

Characteristics of histamine-releasing activity in the sera of patients with chronic idiopathic urticaria

Burton Zweiman, MD, Mary Valenzano, Paul C. Atkins, MD, Tonny Tanus, MD, and John A. Getsy, DMD, DO Philadelphia, Pa.

Background: The serum histamine-releasing activity (HRA) found in a sizable percentage of patients with chronic idiopathic urticaria (CIU) has been partially characterized. However, the variable effect of individual HRA⁺ sera in basophils of different donors and the relationship of HRA to the clinical course require further investigation.

Objective: The study was performed to characterize the HRA found in sera of some members of a sizable group of carefully evaluated patients with CIU.

Methods: Sera of 70 patients with CIU, evaluated with a standard protocol, were screened for increased HRA. HRA⁺ sera were fractionated, heated, and tested on unaltered and altered basophils obtained from a panel of normal donors. HRA levels were compared with concomitant clinical manifestations.

Results: HRA⁺ sera were found in 30% of our patients with CIU. HRA was predominantly in the IgG fraction, sensitive to 56° C heating for 4 hours, and generally reacted more with IgE-stripped basophils. Considerable variation in the degree of response to HRA⁺ sera in the basophils of different normal subjects did not correlate with the degree of response of these cells to heterologous anti-IgE antiserum. Serum HRA levels were generally much lower when symptoms decreased in these patients with CIU.

Conclusion: Serum HRA from patients with CIU appears to bind most commonly to the IgE receptor and may be a marker of clinical disease activity. HRA appears in an IgG-containing fraction of the serum and may contain IgE in some cases. (*J Allergy Clin Immunol* 1996;98: 89-98.)

Key words: Urticaria, histamine release, IgE, IgE receptors, basophils

In most cases of chronic urticaria the cause is not detected even after intensive clinical investigation.¹ Recent reports from one investigative group indicate that sera from up to 60% of patients with chronic idiopathic urticaria (CIU) caused increased amounts of in vitro histamine release from basophils obtained from one or more of a panel of normal donors.^{2,3} The in vivo relevance of these findings is suggested by: (1) reports of decreased blood basophil levels in some patients with CIU,^{2,4} (2) decreased in vitro histamine release induced by

Abbreviations used

| | |
|--------|---|
| CIU: | Chronic idiopathic urticaria |
| HRA: | Histamine-releasing activity |
| PAGCM: | Piperazine-N,N'-bis[2-ethanesulfonic acid] buffer containing albumin, glucose, calcium, and magnesium |

anti-IgE antibodies in the basophils of patients with urticaria,⁵ (3) transient clinical improvement after plasmapheresis in patients with CIU and increased serum histamine-releasing activity (HRA).⁶

Studies by Grattan et al.^{2,7} showed that HRA⁺ sera also induced wheal and flare reactions when injected intradermally in serum donors. These reactions lasted up to 18 hours and were characterized by a mixed accumulation of mononuclear leukocytes, neutrophils, and eosinophils.⁸ HRA⁺

From the Allergy and Immunology Division, Department of Medicine, University of Pennsylvania School of Medicine.

Supported by National Institutes of Health grant RO1 AI 14332 and the Immunology Research Fund.

Received for publication May 15, 1995; revised Sept. 6, 1995; accepted for publication Sept. 12, 1995.

Reprint requests: Burton Zweiman, MD, 512 Johnson Pavilion, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6057.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/1/69404

sera also induced increased histamine release from skin specimens *in vitro*.⁹

The HRA in these CIU sera has been partially characterized, predominantly as being over 100 kd in molecular weight and separating with IgG in an affinity column.² An intriguing finding, not yet clarified, was the variable effects of HRA on basophils of different normal donors. An individual CIU serum may induce prominent, modest, or no increased histamine release from basophils of different donors when tested at the same time. Initial studies suggested that the HRA was an anti-IgE.² However, subsequent investigation revealed that some HRA could induce prominent histamine release from basophils of normal subjects with very low serum IgE levels and presumably very little IgE on their basophils. At present, Hide et al.⁹ believe that the HRA⁺ sera may have varying amounts of IgG antibody activity directed against IgE, as well as against the high-affinity IgE receptor (FcεRI) on the basophils. Thus further characterization of the interaction of HRA and basophils and the relationship of these events to clinical manifestations of CIU would be important.

We report our findings in sera obtained from 70 patients with clinically well-defined CIU who were evaluated in our clinic. Preliminary comparisons of *in vitro* findings and clinical manifestations have been reported elsewhere.¹⁰ Particular attention has been paid to the possible target(s) for HRA on basophils in normal donors.

METHODS

Patient study group

Our original patient population consisted of 85 patients referred consecutively for evaluation of chronic urticaria with or without angioedema. All patients completed a standard questionnaire about their medical background including family, social, and environmental histories. Thorough history and physical examinations were performed, and data were recorded on a standardized form; data included gross characteristics of the urticaria, trigger factors, systemic manifestations, medication history, history of atopic manifestations, evaluation of physical components of the urticaria, and results of previous evaluations such as laboratory studies, skin tests, RAST, skin biopsies, and diet therapies. A symptom score sheet was also used, and pruritus was graded on 4-point scale (0, none; 1, mild; 2, moderate; 3, severe). The approximate number of lesions present at the time of initial evaluation and blood drawing was also graded on a 4-point scale (0, none; 1⁺, 1 to 9; 2⁺, 10 to 19; 3⁺, 20 or more).

