Serum IgG Autoantibodies Directed against the α Chain of FcεRI: A Selective Marker and Pathogenetic Factor for a Distinct Subset of Chronic Urticaria Patients?

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Abstract

While it is well established that acute allergic urticaria is caused by degranulation of skin mast cells occurring after allergen/IgE-dependent cross-linking of high affinity IgE receptors (FcεRI), the pathophysiologic mechanisms operative in chronic urticaria (CU) are less well understood. Some evidence points to the existence of histamine-releasing activity in the serum of CU patients which possibly acts via triggering of FcεRI. In this study, we aimed to better characterize this anti-FcεRIα reactivity of CU patients using affinity-purified, IgE-depleted IgG fractions of such individuals (CU-IgG). Using immobilized, recombinant soluble FcεRIα as a reaction target for Western blot studies, we found that 12/32 (37%) CU-IgG serum samples exhibited IgG autoreactivity against FcεRIα. These findings were confirmed by experiments demonstrating that immunoblot-reactive, but not immunoblot-nonreactive, CU-IgG preparations precipitated the FcεRIα from FcεRIαγ-transfected cells. No anti-FcεRIα reactivity was observed in IgG fractions from atopic dermatitis (AD) patients (0/15) or healthy control individuals (CO: 0/15). As opposed to the selective occurrence of IgG anti-FcεRIα autoantibodies in CU patients, IgG anti-IgE antibodies were detected in all groups investigated (CU: 69%; AD: 73%; CO: 26%). While both types of autoantibodies can exhibit histamine-releasing properties, not all of the autoantibodies proved to be functional in vitro. Our results indicate that the occurrence of IgG anti-FcεRIα reactivity defines an autoimmune-mediated subentity of CU and provide a basis for the development of new diagnostic procedures and, perhaps, therapeutic strategies for this disease. (*J. Clin. Invest.* 1995. 96:2606–2612.) Key words: autoimmunity • IgE receptors • histamine release • mast cell degranulation • pseudoallergic reaction

Introduction

Chronic urticaria (CU)[1] is a common disorder characterized by the eruption of transitory, itchy skin swellings which recur for weeks to years. Several conditions such as bacterial or fungal infections, psychological factors, neoplasms, and intolerance to certain food additives (1, 2) have been found in connection with CU symptoms and, thus, were implicated as etiopathogenetic factors. However, in a given patient, the tedious and laborious search for such conditions is usually not revealing and, even if successful and appropriately dealt with, not necessarily followed by the resolution of symptoms (1).

Since urticaria is the consequence of vasoactive mediator release from skin mast cells (MC) (3), intensive research has focused on the nature of histamine-releasing factors in CU. These studies accumulated evidence for the presence of MC-activating factors within the immunoglobulin (Ig) and/or non-immunoglobulin (e.g., substance P and lipid-derived mediators) fraction of CU serum (2). Classical Ig-mediated type I allergic reactions, i.e., allergen-induced cross-linking of IgE bound to its high affinity receptor (FcεRI) on skin MC (4, 5), are apparently not a common cause for the observed Ig-mediated histamine-releasing activity in CU since allergen-specific IgE can only rarely be identified and total IgE levels are usually normal in CU sera. Evidence for the pathogenetic importance of Ig isotypes other than IgE came from the observation that severe CU episodes can be ameliorated or even abolished after IgG adsorbance by plasmapheresis (6). In fact, serum IgG anti-IgE autoantibodies with MC-activating properties have been described in CU (7, 8). Further evidence for a role of IgG autoantibodies in CU came from a recent study demonstrating histamine-releasing capacity of serum IgG isolates that could be blocked by soluble FcεRIα chain (9). Therefore, it was reasoned that FcεRI can be a target structure recognized by IgG autoantibodies (9). The experimental conditions chosen were not able to determine whether the observed FcεRI-mediated MC degranulation was due to (*a*) in vivo formed IgG/anti-IgE autoantibody complexes (10) which may copurify with the IgG fraction, and/or (*b*) IgE-independent FcεRIα-specific IgG autoantibodies. To distinguish between these two possibilities, we investigated the antigen and epitope specificity of histamine-releasing autoantibodies in IgG fractions devoid of IgG/anti-IgE complexes.

