

Dual Cutaneous Reactions to Anti-Human IgE; No Change in Plasma Level of the Split Product Complement C3d or Effect of Heparin

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The possible dependence of the dual cutaneous reaction (DCR) on activation of the complement and coagulation systems was further elucidated. Heterologous whole anti-human IgE was injected intradermally (i.d.) on the volar aspect of the forearms of 17 drug-free healthy volunteers and venous samples for the selected laboratory test collected from the adjacent cubital vein. Plasma level of C3 split products (C3d) did not change significantly 6 h and 96 h after the injection of anti-IgE inducing immediate cutaneous reactions (ICRs) or DCRs as compared with prechallenge values. Conventional heparin, a compound with anti-coagulant and anti-complementary effects, injected i.d. (200 IE) concomitantly with and by s.c. (1000 IE) infiltration of the whealing 15 and 90 min subsequently to anti-IgE challenge produced no significant inhibition of the resulting ICRs or DCRs. The results suggest that the previously reported involvement of the coagulation process is a secondary phenomenon and do not support the concept that activation of the complement system is necessary for the evolution of DCRs.

Key words: anti-IgE; complement; dual cutaneous reaction (DCR); heparin; immediate cutaneous reaction (ICR); late cutaneous reaction (LCR); late phase reaction (LPR).

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CLINICAL ASPECTS

Sustained reactions (LPR) frequently follow on immediate responses to allergen challenge in atopic skin and airways. Advantage was taken of the fact that anti-human IgE elicits a dual response, i.e. ICRs and LCRs in volunteers, thereby avoiding the possible influence on the results of disease state and previous or concurrent therapy. The view that the two phases of the reactions to anti-IgE are triggered by activation of IgE (ICR) and IgG (LCR) dependent mechanisms was challenged by the present data. The plasma level of the split product complement C3d did not change at a time interval corresponding to the peak of the LCR to anti-IgE. High topical doses of heparin, a compound reported to inhibit IgG-elicited ICRs and with recognized anticoagulant and described anti-complementary effects, had no suppressive action on anti-IgE-induced ICRs or LCRs. The data are in keeping with the concept that the dual response to anti-IgE is elicited without dependence on IgG or activation of the complement or coagulation processes.

So-called dual allergic reactions, immediate (0-30 min) followed by a late phase reaction (LPR) peaking at 6-12 h and with a duration of 24-48 h have counterparts in human skin and airways. They are believed to be due to an IgE-dependent mast cell mediator release reaction with the generation of humoral factors and

inflammatory cells through chemotactic factors (13). Activation of the coagulation process and Factor XII were suggested as possible pathways for mediation of bronchial and cutaneous LPRs (6, 9). The reports on activation of the complement system have been more contradictory and, so far, only studied in association with LPRs in

bronchi. Here, some authors demonstrated occasional appearance of split products (C3d) of factor C3 and complement consumption (1, 20). The present study was designed to further elucidate the pathogenesis of dual cutaneous reactions (DCR) by studying the effect of heparin on the DCR to anti-IgE in human volunteers. This compound interacts with activation of the coagulation and complement process and Factor XII and thus carries a beneficial anti-inflammatory and anti-allergic potential (2, 5, 8, 10, 11, 15). Furthermore, the role of complement factors in the pathogenesis of cutaneous LCRs was studied by determination of the amount of C3d split products in plasma at defined time-intervals in relation to the LCR. Advantage was taken of the fact that anti-IgE elicits a DCR analogously in atopic and non-atopic skin, thereby avoiding possible influence on the results of disease state and previous or concurrent therapy.

MATERIAL AND METHODS

Skin testing and drug treatment

Seventeen drug-free healthy volunteers (13 females, 4 males) aged 21–45 years (mean 28) were injected intradermally (i.d.) with anti-IgE diluted 1/5000 and 1/200 v/v delivered by a micrometer syringe on the volar aspects of the forearms after a certified negative reaction to normal rabbit IgG in corresponding dilution. Preliminary experiments with heparin injected in single therapeutic doses, i.e. 7500 IE s.c. and 5000 IE i.v. immediately prior to anti-IgE challenge, indicated no effect on the DCR in a few subjects. To accomplish a more favourable ratio between dose of agonist and antagonist in the trial, conventional heparin was injected i.d., in 5000 IE/ml concomitantly with anti-IgE 1/200 and 1/5000 v/v and responses compared with those to anti-IgE injected *per se* on opposite arm (control). Administration of heparin produced no obscuring effects in the selected doses, thereby permitting the evaluation of anti-inflammatory properties of the drug. All injection volumes were 0.04 ml and administered in a random fashion. In addition, the whealing to

1/200 anti-IgE was infiltrated in four directions at 15 and 90 min by 0.2 ml of heparin in 5000 IE/ml or vehicle (control). The extents of the immediate reactions were outlined with a marking pen on a transparent film 15 min after injection of anti-IgE for later planimetry. The swelling reaction (LCR) was measured by the perpendicular and vertical diameters (mm) by an independent observer at 6 h and by the test subjects themselves at defined time intervals up to 24 h. The independent observer and volunteers were informed about the aim of the study but not the order of the injections of the different compounds. The subjects gave their informed consent to participate in the study which had been approved by the local Ethical Committee.