Serum was obtained for determination of HRA and, when indicated, total IgE. Other possible causes of the

chronic urticaria were ruled out by extensive laboratory evaluations including a complete blood count with differential, chemistry panel, liver function tests, thyroid function tests, and autoimmune antibody panel consisting of antinuclear antibody, rheumatoid factor, complement levels (C3, C4, CH50), and sedimentation rate. If any of the results of these tests were abnormal, further evaluations were done. If indicated, skin tests, RAST, and skin biopsies were performed.

Basophil histamine release assays

Leukocyte populations containing 0.4% to 1.8% basophils were obtained from a panel of both atopic and nonatopic normal donors with no history of urticaria. Leukocyte populations containing basophils were obtained after dextran sedimentation. These were washed and resuspended in a solution of piperazine-N,N'-bis[2-ethanesulfonic acid] buffer containing albumin, glucose, calcium, and magnesium (PAGCM), as previously described.¹¹ Duplicate cell aliquots (1×10^7 /ml) were incubated with: (1) serum from individual patients with urticaria or normal control subjects in final concentration of 40% vol/vol, (2) an aliquot of pooled normal serum from 30 individuals with no history of urticaria, (3) goat anti-human IgE polyvalent antiserum (0.2 μm/ml; Kirkegaard and Perry, Gaithersburg, Md.) (4) calcium ionophore (0.5 μm/ml) as a positive control, or (5) additional PAGCM (to assess spontaneous histamine release).

After thorough resuspension of the cells and subsequent incubation for 30 minutes at 37° C in a 5% CO₂ incubator, the tubes were centrifuged, and the supernatant was removed for histamine assay. The cell pellet was resuspended in PAGCM and boiled immediately for 10 minutes to release the residual cellular histamine. The cell particles were pelleted, and the supernatant was removed for histamine assay. Histamine levels were assessed by a double-label radioenzymatic assay, as used extensively by us.¹¹ The percent histamine release in individual tubes was expressed as the ratio of histamine released in the first incubation to histamine released in the first incubation plus histamine released by boiling.

In selected experiments aliquots of sera from patients with urticaria and previously demonstrated increased HRA (hereafter called HRA⁺ sera) were reinvestigated after prior heating of the serum at 56° C for 4 hours to reduce the binding of any IgE contained therein to IgE receptors on basophils¹² and separation into IgG-enriched and IgG-depleted fractions by affinity chromatography (Amicon MAC; Amicon Inc., Beverly, Mass.). Serum specimens were exposed to protein A-coated disks in a capsule by repeated passage through the capsule. The serum then remained in the capsule for 15 minutes. Phosphate-buffered saline was passed through the capsule, and the first 3 ml was collected as the IgG-depleted serum fractions. After further irrigation with phosphate-buffered saline, the IgG-enriched fraction was eluted

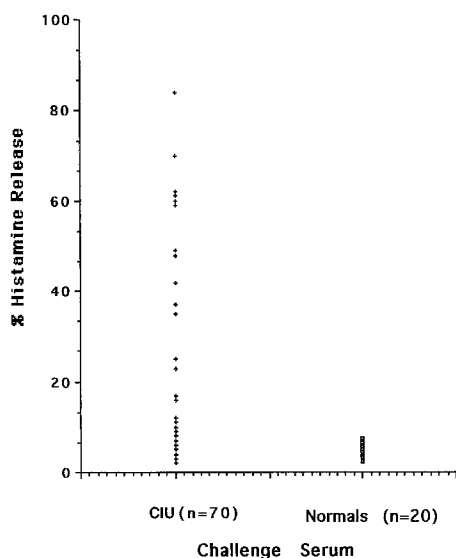


FIG. 1. Percent histamine release induced by serum of 70 patients with CIU at initial evaluation and 20 normal control subjects.

with 0.15 mol/L glycine, collecting the first 3 ml, then neutralized with Tris buffer. The capsule contents were irrigated further and prepared to accept the initial 3 ml (IgG-depleted) fraction for further IgG depletion.

In other selected experiments the effects of HRA⁺ urticaria sera were compared on aliquots of normal basophils that were previously stripped of most of the surface IgE by prior lactic acid treatment, as described by MacDonald et al.¹³; previously incubated for 1 hour in high IgE titer sera from atopic individuals with no history of urticaria (such sera did not contain increased HRA); and stripped of IgE and then incubated with high IgE titer serum.

In some experiments basophils of normal donors, which released relatively little histamine after incubation with known HRA⁺ sera, were stripped of their IgE by lactic acid treatment. Aliquots of such stripped cells were incubated with either autologous serum or serum from a normal volunteer whose basophils had been previously shown to release histamine prominently (50% to 70%) when incubated with the same HRA⁺ sera. These experiments were designed to help determine why sera from patients with urticaria induced prominent histamine release from basophils of some but not other normal donors.

Basophil counts

The percentage of basophils in individual cell populations of the normal donors was determined by an Alcian blue staining technique, modified from that used previously by us.¹⁴ The percentage of basophils in these cell populations varied from 0.4% to 1.8%.

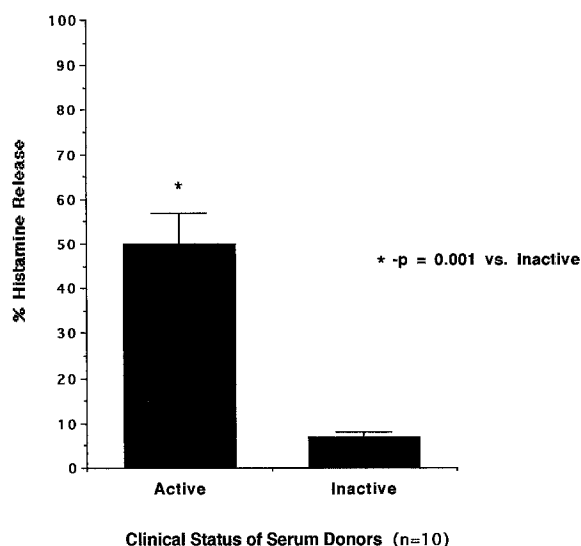


FIG. 2. Mean (\pm SEM) percent histamine release induced in normal basophils by sera obtained from a group of 10 patients with CIU when disease was clinically active and when disease was in clinical remission.