Methods

Patients and controls. 30 patients with CU, defined as recurrent wheals lasting < 24 h and occurring at least twice a week for over 2 mo, were recruited (2, 11). Patients with clinical evidence of urticarial vasculitis or physical urticaria were excluded. None of the patients were taking

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1. *Abbreviations used in this paper:* AD, atopic dermatitis; BSA-NP, nitrophenylacetyl-coupled BSA; CHO, Chinese hamster ovary cells; CO, healthy control individuals; CU, chronic urticaria; FcεRI, high affinity receptor for the Fc part of IgE; FcγRs, receptors for the Fc part of IgG; MC, mast cell.

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steroids or immunosuppressive drugs at the time of venipuncture. In addition, sera from 15 patients suffering from atopic dermatitis (AD) (12) were collected and sera from 15 healthy individuals (CO) served as controls. All sera were stored at −20°C until investigation.

Isolation of IgG fractions. IgG was purified from serum samples by affinity chromatography using protein G columns (Pierce, Rockford, IL) according to the manufacturer's instructions. Eluates containing IgG were adjusted to 10 mg/ml and analyzed for contaminating IgE by ELISA (Pharmacia Biotech, Brussels, Belgium). After running on a 5% SDS-PAGE gel, purity of the IgG fraction was confirmed by protein staining (copper stain kit; Bio-Rad Laboratories, Richmond, CA) and by anti-IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or anti-IgE (Accurate Chemical & Scientific Corp., Westbury, NY) Western blotting. To selectively denature IgE molecules possibly contaminating the protein G eluates, fractions were heated for 3 h at 56°C as described (13, 14).

Immunoaffinity depletion of IgE. Immunoaffinity-purified, polyclonal goat anti-human IgE (Chemicon International, Inc., Temecula, CA) was immobilized on CH Sepharose 4B (Pharmacia) according to the manufacturer's guidelines. Depletion of a myeloma IgE sample (500 μg/ml) was used to establish the IgE-binding capacity of the anti-IgE column. IgE depletion was performed by passing IgG specimens through the affinity column twice.

mAbs used in the study. The anti-FcεRIα mAbs 15-1 and 19-1 were described recently (15). Chimeric human IgE anti-nitrophenylacetyl (NP; cIgE) and myeloma IgE were purchased from Serotec Ltd. (Oxford, United Kingdom). IgG1 and IgG2a isotype controls were obtained from Sigma Chemical Co. (St. Louis, MO).

Cell lines. Chinese hamster ovary (CHO) cells were obtained from the American Type Culture Collection (Rockville, MD). Stable transfectants coexpressing the human FcεRIα- and FcεRIγ chain (CHOαγ cells) have been described previously (15).

Human recombinant soluble FcεRIα. The gene segment encoding the extracellular portion of human FcεRIα (16) was cloned into the baculovirus vector pVL941. Recombinant baculovirus was generated in insect cells (Invitrogen, San Diego, CA) using the Baculogold transfection kit (PharMingen, San Diego, CA) according to the manufacturer's instructions. The soluble receptor molecule was purified from supernatants of infected insect cells by a two-step chromatography protocol. First, the material was immunoaffinity-purified on a Sepharose 4B–coupled (Pharmacia Biotech, Uppsala, Sweden) mouse anti-human FcεRIα mAb. Final purification was achieved by anion exchange column chromatography (Mono Q; Pharmacia Biotech). The purification product migrated as a single 32-kD band as judged by silver staining of a denaturing polyacrylamide gel.

