

Inhibition of the Late Phase Reaction to Anti-IgE by Previous Mast Cell Activation with Compound 48/80

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The concept of an obligate association between mast cell activation and development of a late phase reaction (LPR) to various agents in human skin was further elucidated. Skin sites were treated four times at 24 h intervals with non-LPR-inducing doses of the histamine liberator compound 48/80 in 10 volunteers. The previously compound 48/80-challenged sites responded with an approximately 40% attenuated early response ($P < 0.01$) and a 70% reduction of the LPR ($P < 0.001$) to subsequently injected anti-IgE as compared with simultaneous reactions at control sites. The data suggest that mediators from the cutaneous mast cells are necessary for the final expression of an LPR.

Key words: anti-IgE; challenge; compound 48/80; LPR; mediator release.

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CLINICAL ASPECTS

The late phase reaction (LPR) to allergen is a well-known phenomenon at clinical skin testing. However, the pathogenesis of this reaction is still unclear. In healthy individuals an LPR with similar manifestations as those produced by allergen may experimentally be elicited by anti-human IgE. The present study showed skin sites treated with a synthetic histamine liberator to respond with reduced early and markedly diminished late reactions to subsequently injected anti-IgE as compared with control sites. The data add substantial weight to the concept that mast cell activation is obligate for the expression of an LPR.

The frequency and magnitude of the late phase reaction (LPR) in skin to allergen challenge is closely associated with clinical sensitivity (1, 15). The LPR is characterized by a diffuse oedematous lesion following the initial wheal and flare reaction with a peak around 6 to 10 h and gradually subsiding and disappearing within 24 to 48 h. The pathogenesis of the LPR to allergen and anti-human IgE is unclear. It is generally believed to involve a strong IgE-dependent release of mast cell-derived primary and secondary mediators with activation of various pathways with the final expression of the LPR (5). The purpose of the present study was to find further support for this concept by pre-treating skin sites with non-LPR-inducing doses

of the histamine liberator compound 48/80 followed by challenge with anti-IgE.

MATERIAL AND METHODS

Antiserum and chemicals

Anti-human IgE and compound 48/80 were kindly supplied by Prof. Gunnar Johansson and Anita Sydbom, Dept. of Clinical Immunology, Karolinska Hospital and Dept. of Pharmacology, Karolinska Institute respectively, Stockholm, Sweden. The antiserum was raised in normal rabbits against Fc fragments from a human IgE myeloma protein. The serum was precipitated and washed with 18% Na_2SO_4 ,

dissolved and dialyzed against sodium chloride (0.15 mol/l) to 1/20 (v/v) containing 0.02 g/l specific antibody activity, passed through a millipore filter (0.45 μ m) and stored at +4°C. The anti-IgE, normal rabbit IgG diluted 1/10, compound 48/80 and histamine dihydrochloride were taken on the day of each experiment and further diluted with physiological saline and 0.3% phenol. For specificity and ethical reasons it was also certified that none of the test subjects had any via RIA-technique detectable serum level of IgG antibodies towards rabbit IgG.

Skin testing

Skin testing was performed in 10 healthy drug-free volunteers (7 females, 3 males), 20–54 years of age (mean 31.3). A skin site on the upper or lower volar aspect of each forearm was injected with 50 μ l of compound 48/80 (1 μ g) followed by re-challenge at approximately 24 h-intervals with increasing doses of the compound (2.5–12.5–12.5 μ g). The dose was successively increased in order to avoid a possible LPR to the compound which occurs irregularly in the dose-range ≥ 50 μ g (2, 3, 13). Anti-IgE diluted 1/333 (v/v) and histamine, 3 μ g, were then administered in 30 μ l with a micrometer syringe at the pretreated sites 24 h subsequently to the last injection of compound 48/80. Comparison of the resulting reactions to anti-IgE and histamine were made with control reactions, i.e. the responses to the same agonists injected simultaneously at sites pretreated with analogous time-intervals with saline. Histamine was included in order to ascertain that repeated treatment of skin sites with compound 48/80 would not significantly diminish the vascular reactivity. For specificity reasons normal rabbit IgG diluted 1/333 was administered. The immediate reactions to the agonists were outlined with a making pen 15 min after injection of agonists for later planimetric measurement. The compound 48/80-challenged sites were carefully observed 6 h after injecting the compound on days 1 to 4 as to possible evolution of an LPR (infiltrate $\geq 10 \times 10$ mm). The diameter of the swelling reaction (LPR) was mea-