Serum IgE

Serum IgE levels were determined in patients with CIU and some of the normal cell donors by an ELISA (The Binding Site Co., Birmingham, U.K.).

RESULTS

Clinical assessment

In 70 of the 85 patients, no reasonable cause for the chronic urticaria could be found after clinical and laboratory evaluation. These 70 individuals were classified as having CIU.

Prevalence of increased serum HRA in CIU

Initial quality control studies with the sera of 20 normal subjects without urticaria showed that a 40% vol/vol final serum concentration induced a mean \pm 2 SD histamine release of 4.7% \pm 4.1% (range, 2.0% to 9.0%) from basophils of a panel of normal donors (Fig. 1). The histamine release induced by sera of atopic control subjects was quite similar to that induced by the sera of nonatopic control subjects (mean \pm 2 SD: 5.4% \pm 3.0% for nonatopic subjects and 4.9% \pm 3.2% for atopic subjects). Therefore histamine release of 10% or greater induced by individual sera in a 40% vol/vol concentration was considered to be evidence of increased HRA.

With these criteria, sera of 30% of the patients with CIU were found to have increased HRA (HRA⁺), with the degree of histamine release

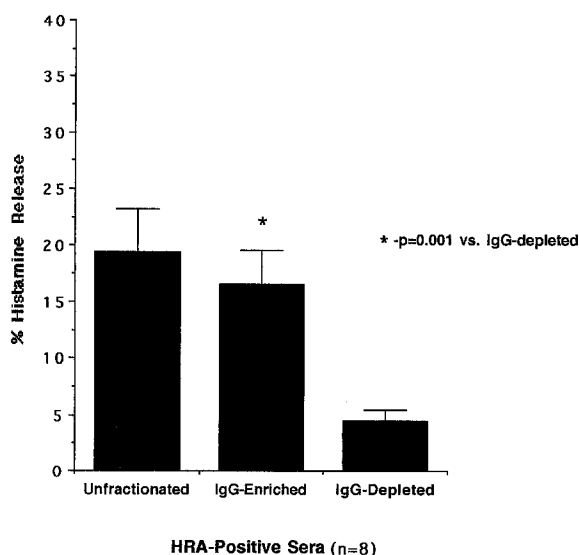


FIG. 3. Mean (\pm SEM) percent histamine release induced by HRA⁺ sera, which were unfractionated, enriched for IgG by affinity column separation, and depleted of IgG in such separation.

ranging from 11% to 84% (Fig. 1). Basophils of three normal donors, which released more than 10% of their histamine after incubation with all the HRA⁺ sera in initial studies, were used for all subsequent screening studies of new CIU sera.

Comparison of serum HRA and clinical manifestations in CIU

There was no apparent difference in the patterns of clinical manifestations in the patients with CIU whose sera had increased (vs normal) HRA. These findings confirmed our impression from an earlier preliminary report of a smaller group of subjects with CIU.¹⁰ However, in serial studies of 10 patients with CIU and HRA⁺ sera, serum HRA levels were significantly lower in specimens obtained after the clinical manifestations of urticaria had diminished significantly ($p = 0.001$, paired t test) (Fig. 2). During the latter period, 50% of the patients had been receiving no medication for at least 1 week.

Presence of HRA in different serum fractions

Eight of the HRA⁺ urticaria sera (mean HRA = $20\% \pm 4\%$ for the group) were separated into IgG-enriched and IgG-depleted fractions by using affinity chromatography. The fractions were then adjusted to a similar total protein concentration. There was only about 5% of residual IgG in the IgG-depleted fraction, as assessed by nephelometry. The IgG-enriched fraction contained most of

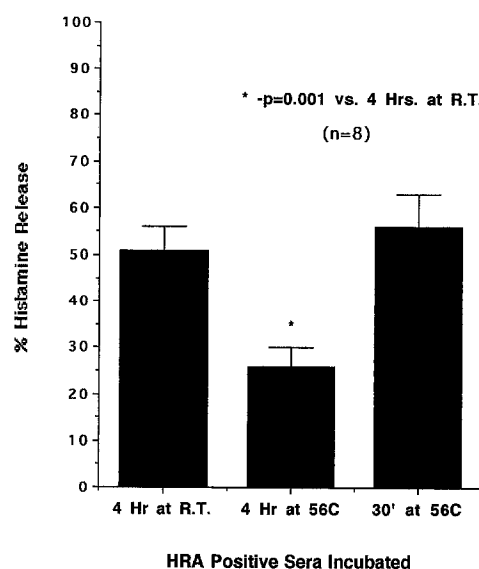


FIG. 4. Mean (\pm SEM) percent histamine release induced in normal basophils by aliquots of eight HRA⁺ sera which had been incubated for 4 hours at room temperature (R.T.), for 4 hours at 56° C, and for 30 minutes at 56° C.

the HRA ($17\% \pm 3\%$), whereas the IgG-depleted fraction released only $4\% \pm 1\%$ of the histamine from basophils obtained from several normal donors (Fig. 3).

