4-6 animals were sensitized by a single injection of 0.1 ml antigen in Freund’s complete (FCA) or incomplete adjuvant (FIA) in both hind footpads. Equal volumes of homogenate and adjuvant were thoroughly mixed by ultrasonication to final concentrations of 100 and 500 µg homogenerate protein per 0.2 ml. Protein was determined by the method of Lowry et al. [19], using bovine serum albumin as a standard. Control animals were injected with a mixture of equal volumes of FIA or FCA and 0.9% sodium chloride.

The animals were shaved on 1 flank and then challenged intracutaneously at different times after sensitization with 20, 50, and 100 µg homogenate protein in 0.1 ml saline. Some guinea pigs were repeatedly tested on alternate flanks. Skin infiltration was measured with a caliper prior to and 2, 6, 12, 24, 48, 72, and 96 hr after the application of the antigenic mixture. In later experiments skin reactions were only measured at 24 and 48 hr after challenge.

RESULTS

In the first experiment, animals were sensitized with 100 or 500 µg homogenerate protein in FIA or FCA. Challenge and estimate of skin swelling was carried out 14 days later as described above. The results indicate that delayed-type hyper-
sensitivity was induced with staphylococcal antigens in both FIA and FCA. Stronger reactions were obtained with FCA, but the controls also showed stronger indurations with FCA and in the case of sensitization with 500 µg in FCA the differences between test animals and controls were even smaller than in the FIA-group (Fig 1 and 2). However, sensitization with the higher dose (500 µg) of antigen in general resulted in a higher level of hypersensitivity. Positive test reactions were obtained with all 3 concentrations (20, 50, and 100 µg homogenate protein). The skin infiltration in both sensitized and control animals increased quickly during the first 12 hr and remained stable or increased further in sensitized guinea pigs for up to 72 hr, whereas in nonsensitized controls the reaction declined after 12 hr. If the guinea pigs received 2 sensitizing injections within a 14-day period and were challenged after a total of 28 days, the reactions were stronger and skin infiltration increased faster, resulting in a maximum at 2 hr, than in guinea pigs subjected to a single injection (Fig 3). Again the high dose of antigen in FCA gave the strongest reactions, but in this experiment too, the differences between test- and control-animals were smaller in the FCA- than in the FIA-group.

In order to assess the persistence of the delayed-type hypersensitivity, the animals which had received only 1 sensitizing injection were retested 3 mo later and reactions read after 24 and 48 hr. In contrast to the former experiments, in the FCA-group positive skin reactions were observed in guinea pigs sensitized either with 500 or 100 µg homogenate protein, whereas in the FIA-group only the 500 µg dose gave significantly stronger reactions than the controls (Fig 4).

To find the optimal time for challenging, 3 groups of 6 animals each (weighing 300-450 gm) were sensitized with 500 µg in FIA or with 500 µg in FCA. The 3 groups were challenged the first time after 14, 28, or 84 days and then another 3 times at weekly intervals at opposite flanks. Because the course of reactions with 50 and 20 µg homogenate protein was not different, only the results after challenge with 100 µg homogenate protein are reported here. With FIA the strongest reactions were observed in the group challenged for the first time 14 days and with FCA in the group challenged for the first time 28 days after sensitization (Fig 5). Among the repeated challenges in these 2 groups the strongest response was obtained at the time of the third test. During this repeated weekly testing, earlier test sites on the opposite flank were reactivated. When the test procedure was begun on days 28 and 84 in the FIA-group and on days 14 and 84 in the FCA-group no significantly positive reactions were observed. According to these results 2 groups of older animals (weighing 500-800 gm) were sensitized with 500 µg homogenate protein in FIA or FCA. Challenging the FIA-group 14 days and the FCA-group 28 days after the sensitization, we obtained more significant results with these older guinea pigs compared to those observed in younger animals (Fig 6).

**DISCUSSION**

The experiments showed that delayed-type hypersensitivity against staphylococcal antigens can be induced in guinea pigs
Fig. 4. Skin test reactions estimated 24 and 48 hr after challenging with 100, 50 and 20 μg homogenate protein, elicited 3 mo after the first testing (Fig 1 and 2). Sensitization with 100 μg homogenate protein (a) or with 500 μg homogenate protein (b). □ Test animals, □ control animals, FIA, Freund's incomplete adjuvant.