sured in two perpendicular directions by an independent observer 6 h after injecting anti-IgE. Measurements of the LPR at other time intervals were made by the test subjects themselves after thorough instructions. The independent observer and the test subjects were informed about the aim of the study but not the order of injections of the different compounds. The reactions to anti-IgE and histamine at compound 48/80-pretreated and control sites were then compared in each individual and differences evaluated by the aid of paired Student's *t* test. The testing technique and statistical formulas used have been described in detail elsewhere (8). All subjects gave informed consent prior to participation in the study.

RESULTS

Intradermal (i.d.) injection of 1/333 normal rabbit IgG caused no reaction in excess of that to saline in 10 test subjects exhibiting a dual (immediate and late) reaction to 1/333 anti-IgE at control site. Compound 48/80 and histamine elicited no LPR in the dose-range used. The reproducibility expressed as mean coefficient of variance (%) for skin reactions to 1/333 anti-IgE simultaneously injected at approximately the same level of each forearm in four individuals was 8% (flare), 14% (wheal) and 16% (LPR at 6 h).

Fig. 1 shows the immediate flare and wheal reactions at skin site repeatedly challenged with increasing doses of compound 48/80 on days 1 to 4. The reactions to the final injection of compound 48/80 on day 4 are reduced by approximately 90 (flare) and 55% (wheal) as compared with responses induced on day 1 ($P < 0.001$). The resulting immediate responses to 1/333 anti-IgE on day 5 at compound 48/80-pretreated site were significantly inhibited by about 40% ($P < 0.01$) as compared with simultaneously induced reactions at control site. In Fig. 1 the size of the wheal responses has not been corrected for the initial bleb caused by the injection volume of the various compounds.

The flare and wheal responses to histamine, 3 μ g, were not significantly influenced by pre-

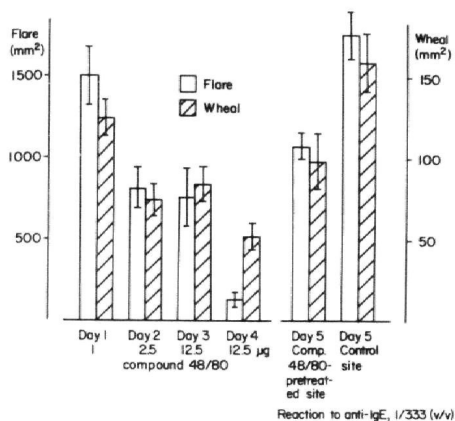


Fig. 1. Immediate flare and wheal reactions (mm^2) at skin site repeatedly challenged days 1–4 with compound 48/80 1 – 2.5 – 12.5 – 12.5 μg , and resulting early responses to anti-IgE injected on day 5 at compound 48/80-pretreated site as compared with responses to anti-IgE injected simultaneously at control site (mean $\pm$ SEM; $n = 10$).

vious compound 48/80-treatment as compared with responses at control site.

The LPR to anti-IgE at compound 48/80-pretreated site was significantly inhibited throughout the observation period ($P < 0.001$), at 6 and 10 h by about 60 and 70% respectively, as compared with simultaneously elicited response at control site (Fig. 2). At 24 h the LPR was completely resolved in 3/10 individuals at control site, therefore statistical analysis is less reliable at this point of the reaction.