It was not clear whether the presence of HRA in the IgG-containing fraction of CIU sera represented an antibody against a cellular component, such as the FcεRI,³ or a circulating component in the form of an immune complex also containing IgE. IgG anti-IgE antibodies have been reported in the sera of patients with CIU.¹⁵ Therefore we investigated the effects of prior incubation of eight HRA⁺ sera at 56° C for 4 hours. Such heating reduces the capacity of IgE molecules to bind to the FcεRI on mast cells and basophils.¹² The mean percent histamine release induced by this group of sera was reduced significantly from $50\% \pm 5.9$ to $26\% \pm 4.9\%$ ($p = 0.001$, paired t test) by such prior 56° C incubation as compared with incubation at room temperature for 4 hours (Fig. 4). However, prior incubation of the HRA⁺ sera at 56° C for 30 minutes to eliminate most classical complement pathway biologic activity¹² did not significantly alter HRA (Fig. 4). Incubation of goat anti-IgE antiserum at 56° C for 4 hours did not affect its HRA on normal basophils.

HRA effects on IgE-stripped and passively incubated basophils

As noted previously, Grattan et al.² had concluded that the HRA in CIU sera had the func-

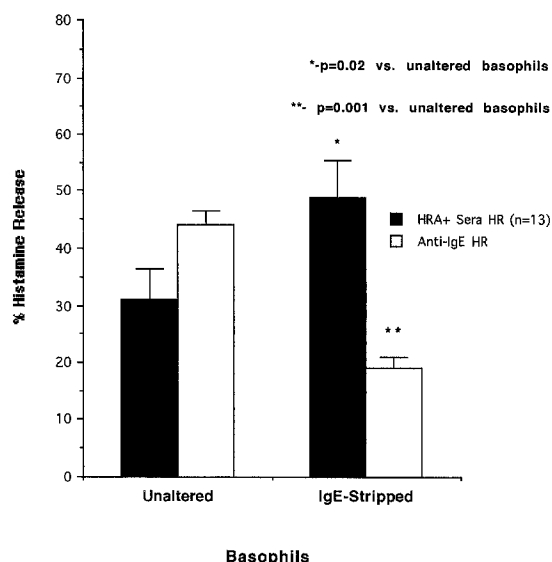


FIG. 5. Mean (\pm SEM) percent histamine release induced by aliquots of 13 HRA⁺ sera and anti-IgE antiserum in aliquots of normal basophils without any prior treatment and aliquots of the same basophil populations previously stripped of most surface IgE.

tional properties of an anti-IgE, in part on the basis of their findings that HRA⁺ sera induced much less histamine release from basophils previously stripped of surface IgE by lactic acid treatment. In some cases they restored HRA⁺ reactivity on IgE-stripped basophils, which were then incubated with human IgE. The same group later concluded that HRA sometimes represented IgG reactivity against Fc ϵ RI.³

We investigated these matters, using similar approaches, in sera from 13 of our patients with HRA⁺ sera. For the group as a whole, the mean percent histamine release was greater in basophil aliquots that had been previously stripped of most of their surface IgE by lactic acid treatment than in unaltered aliquots of these cells ($48\% \pm 5.8\%$ vs $32\% \pm 4.7\%$; $p < 0.05$, paired t test) (Fig. 5).

Eleven of these 13 HRA⁺ sera induced greater histamine release from the IgE-stripped basophils than from unaltered, normal basophils. One of the other two sera stimulated much less histamine release in the IgE-stripped aliquots, and the other serum induced very similar responses in IgE-stripped and unaltered basophils. At the same time, the histamine release induced by goat anti-IgE antiserum was reduced by 57% in other aliquots of these IgE-stripped basophils compared with the anti-IgE-stimulated histamine release seen in unaltered basophils ($19\% \pm 1.9\%$ vs $44\% \pm 2.5\%$) (Fig. 5).

The effects of 15 HRA⁺ sera were also compared by using aliquots of normal basophils previ-

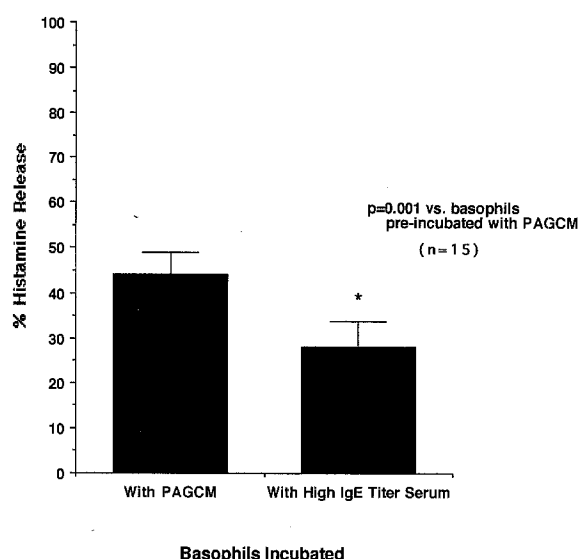


FIG. 6. Mean (\pm SEM) histamine release induced by 15 aliquots of HRA⁺ sera in aliquots of normal basophils previously incubated for 60 minutes in PAGCM (see text) and aliquots of the same basophil population incubated previously in a high IgE titer serum for 60 minutes.

ously incubated for 1 hour with high IgE titer (1000 IU/ml) serum obtained from a highly atopic individual with no history of urticaria and aliquots of the same cells incubated in buffer under similar conditions. The overall histamine release induced by the group of HRA⁺ sera in basophils previously incubated in IgE-containing serum was significantly less than that seen after incubation with basophils previously incubated in buffer ($28\% \pm 4.3\%$ vs $44\% \pm 4.2\%$; $p = 0.001$, paired t test) (Fig. 6). At the same time, there was no significant difference in the histamine release induced by anti-IgE antiserum in other aliquots of these basophils previously incubated in IgE-rich serum versus the release induced in basophils incubated in buffer ($46\% \pm 4.7\%$ vs $51\% \pm 2.7\%$; $p = \text{NS}$).