Fig. 5. Skin indurations in guinea pigs, elicited 14, 28 or 84 days after sensitization with 500 μg homogenate protein in Freund's incomplete adjuvant (FIA) or Freund's complete adjuvant (FCA). Animals were tested intracutaneously with 100 μg homogenate protein 4 times in weekly intervals. Skin reactions were read 24 and 48 hr after challenging. □ Test animals, □ control animals.

Fig. 6. Skin test reactions estimated 24 and 48 hr after challenge with 100, 50 and 20 μg homogenate protein. The animals were sensitized with 500 μg homogenate protein in FIA or FCA and tested 14 (Freund's incomplete adjuvant [FIA]) or 26 days (Freund's complete adjuvant [FCA]) later. □ Test animals, □ control animals.

After a single injection with staphylococcal homogenate in FIA as well as in FCA. Comparing the data among the various experiments, we found stronger reactions in older animals (weighing 500–800 gm) than in guinea pigs weighing less than 500 gm. This could explain the relatively small differences between the indurations of control and sensitized animals (net reactions) in the experiment presented in Fig 1.

In the experiments presented in Figs 1 and 2 the net reactions between test- and control-animals were weaker in the FCA-group than in the FIA-group, using the higher antigen dose (500 μg) for sensitization. This was due to the relatively strong control reactions. In this case, both, FIA and FCA animals, were challenged 14 days after sensitization which is, as seen later, not optimal for the FCA group (Fig 5). Even in animals which had received two sensitization injections, we observed stronger net reactions in the FIA-groups, challenged 14 days after the second sensitization. These relatively strong reactions in the controls may be explained by a nonspecific test response, caused by the injection with FCA, which declines before the 28th day. That indeed the strongest net reactions are obtained in FCA-sensitized animals is shown in Fig 4 and 6. The skin induration which had developed at 12 hr (Fig 1 and 2) was probably due to a nonspecific effect after challenge with staphylococcal homogenate or to the occurrence of natural antibodies against staphylococcal antigens, because it was also observed in control animals, injected with 0.9% saline in adjuvant. Immunological cross-reactions between mycobacteria and staphylococcal antigens can be excluded, because this phenomenon was also found in guinea pigs sensitized with FIA.

Although staphylococcal antigens both in FIA and FCA induced delayed-type hypersensitivity in guinea pigs, a significant difference seems to exist between these 2 modes of sensitization. It is known that the injection of guinea pigs with a protein in FIA within a week leads to "Jones-Mote" hypersensitivity (cutaneous basophil hypersensitivity), which is characteristic by very slight induration and infiltration with basophilic leukocytes. It persists only in the absence of circulating antibodies and can therefore be elicited only at early intervals after sensitization [20–24]. In the experiments described in this paper, animals in the FIA-group showed less induration and less persistent delayed-type hypersensitivity than animals in the FCA-group. However these differences were not as pronounced as those described in other reports about Jones-Mote hypersensitivity [20–24]. Therefore we assume that certain staphylococ-
cal antigens may act in the same manner as mycobacteria in FCA, inducing a type of hypersensitivity somewhere between Jones-Mote and classical delayed-type hypersensitivity. It remains to be proved whether an antagonism exists between delayed-type hypersensitivity and antibody level and whether an infiltration with basophilic leukocytes occurs as observed in rabbits sensitized with heat-killed staphylococci in FIA [25].

REFERENCES


Announcement

The Society for Investigative Dermatology and the European Society for Dermatological Research will have a Joint Meeting in Amsterdam, The Netherlands, June 10-13, 1979. Abstract forms will be mailed to all members in October 1978, or may be obtained from the Secretary-Treasurer. Deadline for receipt of abstracts is January 15, 1979. Write to: W. Mitchell Sams, Jr., M.D., Secretary-Treasurer, The Society for Investigative Dermatology, Inc., Department of Dermatology, N. C. Memorial Hospital, Chapel Hill, North Carolina 27514.