SDS-PAGE and immunoblotting. Soluble recombinant FcεRIα (200–400 ng/lane) or precipitated samples were submitted to electrophoresis on 10–13% gels and blotted onto nitrocellulose membranes (Bio-Rad Laboratories) which were then blocked with 5% dry milk/0.05% Tween 20 (Sigma Chemical Co.)/PBS for at least 6 h. Reactivities of FcεRIα-specific mAb 19-1 and the IgG2a isotype control mAb (200 ng/ml) were detected with a goat anti- mouse horseradish peroxidase conjugate (1:40,000) (Bio-Rad Laboratories). Binding of the biotinylated mAb 19-1 and the biotinylated isotype control mAb were detected using a streptavidin horseradish peroxidase conjugate (1:2,000) (Amersham International, Buckinghamshire, United Kingdom). For the detection of FcεRIα-reactive serum Abs, membranes were reacted with purified IgG specimens diluted 1:25 or 1:10 (i.e., 400 or 1,000 μg IgG/ml). As controls, binding of native and heat-denatured myeloma IgE (10 μg/ml) to membrane-bound soluble FcεRIα was investigated. Membrane-bound first-step antibodies were detected with either rabbit anti-human IgG (1:50,000) or rabbit anti-human IgE horseradish peroxidase conjugates (1:30,000). Membranes were incubated with ECL developing solution (Amersham International) and exposed to Kodak X-Omat S films. For the detection of autoantibodies with anti-IgE properties the following procedure was used: 200–400 ng/lane NP-conjugated BSA (BSA-NP) (17) were electrophoresed and blotted onto nitrocellulose membranes that were blocked with 5% dry milk/0.05% Tween 20/PBS before exposure to cIgE (10 μg/ml). The subsequent detection of membrane-bound IgG anti-IgE reactivity was performed as described above.

Immunoprecipitation. CHOαγ cells were solubilized in 1% NP-40 Tris-lysis buffer as described (18). Before immunoprecipitation, lysates were precleared with protein G beads (Sigma Chemical Co.) for 4 h. Lysates of 1×10^5 cells were incubated with 40 μl of the serum IgG specimens for 2 h at 4°C. Next, protein G beads were added and samples were rotated for an additional hour at 4°C. Precipitated proteins were eluted, electrophoresed, and immunoblotted with FcεRIα-specific mAb 19-1 as described above.

Biotinylation of IgG fractions. IgG fractions were dialyzed against buffer (0.1 M $NaHCO_3$, 1 M NaCl) overnight and, thereafter, reacted with Biotin-X-NHS (Calbiochem-Novabiochem Co., La Jolla, CA) 15–30 mg/ml/mg protein for 1 h at room temperature.

Flow cytometric studies. The binding of biotinylated Abs to FcεRIαγ-transfected and untransfected CHO cells was visualized by incubating the cells with 1 μg/ml streptavidin-PE (Becton Dickinson & Co., Mountain View, CA). Cellular fluorescence was analyzed on a FACScan® flow cytometer supported by Lysis II software (both from Becton Dickinson & Co.). In the blocking studies, cells were incubated with 50 μg/ml of mAb 15-1, mAb 19-1, mAb 15-1, and mAb 19-1, or 20–200 μg/ml cIgE before exposure to the biotinylated cIgE or serum IgG fractions.

Histamine release. Histamine release assays were performed with basophil-enriched peripheral blood cells from two unrelated healthy volunteers. Removal of in vivo bound IgE from basophils was performed after dextran sedimentation as described (19). Where indicated, basophils were reconstituted with cIgE (10 μg/ml, 30 min, 4°C). Basophil-enriched cell fractions were incubated for 40 min at 37°C in buffer containing IL-3 (20) and 1:2, 1:5, 1:10, or 1:20 dilutions of serum IgG fractions from patients or healthy controls. Monoclonal anti-human IgE antibody was used as a positive control. Histamine release was measured with RIA (Immunotech International, Marseille, France) and expressed as a percentage of total cellular histamine. Spontaneous release accounted always for $< 5\%$ of total histamine.

Results

Protein G isolates from serum of CU patients react with recombinant FcεRIα. In a first set of experiments we aimed to gain biochemical evidence for the occurrence of anti-FcεRIα immunoreactivity in CU sera. Our test system uses recombinant FcεRIα protein that has been expressed in baculovirus-infected insect cells as a truncated non-fusion protein lacking the transmembrane/intracytoplasmic domain. Western blot experiments revealed that the soluble FcεRIα is recognized by the FcεRIα chain-specific mAb 19-1 as a single protein band of 30–35 kD and, to a lesser extent, as a dimer of ~ 65 kD (Fig. 1 *A*, *left panel*) that disappears under reducing conditions (data not shown). The availability of this recombinant material as immunoblotted reaction target for serum antibodies enabled us to investigate large numbers of serum samples for the presence of the postulated anti-FcεRIα immunoreactivity. Fig. 1 *A* depicts a representative immunoblot experiment showing that the protein G-binding fraction of a selected CU serum contains FcεRIα-binding activity that can be detected with anti-human IgG Abs. The antigenic specificity of the phenomenon was demonstrated by the inhibition of the binding of FcεRIα-specific moieties by incubation of CU-IgG fractions with soluble α-protein before the reaction with the immunoblotted material (Fig. 1 *A*). To definitively prove that FcεRIα is recognized as a nondenatured protein by CU-IgG fractions, immunoprecipitation experiments using FcεRIαγ-transfected CHO cells were performed. Therefore, IgG fractions from 24 CU patients were comparatively analyzed for their capacity to precipitate FcεRIα from transfectants and to react with soluble FcεRIα in Western