Thirteen of these 15 HRA⁺ sera induced less histamine release from the serum-incubated basophils. Of note, one of the other two HRA⁺ sera was the same one that stimulated less histamine release from IgE-stripped basophils (as described previously).

Also, in three experiments aliquots of normal basophils, previously stripped of surface IgE by lactic acid treatment, were then incubated with either the high IgE titer serum or buffer before exposure to HRA⁺ sera. The latter sera had previously been shown to induce more histamine release in IgE-stripped basophils. The aliquots of IgE-stripped cells incubated with buffer released

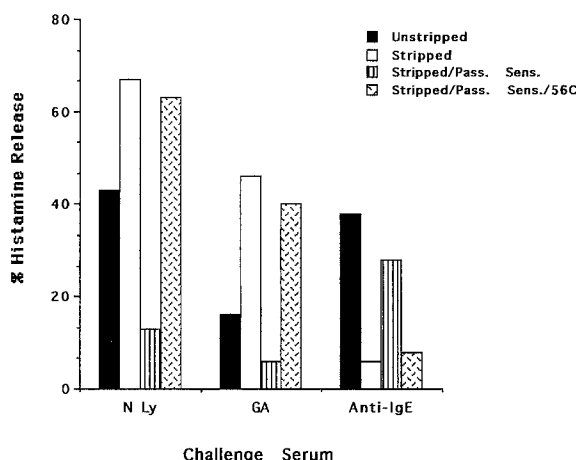


FIG. 7. Percent histamine release stimulated by two HRA⁺ urticaria sera (N Ly and GA) 40% vol/vol and goat anti-human IgE antiserum (0.2 µg/ml) in aliquots of basophils unstripped (*solid columns*); stripped of most IgE (*open columns*); IgE-stripped and then incubated in high IgE titer serum (stripped/Pass. Sens, *vertical striped columns*); and IgE-stripped and then incubated in high IgE titer serum, which had been previously heated at 56° C for 4 hours (stripped/Pass. Sens./56° C, *cross-hatched columns*). Pass. Sens., Passively sensitized.

more histamine when incubated with HRA⁺ sera than did unstripped basophils. In contrast, the IgE-stripped cells, then incubated with IgE-rich serum, subsequently released less histamine than did unstripped basophils in response to the HRA⁺ sera. However, the reduced histamine release seen in this latter group was not observed when the IgE-stripped cells were preincubated with IgE-rich serum, which itself had been previously heated at 56° C for 4 hours (Fig. 7). This suggests that the reduced HRA⁺ serum-induced histamine release from IgE-stripped basophils previously incubated with high IgE titer serum was the IgE in the latter serum. Thus it appears that removing IgE from the surface of normal basophils enhances their histamine-releasing responsiveness to most HRA⁺ urticaria sera, whereas placing more IgE on the surface reduces such reactivity.

Variable histamine-releasing responses of basophils from different normal donors

We confirmed the findings of Grattan et al.² that there was sometimes considerable variability in the degree of increased histamine release induced in the basophils of different normal subjects by individual HRA⁺ sera. Our initial impression was that responses tended to be greater in the basophils of atopic individuals (without urticaria) than in the cells of nonatopic subjects. However, there was no

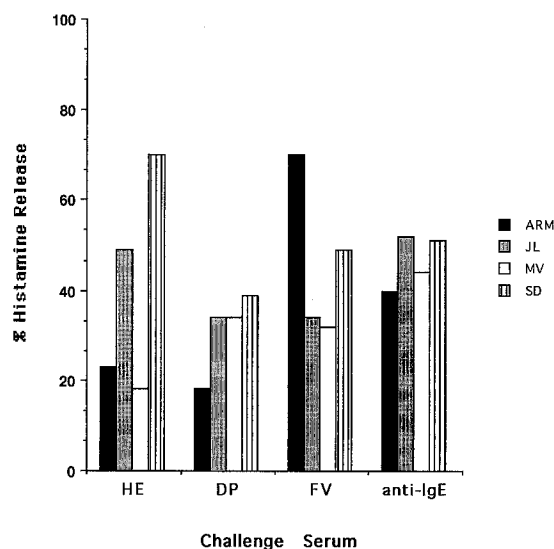


FIG. 8. Varying degrees of histamine release induced in the basophils of four normal donors: ARM (*solid black column*), JL (*gray column*), MV (*open column*), and SD (*striped column*) when incubated with the HRA⁺ serum of patients with urticaria (HE, DP, FV) and with anti-IgE antiserum.

overall correlation between the percent histamine release stimulated by individual HRA⁺ sera and the degree of concomitant anti-IgE-induced histamine release in the basophils of individual normal donors. The variability in histamine release responses in basophils of various cell donors is shown in Fig. 8. Depicted is the histamine release from basophils from four normal donors (ARM, JL, MV, and SD; two of whom are atopic) in response to each of three different HRA⁺ sera (HE, DP, and FV) and to goat anti-IgE antiserum. It can be seen that there was sometimes considerable variation in histamine-releasing responses of the basophils from the four cell donors to the same HRA⁺ serum. However, there was no consistent pattern of such variable responses (of individual cell donors) to the three HRA⁺ sera studied. At the same time, the mean anti-IgE-induced histamine release varied relatively little among the basophils of these four cell donors.