Complement analysis

Venous samples were collected by repeated puncture of the adjacent cubital vein immediately before and 6 h and 96 h after a challenge with anti-IgE in concentrations inducing isolated ICRs and DCRs. The time intervals 6 h and 96 h were chosen with respect to the peak of the LCR (6 h to 10 h) and to the complete resolution of the infiltrate (48 h to 72 h). The total amounts of C3 and C4 analysed at the defined time intervals yielded no evidence of complement involvement in a pretrial test performed in five subjects and henceforward were not systematically studied. The blood samples were centrifuged within 4 h and the plasma removed and stored at -20°C until tested. Complement split products C3d were quantitated by a modification of the double-decker rocket immunoelectrophoresis of Brandslund (3). Each sample was analysed in duplicate.

Statistical analysis

Immediate cutaneous reactions (ICRs) (mm^2) and LCRs (sum of arithmetic mean diameter at 1–24 h) at skin sites treated with heparin and vehicle (control) were compared, and the amount of C3d split products during ICRs and DCRs analysed vs. no reaction to normal rabbit

IgG. Student's *t*-test for paired observations was employed for statistical within-subject analysis in this respect.

Antiserum and chemicals

Heterologous whole anti-human IgE was raised in rabbits against a mixture of isolated Fc fragments from the epsilon chain from a human IgE myeloma protein, and any activity to other serum protein than IgE was removed by absorption with normal human serum. The antiserum was precipitated and washed with 18 % Na₂SO₄, dissolved and dialysed against sodium chloride (0.15 mol/l) to 1/20 (v/v), and the resulting solution was equivalent in gel diffu-

sion to 0.02 g/l IgE. The antiserum was passed through a millipore filter (0.45 µm) and stored at +4°C until used. The anti-IgE, normal rabbit IgG, prepared analogously and diluted 1/20 (v/v) and heparin (Heparin®, 5000 IE/ml, AB Kabi Vitrum, Stockholm, Sweden) were taken on the day of each experiment and further diluted in physiological saline containing 0.3 % phenol.

RESULTS

Complement determination

The plasma levels of C3 split products C3d 6 h and 96 h after injections of anti-IgE and normal rabbit IgG are presented in Table 1. Isolated

Table 1

Amounts of complement split products C3d in plasma expressed in per cent of prechallenge values (mean ± SEM)*

	<i>n</i>	Dilution (v/v)	6 h	96 h
No reactions (normal rabbit IgG)	4	1/200	100±0	100±0
Isolated immediate reactions	4	1/10000	93.9±4.0 (NS)	108.2±11.7 (NS)
Dual reactions	9	1/200	106.1±5.7 (NS)	106.0±4.9 (NS)

* Isolated immediate and dual reactions after challenge with anti-human IgE were compared to conditions when no responses were observed after injections of rabbit IgG in healthy subjects. Samples were taken 6 h and 96 h after injection.

NS = non-significant as compared with prechallenge values.

Table 2

Effect of heparin injected in different doses and by various routes on the immediate reaction and the LCR to anti-IgE (mean±SEM)

No. of sub- jects	Anti-IgE concentration (v/v)	Heparin dosage (IE)	Route of heparin admini- stration	Time interval between injection of anti-IgE and heparin (min)	Flare		Wheal		LCR	
					Drug	Control	Drug	Control	Drug	Control
4	1/200	200	i.d.	0	2075±210 (NS)	1960±135	135±14 (NS)	150±15	45±5 (NS)	49±5
7	1/5000	200	i.d.	0	1310±150 (NS)	1375±105	69±4 (NS)	72±6	-	-
13	1/200	2000	s.c. infil- tration	15,90	2165±115	2025±160	138±9	150±11	55±5 (NS)	55±4

The flare and wheal responses (mm²) and the LCRs (mean diameter, mm) were measured 15 min and 6 h after challenge with anti-IgE.

i.d.: intradermal; s.c.: subcutaneous; NS: non-significant as compared with control reactions.

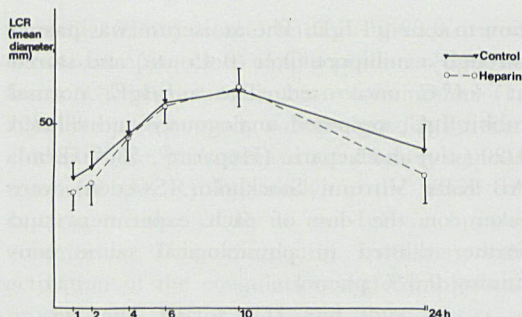


Fig. 1. Effect on the late cutaneous reaction (LCR, arithmetic mean diameter, mm) to anti-IgE of heparin (1000 IE) infiltrated subcutaneously 15 and 90 min after anti-IgE challenge (mean $\pm$ SEM; $n = 13$).