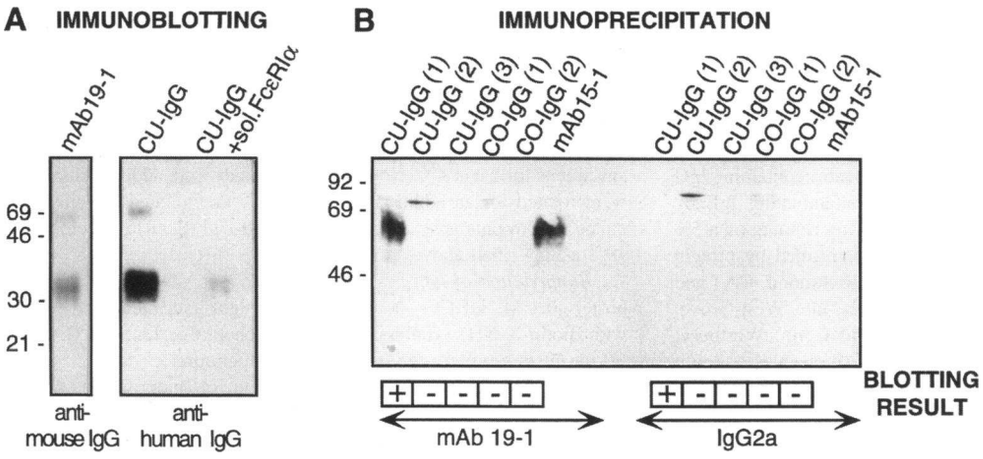

Figure 1. Serum IgG preparations of CU patients (CU-IgG) react with recombinant FcεRIα. (*A*) Recombinant FcεRIα was electrophoresed and blotted onto nitrocellulose. Membranes were reacted with FcεRIα-specific mAb 19-1 and CU-IgG in the presence or absence of excess soluble FcεRIα. Ab-binding was visualized with goat anti-mouse and rabbit anti-human IgG Abs, respectively. (*B*) CU-IgG preparations [CU-IgG (1–3)], IgG specimens from healthy controls [CO-IgG (1) and CO-IgG (2)], or the anti-FcεRIα specific mAb 15-1 was reacted with lysates of FcεRIαγ-transfected CHO cells. Precipitated proteins were blotted and reacted with biotinylated mAb 19-1 or a biotinylated IgG2a control mAb. Membrane-bound first-step mAbs were detected with streptavidin peroxidase. Molecular mass calibration is shown on the left in kilodaltons.

blot experiments. We found that the same 8/24 CU-IgG specimens that were reactive in immunoblot experiments precipitated FcεRIα [50–70 kD (5)] from CHOαγ lysates [e.g., CU-IgG (1); Fig. 1 *B*]. No signals were obtained when either Western blot–nonreactive CU-IgG [(16/24), e.g., CU-IgG (2) and CU-IgG (3); Fig. 1 *B*], IgG from healthy controls [e.g., CO-IgG (1) and CO-IgG (2); Fig. 1 *B*], or lysates from untransfected CHO cells (data not shown) were used for precipitation.