This similarity in anti-IgE-induced histamine release seen in Fig. 8 was not simply a reflection of the necessary use of the mean values for anti-IgE-stimulated responses in multiple experiments. It turned out that there was relatively little variation in the degree of anti-IgE-induced histamine release from basophils in blood specimens obtained from individual donors on repeated occasions over a period of months. (Fig. 9). Thus it appears that

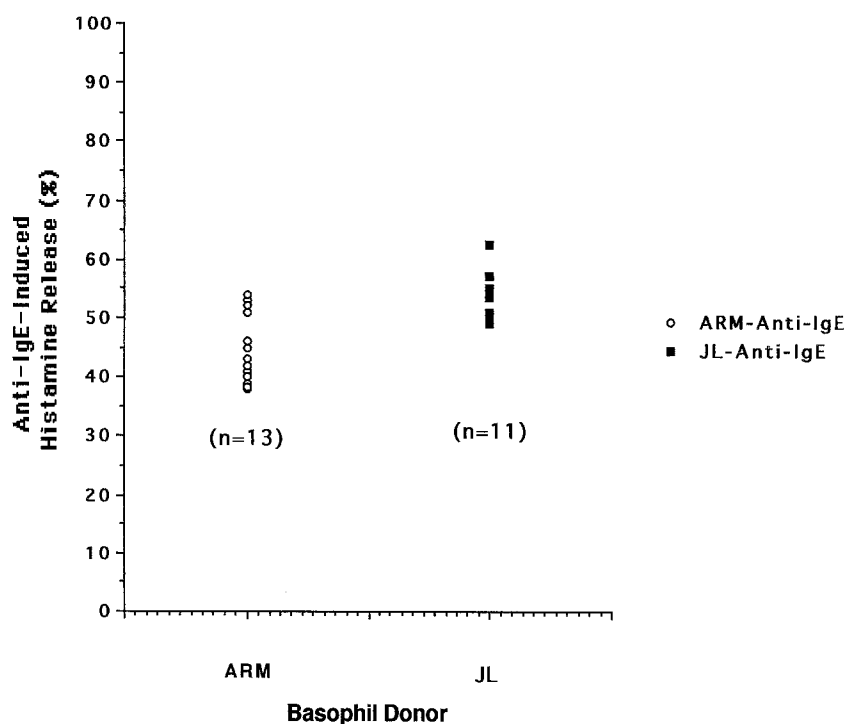


FIG. 9. Variability in the degree of anti-IgE-induced histamine release in basophils of two individual normal donors in blood specimens obtained sequentially over a period of 1 year (donor ARM, 13 specimens; donor JL, 11 specimens).

the variability in the degree of HRA-induced histamine release in the basophils of different normal donors was not simply a correlate of concomitant anti-IgE-induced histamine release.

To investigate these variable target responses further, experiments were designed to determine whether the nature of the IgE or other serum factors that could bind to basophils might play a role in the degree of HRA-induced histamine release from the basophils of a particular donor. Leukocytes were obtained from a normal cell donor (ARM) whose cells had previously released relatively modest amounts (15% to 30%) of histamine during incubation with most HRA⁺ sera (including the two sera to be used in these challenge experiments). These cells were then divided into 2 aliquots. Aliquot A was stripped of most IgE by lactic acid treatment, washed, and then incubated for 1 hour at 37° C with the serum of another normal cell donor whose basophils had previously released much more histamine (35% to 60%) when incubated with most HRA⁺ sera. Aliquot B was stripped of most IgE and was then incubated for 1 hour at 37° C with autologous serum.

Both cell aliquots were washed once with PAG at room temperature and then resuspended in PAGCM. They were then subdivided into aliquots

incubated with each of two HRA⁺ sera, HF and LCD, (40% vol/vol); anti-IgE, or additional PAGCM (to assess spontaneous histamine release).

As seen in Fig. 10, there was no significant difference in the HRA-induced histamine release from basophils of the individual donor (ARM), whether these cells had been previously incubated with the serum of a "high release" cell donor (JL) or with autologous serum. Approximately 17% histamine release was induced in both cell aliquots by serum HF, and 38% release was stimulated by serum LCD. These findings are similar to those observed when the basophils of donor ARM were incubated with the sera from HF and LCD without any previous stripping or serum incubation (23% and 41%, respectively). These findings also suggested that the variable degree of HRA-induced histamine release from basophils of different cell donors was not a reflection of factors present in the donor serum, which might bind to the target basophils.

DISCUSSION

The findings reported confirm the earlier observation of Grattan et al.² and Hide et al.³ that one or more factors present in the serum of a sizable percentage of patients with CIU induce histamine

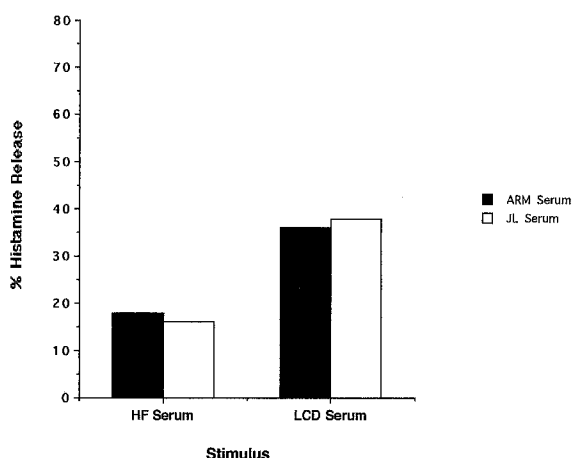


FIG. 10. Histamine release induced by two HRA⁺ sera in the basophils of normal donor ARM (that had previously responded modestly to these two sera), which had been IgE-stripped and then incubated in autologous serum and serum of normal donor JL whose own basophils were more reactive to these two HRA⁺ sera.