DISCUSSION

The pathogenesis of the LPR remains unclear. There is strong evidence that the reaction is dependent on an initial activation of mediator-containing cells, i.e. mast cells and basophils, by an intense immunological or non-immunological stimulus (5). Anti-IgE reacts with mast cell-bound IgE whereas compound 48/80 is a synthetic histamine liberator known to trigger mast cell degranulation in a way closely related to that of allergen (9). The occurrence and magnitude of the LPR to various agents seem to depend on the size of the immediate response, thus an LPR may be expected if a wheal reaction ≥ 10 –15 mm in diameter is elicited (1, 2, 13). Furthermore, inhibition of the early response to anti-IgE by previous i.d. application of a β_2 -adrenoceptor stimulant, with possible action through antagonism of the mast cell release reaction (7, 14), was followed by attenuation of the LPR (8). These observations support an interrelationship between the two phases of the reaction. An LPR defined as above has not been reported subsequently to a negative wheal and flare reaction. However, the occasional clinical phenomenon of late-onset reactions occurring 12 to 24 h after a routine negative i.d. skin test has been described (6, 12). The clinical significance of this reaction is unknown and would be expected to have a pathogenesis different from that of the LPR described above.

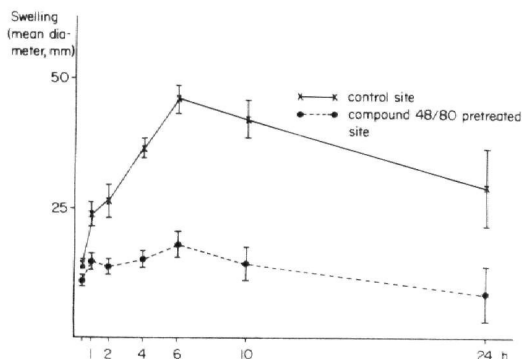


Fig. 2. Swelling (LPR mm, arithmetic mean diameter) induced by anti-IgE diluted 1/333 (v/v) at skin site pretreated with compound 48/80 as compared with simultaneous injection at control site (mean $\pm$ SEM; $n = 10$).

In the present investigation skin sites treated with compound 48/80 responded with an attenuated immediate and a markedly diminished LPR to subsequent challenge with anti-IgE. The intention was to abolish completely the early, and thereby hypothetically the late, phase of the reaction to anti-IgE following sufficient previous mast cell mediator depletion by compound 48/80. However, this was not possible, which may be due to persisting mast cell storage of mediators after compound 48/80 challenge. Thus, the wheal response to the compound was not fully reduced by the end of the pretreatment period. Alternatively, additional mast cells might have been involved in the reaction to anti-IgE due to technical problems of injecting anti-IgE into exactly the same tissue compartment as compound 48/80. Different mechanisms of action of these agents with possible generation by anti-IgE of additional mediators could be an alternative explanation. Finally, it is well recognized that the dose-response curve of anti-IgE-induced LPR is steeper than that of the immediate whealing (5). This might explain why the moderate compound 48/80-induced reduction of the following wheal reaction to anti-IgE resulted in a major inhibition of the LPR to anti-IgE.

Refractory states have been reported to immediate allergic reactions, supporting the occurrence of specific desensitization in addition to mediator depletion (4, 7, 10). Therefore, different triggering agents, i.e. compound 48/80 followed by anti-IgE, were administered in this study. A local refractory state to LPR is induced after initiation of an LPR (3, 11). Consequently, compound 48/80 was injected in limited doses in order to avoid an LPR to this agent. However, the possibility remains that compound 48/80 produced clinically non-detectable LPRs thereby generating a relative refractory state to the following LPR to anti-IgE.

Previous data indicated an increasing permeability of histamine promoting the participation of other factors and cells in the evolution of the LPR (8). Therefore, it was ascertained that the vascular reactivity of the tissue after treatment with compound 48/80 was not significantly reduced by comparing the response to histamine

at pretreated vs. control site. This argues against the possibility that a histamine liberator-induced diminution of vascular response contributes to the demonstrated attenuation of anti-IgE-induced immediate and late reactions.

In conclusion the present data point to an obligate role for the mast cell in the generation of an LPR in human skin.

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