Since IgG anti-IgE serum antibodies would result in the copurification of IgE and IgG when applied to protein G columns, we had to determine whether the observed anti-FcεRIα immunoreactivity derives from true IgG anti-FcεRIα autoantibodies or, alternatively, may be due to the occurrence of IgG anti-IgE/IgE immune complexes. When we compared serum and protein G fractions for IgG- and IgE-mediated Ig-binding to immobilized FcεRIα we found that unfractionated CU sera exhibited not only IgG but also IgE reactivity to the FcεRIα protein whereas no IgE-binding was detectable with protein G–purified IgG fractions (Fig. 2, *Exp. I*). Since the remote possibility still existed that minute quantities of IgE had mediated IgG (anti-IgE)-binding to FcεRIα molecules, protein G–IgG fractions were further subjected to selective heat denaturation of IgE and/or anti-IgE immunoaffinity depletion. To see whether these treatment modalities or the combination thereof are/is effective in prevention of IgE-binding to immunoblotted FcεRIα, their efficacy was tested on purified myeloma IgE. As demonstrated in Fig. 2, *Exp. II*, heat denaturation clearly reduces IgE-binding to FcεRIα but does not influence the anti-FcεRIα reactivity of CU-IgG fractions. The occurrence of FcεRIα-bound myeloma IgE was completely abrogated by selective heat denaturation of the protein G fraction combined with two rounds of anti-IgE immunoaffinity depletion. In contrast to this loss of myeloma IgE–mediated immunoreactivity, CU-IgG–mediated anti-FcεRIα reactivity remained unchanged under identical treatment conditions (Fig. 2, *Exp. III*). Taken together, these experiments demonstrate that the serum of certain CU patients contains IgG autoantibodies which recognize the IgE-binding a chain of FcεRI in an IgE-independent manner.

FcεRIα-specific IgG autoantibodies recognize epitopes closely related to the IgE-binding site of the high affinity receptor for IgE. Next, we investigated whether serum IgG from CU patients can bind to native FcεRIα protein expressed on cell surfaces. As shown in Fig. 3 *A*, heat-denatured and IgE-depleted CU-IgG was found to bind to FcεRIαγ-transfected, but not untransfected, CHO cells, indicating that under native conditions the extracellular part of FcεRIα is a specific target for serum autoantibodies in CU. The question of whether these IgG autoantibodies recognize epitopes related or unrelated to the IgE-binding site of the high affinity IgE receptor was addressed by blocking studies using anti-FcεRIα–reactive mAbs recognizing epitopes related to the IgE-binding site of FcεRIα (mAb 15-1 and mAb 19-1) or cIgE. Fig. 3 *B* shows that the binding abilities of biotinylated CU-IgG were modified by preincubation with either mAb 15-1 or mAb 19-1. Control experiments demonstrated that IgE-binding to FcεRIαγ-transfected CHO cells can be prevented by preincubation of the cells with mAb 15-1 or cIgE, but, possibly due to the low binding affinity of mAb 19-1 (Kinet, J.-P., unpublished observation), is hardly inhibited by this mAb. Nevertheless, the combination of mAb 15-1 and mAb 19-1 resulted in additive and, therefore, almost complete inhibition of CU-IgG–binding to FcεRIα (Fig. 3 *B*). A virtually identical inhibitory effect on autoantibody-binding was observed when the FcεRIαγ-transfected cells had been preincubated with cIgE (Fig. 3 *B*). These findings strongly suggest that the anti-FcεRIα autoreactivity present in CU sera is directed against IgE-binding epitopes and/or epitopes closely related to the IgE-binding site of FcεRIα. Surprisingly, high concentrations (200 μg/ml) of cIgE were needed to completely prevent IgG autoantibody-binding to the transfectants (Fig. 3 *B*). Preincubation of the cells with 20 μg/ml cIgE was largely ineffective (data not shown). Taken together these data imply that FcεRIα autoantibodies are present in considerably high concentrations and/or are affinity-matured, high affinity Abs as suggested by their IgG nature.

Serum IgG specimens from CU patients contain IgG autoreactivity directed against IgE and/or FcεRIα. Since anti-IgE autoantibodies have been described in CU and have been implicated as a trigger mechanism of this disease, we decided to investigate the prevalence of the (co-)occurrence of anti-IgE and anti-FcεRIα autoantibodies in CU patients. For the detection of anti-IgE Abs, monoclonal IgE anti-NP bound to immobilized BSA-NP was used as a target molecule. The specific IgG anti-IgE autoantibody-binding to membranes was visualized with an anti–human IgG reagent (Fig. 4, representative experi-