release from the basophils of normal individuals without urticaria. As reported by those earlier observers, we also found the HRA to be localized in the IgG fraction of the CIU sera. We have used the term HRA to describe this activity because we do not yet know whether the biologic effects we have observed are due to one or more discrete factors. However, the HRA reported here does not appear to be the same as the lower molecular weight (15 to 35 kd) factors released from stimulated cultured blood mononuclear cells, originally described by Thuesen et al.¹⁶ as HRA.

However, there were some important differences in our findings compared with the earlier reports. Grattan et al.² originally concluded that the HRA in CIU sera behaved like an anti-IgE antibody. Later studies from this group showed that five of the 17 HRA⁺ sera (about 30%) had properties of an anti-IgE antibody.³ Another five sera (30%) reacted significantly more with basophils with very little endogenous surface IgE. The rest of these HRA⁺ sera did not induce significantly different histamine release in basophils with ample surface IgE compared with basophils with very little surface IgE. They provided other evidence to suggest that the HRA⁺ sera inducing histamine release predominantly from the "low surface IgE" basophils were binding directly to the FcεRI, possibly as an anti-FcεRI antibody.³

In contrast, we found that only one of 13 HRA⁺

sera studied behaved like an anti-IgE antibody, inducing significantly less histamine release from normal basophils previously stripped of surface IgE. Eleven of the other HRA⁺ sera did the opposite—stimulated more histamine release from IgE-stripped basophils, with diminution of the histamine release of the normal basophils that had been incubated with IgE-rich sera from atopic subjects without urticaria. Thus almost all the HRA⁺ sera that we studied appear to act in good part by binding directly to the IgE receptor, in contrast to the findings of Hide et al.³ of such activities in about 30% of their sera.

Our studies turned up additional new findings. We found that incubating the HRA⁺ sera at 56°C for 4 hours reduced their subsequent HRA by approximately 50%. This suggests that IgE may be a constituent, along with IgG, in the serum component(s) inducing histamine release from normal basophils. Such heating at 56°C for 4 hours is thought to alter binding of IgE to its receptors.¹² Prior incubation of anti-IgE antisera at 56°C for 4 hours did not reduce the HRA of these antisera in our studies. Of note, there was no reduction by prior incubation at 56°C for 4 hours in the HRA of the one serum in our HRA⁺ serum group, which had otherwise behaved like anti-IgE antiserum.

Prior incubation at 56°C for 30 minutes did not significantly reduce the HRA of any of the HRA⁺ sera tested, suggesting that the classical complement pathway is not involved. Also, no decreased serum levels of C3 and C4 were found in these HRA⁺ sera.

We do not yet know the nature of this partially heat-sensitive component in HRA⁺ sera. An IgG-anti-IgE immune complex could bind to and possibly activate the FcεRI. Hide et al.³ found that the histamine release induced by HRA⁺ sera predominantly in "low surface IgE" basophils could be inhibited by the soluble fragment of the FcεRI. It is conceivable that this inhibition could be due to decreased binding of an IgE-containing immune complex, as well as to decreased binding of an IgG anti-FcεRI antibody. IgG anti-IgE antibodies have been detected by binding assays in the sera of patients with several types of dermatoses,^{15, 17, 18} but their clinical relevance is uncertain.⁹ Further investigation is required to answer these questions.

Our study has also provided new information about the interaction between HRA⁺ sera and the target basophil. We confirmed the earlier observation of Grattan et al.² that there is considerable variability in the degree of histamine release induced by individual HRA sera by the basophils of

different normal donors. Because the reasons for this variability have not been established, we carried out additional investigations. There were definite, though variable, levels of serum IgE in all the basophil donors. There was a trend toward greater responses to HRA⁺ sera in basophils of atopic donors. However, there was no significant correlation between the degree of histamine release induced by HRA⁺ sera compared with release induced by anti-IgE on the basophils of individual donors. Of note, we found that the basophils of occasional normal donors became transiently unresponsive to stimulation by HRA⁺ sera, only to become fully reactive several months later. Interestingly, this unresponsiveness of certain basophil populations to HRA⁺ sera was generally accompanied by a markedly diminished histamine release induced by anti-IgE antiserum (but unaffected responses to calcium ionophore). A pattern of transient unresponsiveness of some normal basophils to anti-IgE antisera has been described by MacGlashan.¹⁹

In addition, "lower responding" basophils could not be converted into higher response to HRA⁺ sera by prior stripping and then incubation in the sera of "higher responding" cell donors. Thus it appears unlikely that the variability in basophil responses is due to differences in levels of serum factors, which may bind to receptors or other surface determinants on the basophils.

Nevertheless, our findings support the impression that HRA that we observed in some CIU sera is related somehow to the clinical manifestations. In those patients who had HRA⁺ sera when they had symptoms, comparative studies of sequentially obtained serum specimens showed that levels of HRA decreased markedly, frequently becoming undetectable, when the patients became free of symptoms. This decrease in HRA does not appear to be due to any in vitro effects of any medication, which might be present in the sera of our treated patients with CIU because similar decreases in HRA were found in sera of HRA⁺ patients who became free of symptoms and were then receiving no treatment when the follow-up blood specimen was obtained. It is not known whether HRA is pathogenic in these patients or just a marker of disease activity. Greaves²⁰ has recently concluded that autoantibodies are important in explaining why some patients have severe, unremitting, chronic urticaria.