early flare and wheal reactions to 1/10000 anti-IgE (1495 ± 90 and 69 ± 4 , mm² mean $\pm$ SEM) and DCR to 1/200 anti-IgE with ICR (2155 ± 65 and 139 ± 10) and LCR (57 ± 3 , mean diameter, mm) were not associated with a significant change in the amount of split products of C3 as compared with prechallenge values or corresponding values after injection of normal rabbit IgG. Analysis of C3d at 2 h in a few subjects yielded analogous results (not shown). All values were within the normal range (≤ 1.0 arbitrary units). The mean coefficient of variation for C3d in samples analysed simultaneously in duplicate and with ≥ 1 week interval was 2 and 7 % respectively. Anti-IgE 1/200 induced LCRs in all test subjects with the typical recognized appearance, i.e. a gradually increasing diffuse asymptomatic edema, which peaked at 6–10 h and required up to 48 h to resolve completely.

Heparin

The influence of heparin administered by various routes is demonstrated in Table 2. Heparin (200 IE) injected i.d. concomitantly with 1/200 anti-IgE had no significant effect on the DCR. Decreasing the ratio between agonist and antagonist by using 1/5000 anti-IgE and heparin 200 IE had no further effect on the resulting isolated ICR. Infiltration of the whealing at 15 and 90 min with heparin in the total amount of 2000 IE produced no significant attenuation of the LCR to 1/200 anti-IgE (Fig. 1). 2/13 subjects

exhibited a slight bleeding at the heparin-treated site observed at 2–4 h.

DISCUSSION

Mediator-containing cells are believed to be necessary for the expression of DCRs (13). Anti-IgE triggers mast cell degranulation via the so-called reversed type reaginic reaction in healthy and atopic subjects, and the reaction is argued to be entirely comparable to allergen-induced responses (7). Previous extensive studies have suggested isolated IgE-mediation of DCRs to anti-IgE and allergen in human skin without dependence upon IgG or activation of the complement cascade (7, 19, 22). Thus, intact anti-IgE and its (Fab')₂-fragments induce analogously DCRs (7). However, this view was challenged by the finding that mast cell activation through anti-IgG, staphylococcal protein A, or anaphylatoxin, produced inflammatory DCR lesions of an essentially similar appearance compared with those induced by anti-IgE IgE in healthy volunteers (21). Moreover, anti-IgE might hypothetically activate the complement system through various pathways, e.g. via aggregated IgG (17), by preformed antibodies of the recipient towards rabbit immunoglobulin (7), by formation of anti-IgE IgE complexes (17), or via products derived from infiltrating cells (16). Finally, tranexamic acid, widely used as an antifibrinolytic drug but with additionally described anticomplementary property, suppressed the LCR to anti-IgE in volunteers (14).

These observations prompted me to evaluate the possible association between activation of the complement system and the expression of DCRs. The present findings on DCRs to anti-human IgE are not consistent with such an assumption. Thus, the amount of the split product C3d measured in a sample taken from a vein adjacent to the challenged skin site at a time interval corresponding to the peak of the LCR was unchanged as compared with prechallenge values. Factor C3 is the central component in the complement system. The level of C3d split products reflects the extent of complement activation, both by the classical

and the alternative pathways. Since C3d has been argued to diffuse rapidly into the plasma from sites of extravascular activation of complement (4) changes should have been detectable. However, an activation of the complement system during the anti-IgE-elicited DCR might hypothetically be limited to the skin site, and changes in plasma samples therefore too small for detection with the present method.

More convincing data against the thesis of dependence on IgG-induced complement activation and possible formation of immune complexes during the DCR to anti-IgE were that high-dose topical conventional heparin lacked inhibitory capacity on this reaction. Heparin has been reported to counteract anaphylaxis and suppress bronchospasm in asthmatic subjects, and the anti-complementary action of the drug is one among a number of proposed beneficial mechanisms of action in various allergic states (2, 5, 10). Thus, heparin interferes with several steps in the classical as well as the alternative pathway activation of complement (11). In contrast to non-heparin responding IgE-dependent reactions, this compound inhibited IgG-induced early and late phase skin responses. Thus, in a controlled study in normals, heparin, injected i.d. in a 100-fold lower dose than that used in the present study, produced a clear-cut inhibition of the ICRs to heat-aggregated IgG without effect on responses to histamine, compound 48/80, or trypsin. This strongly suggested an interaction by the drug with the complement cascade (12 and Ekre, H-PT, personal communications). Furthermore, a 14-day regimen with heparin counteracted the late i.d. reactions to tobacco antigens, considered as dependent on IgG and activation of complement factors, in smoking patients with coronary artery disease (18). Taken together, the data add indirect support to the interpretation that anti-IgE elicits the DCR without dependence on IgG and activation of the complement cascade.

The coagulation process was reported to be activated in skin blister fluid samples obtained after allergen skin testing in atopic subjects with LCRs (9). The present study demonstrated no antagonistic effect on LCRs to anti-IgE of

heparin, widely used as anticoagulant and injected in single therapeutic as well as considerably high topical doses. This suggests that the previously reported (9) activation of the coagulation system during IgE-dependent LCRs is a secondary phenomenon.

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