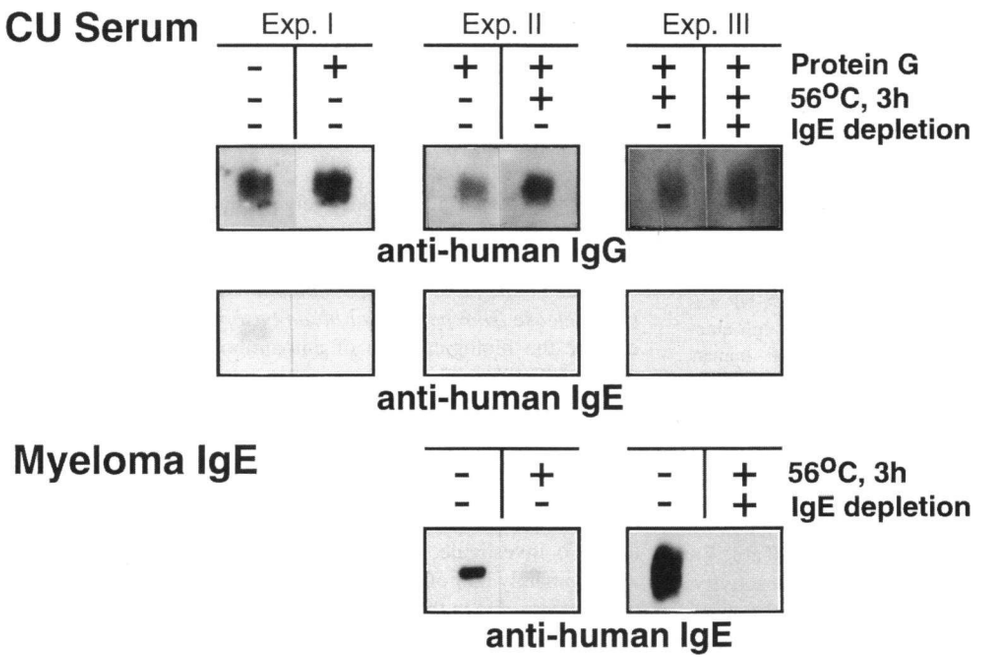

Figure 2. Elimination of residual IgE from CU-IgG isolates does not affect IgG-mediated anti-FcεRIα immunoreactivity. In three independent experiments, the effect(s) of protein G fractionation of serum (*Exp. I*), heat denaturation of protein G fractions (*Exp. II*), and anti-IgE immunoaffinity depletion of heated protein G fractions (*Exp. III*) on Ig-mediated FcεRIα reactivity was (were) investigated. Unfractionated serum (*Exp. I*) and protein G isolates either mock-treated (*Exps. I and II*), or subjected to heat treatment (56°C, 3 h) and/or anti-IgE immunoaffinity depletion (*Exps. II and III*) were allowed to react with immobilized FcεRIα. FcεRIα-bound IgG and IgE were detected with anti-human IgG and anti-human IgE Abs, respectively. The efficacy of heat treatment and/or IgE depletion procedures to abolish IgE-binding to immobilized FcεRIα is demonstrated using myeloma IgE (*Exps. II and III*). Representative experiment ($n = 4$).

ment). For control purposes, the binding of cIgE to BSA-NP was detected with an anti-IgE reagent (Fig. 4). BSA-NP in the absence of IgE anti-NP was used to demonstrate the anti-IgE specificity of the observed serum IgG-binding (Fig. 4). The comparison of anti-FcεRIα reactivity (Fig. 5 *A*) with anti-IgE reactivity (Fig. 5 *B*) revealed that both autoantibodies can, but do not necessarily, co-occur in CU sera.