However, we did find decreased frequency of basophils in some of the patients with positive HRA, as did Hide et al.³ We do not yet have

sufficient sequential measurements in these patients to determine whether the basophil levels returned to the normal range when these patients became free of symptoms.

Nevertheless, these and other studies strongly suggest that HRA plays a pathogenic role in some patients with urticaria, especially because preliminary trials of plasmapheresis in a few patients with HRA⁺ sera have been associated with an impressive transient clinical improvement.⁶ Preliminary trials by the same group in patients with severe chronic urticaria and autoantibodies indicate that intravenous immunoglobulin and cyclosporine were associated with remission in some cases.²⁰ These provocative findings should stimulate further investigation of possible pathogenic mechanisms in CIU.

REFERENCES

1. Champion RH, Roberts SOB, Carpenter RG, Roger JH. Urticaria and angioedema. A review of 554 patients. *Br J Dermatol* 1969;81:588-97.
2. Grattan CEH, Francis DM, Hide M, Greaves MW. Detection of circulating histamine-releasing auto antibodies with function properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1992;21:695-704.
3. Hide M, Francis DM, Grattan CEH, et al. Auto-antibodies against the high affinity IgE receptors as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-604.
4. Rorsman H. Basophilic leucopenia in different forms of urticaria. *Acta Allergol* 1962;17:168-84.
5. Kern F, Lichtenstein LM. Defective histamine release in chronic urticaria. *J Clin Invest* 1976;57:1369-77.
6. Grattan CEH, Francis DM, Slater NGP, Barlow RJ, Greaves MW. Plasmapheresis for severe unremitting chronic urticaria. *Lancet* 1992;339:1078-80.
7. Grattan CEH, Wallington TB, Warin RP, Kennedy CTC, Bradfield JW. A serological mediator in chronic idiopathic urticaria: a clinical, immunological and histological evaluation. *Br J Dermatol* 1986;114:583-90.
8. Grattan CEH, Boon AP, Eady RAJ, Winklemann RK. The pathology of the autologous serum skin test response in chronic urticaria resembles IgE-mediated late phase reaction. *Int Arch Allergy Appl Immunol* 1990;93:198-204.
9. Hide M, Francis DM, Grattan CEH, Barr RM, Winklemann RK, Greaves MW. The pathogenesis of chronic idiopathic urticaria: new evidence suggests an auto-immune basis and implications for treatment. *Clin Exp Allergy* 1994;24:624-7.
10. Tanus T, Valenzano M, Zweiman B, Atkins PC. Serum basophil histamine releasing activity in chronic urticaria [Abstract]. *J Allergy Clin Immunol* 1994;93:277.
11. Atkins PC, von Allmen C, Valenzano M, Olson R, Shalit M, Zweiman B. Determinants of in vivo histamine release in cutaneous allergic reactions in humans. *J Allergy Clin Immunol* 1990;86:371-9.
12. Dorrington KJ, Bennich HH. Structure-function relationships in human immunoglobulin E. *Immunol Rev* 1978;41:3-28.

13. MacDonald SM, White JM, Kagey-Sobotka A, MacGlashan DW, Lichtenstein LM. The heterogeneity of human IgE exemplified by the passive transfer of D₂O sensitivity. Clin Exp Allergy 1990;21:133-8.
14. Dunskey EH, Zweiman B, Fischler E, Levy DA. Early effects of corticosteroids on basophils, leukocyte histamine and tissue histamine. J Allergy Clin Immunol 1979;63:426-32.
15. Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE auto-antibodies in urticarial syndromes. J Invest Dermatol 1989;93:246-52.
16. Thuesen DO, Speck LS, Lett-Brown MA, Grant JA. Histamine releasing activity (HRA). I. Production by mitogen or antigen-stimulated human mononuclear cells. J Immunol 1979;123:626-33.
17. Marone G, Casolaro V, Paganelli R, Quinti I. IgG anti-IgE from atopic dermatitis induces mediator release from basophils and mast cells. J Invest Dermatol 1989;93:246-52.
18. Twena DM, Marshall JS, Haeney MR, Bell EB. A survey of non-atopic and atopic children and adults for the presence of anti-IgE auto antibodies. Clin Immunol Immunopathol 1989;53:40-51.
19. MacGlashan D Jr. Signal transduction: mechanisms in basophils. J Allergy Clin Immunol 1994;94:1146-51.
20. Greaves MW. Chronic urticaria. N Engl J Med 1995;332:1767-72.

ON THE MOVE?

Send us your new address at least six weeks ahead

Don't miss a single issue of the journal! To ensure prompt service when you change your address, please photocopy and complete the form below.

Please send your change of address notification at least six weeks before your move to ensure continued service. We regret we cannot guarantee replacement of issues missed due to late notification.

JOURNAL TITLE:

Fill in the title of the journal here. _____

OLD ADDRESS:

Affix the address label from a recent issue of the journal here.

NEW ADDRESS:

Clearly print your new address here.

Name _____

Address _____

City/State/ZIP _____

COPY AND MAIL THIS FORM TO:

Journal Subscription Services
Mosby-Year Book, Inc.
11830 Westline Industrial Dr.
St. Louis, MO 63146-3318

OR FAX TO:

314-432-1158

OR PHONE:

1-800-453-4351
Outside the U.S., call
314-453-4351

 **Mosby**