In an extended patient study, we evaluated the prevalence

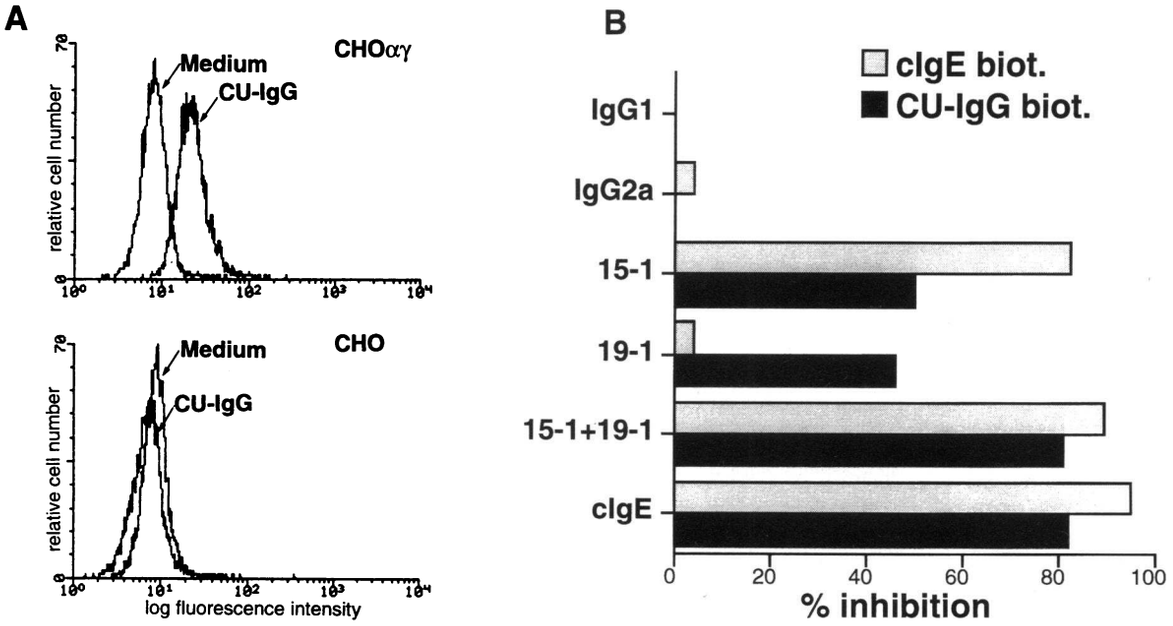

Figure 3. IgE-depleted CU-IgG binds to cell surface–expressed FcεRIα. (*A*) Binding of biotinylated CU-IgG to CHOαγ and to CHO cells was visualized by streptavidin-PE and analyzed by flow cytometry. Mean fluorescence intensity is given on the abscissa and the relative cell number on the ordinate. Representative experiment out of seven. (*B*) Reactivity of biotinylated cIgE or biotinylated CU-IgG with CHOαγ cells after preincubation of the cells with mAbs 15-1, 19-1, 15-1 plus 19-1, cIgE, or IgG1 and IgG2a control mAbs. Abscissa shows the mAb-induced inhibition (%) of cIgE- and CU-IgG–binding.

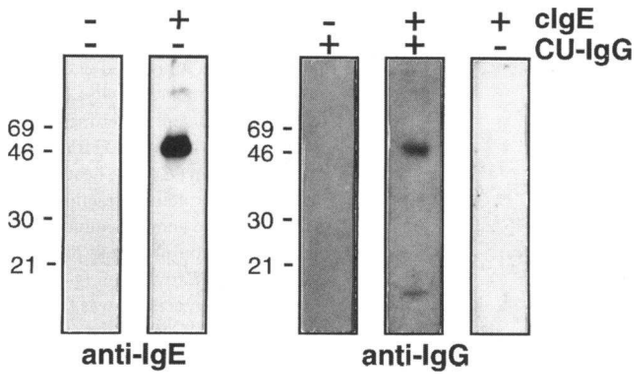

Figure 4. Biochemical demonstration of anti-IgE autoreactivity in CU-IgG preparations. cIgE (IgE-anti NP)-binding to blotted BSA-NP was confirmed by anti-human IgE immunoreactivity (*left*). Anti-IgE autoantibodies in CU IgG fractions were detected with rabbit anti-human IgG Abs (*right*). Omission of either cIgE or CU-IgG results in loss of anti-human IgG reactivity. Molecular mass calibration is shown on the left in kilodaltons.

of anti-FcεRIα and anti-IgE autoreactivity in IgG fractions from CU patients, AD patients, and healthy controls (CO) (Table I). 37% of the CU-IgG samples exhibited anti-FcεRIα reactivity, whereas none of the AD-IgG samples or IgG samples from healthy volunteers reacted. Anti-IgE reactivity was found in 69% of the CU patients, in 73% of AD-IgG samples, and in 26% of healthy controls. This observed prevalence of IgG anti-IgE autoreactivity might even be underestimated because the experimental conditions used do not allow the detection of anti-idiotypic or IgE-saturated IgG anti-IgE autoantibodies. IgG anti-FcεRIα and IgG anti-IgE autoreactivity co-occurred in 22% of the CU patients but in none of the AD patients or controls. Our data clearly show that, in contrast to anti-IgE autoreactivity,

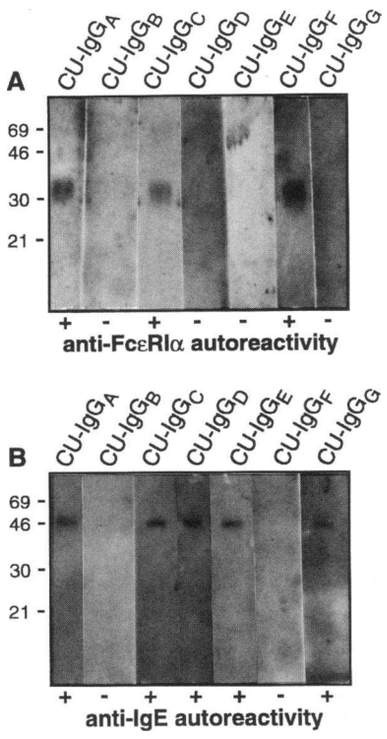

Figure 5. Comparative analysis of anti-FcεRIα (*A*) and anti-IgE (*B*) autoreactivity in CU-IgG fractions. Sera of CU patients can contain IgG autoreactivity directed against either FcεRIα (CU-IgG$_F$), IgE (CU-IgG$_D$, CU-IgG$_E$, CU-IgG$_G$), or both FcεRIα and IgE (CU-IgG$_A$, CU-IgG$_C$). In one case (CU-IgG$_B$) neither antigen is recognized. Molecular mass calibration is shown on the left in kilodaltons.

Table I. Patient Study

	CU-IgG	AD-IgG	CO-IgG
Anti-FcεRIα	37% (12/32)	0% (0/15)	0% (0/15)
Anti-IgE	69% (22/32)	73% (11/15)	26% (4/15)
Either/or	81% (26/32)	73% (11/15)	26% (4/15)
Both	22% (7/32)	0% (0/15)	0% (0/15)

anti-FcεRIα reactivity is selectively present in CU patients and —within this disease entity—defines a subgroup of such patients.

Anti-FcεRIα and anti-IgE autoantibodies can induce histamine release from peripheral blood basophils. In an attempt to explore the biological role of autoantibodies against FcεRIα and/or IgE, CU-IgG specimens were analyzed for their capacity to release histamine from basophils of healthy donors. Exposure to anti-FcεRIα autoantibody-positive IgG fractions revealed a substantial release of endogenous histamine in four out of eight cases (Fig. 6, representative experiment, $n = 3$). The magnitude of this response was comparable with positive control stimulation. To investigate whether the lack of mediator release in approximately half of the CU patients with anti-FcεRIα autoantibodies was due to the receptor blockade by in vivo bound IgE, IgE was eluted from basophils by lactic acid stripping before exposure to IgG fractions. Neither the removal of surface-bound IgE (data not shown) nor the reconstitution of the stripped cells with 10 μg/ml monomeric IgE changed the histamine-releasing capacity of anti-FcεRIα-containing specimens (Fig. 6 *A*). Importantly, two of three anti-IgE-containing IgG fractions induced histamine release from basophils reconstituted with saturating doses of IgE (Fig. 6 *A*, representative experiment, three independent experiments). To investigate whether the failure of certain FcεRIα-reactive IgG specimens to release histamine was due to low concentrations of autoantibodies present in these preparations, basophils were incubated with CU-IgG concentrations up to 5 mg/ml (dilution: 1:2). Fig. 6 *B* demonstrates that certain FcεRIα-reactive CU-IgG specimens, even when applied in high concentrations, did not result in induction of histamine release, whereas others activated basophils irrespective of the concentration used. Importantly, FcεRIα-nonreactive IgG from CU or CO did not induce histamine release at any of the concentrations tested (Fig. 6 *B*). Although not all of the anti-FcεRIα and/or anti-IgE autoantibody-containing IgG fractions were able to release histamine from peripheral blood basophils (Fig. 6, *A* and *B*), our data show that humoral autoimmune phenomena can be observed in the majority of CU patients and that, at least in a group of these patients, in vitro histamine release can be ascribed to the presence of autoantibodies.

Discussion

It is well known that serum of certain CU patients contains MC-activating properties as evidenced by its ability to elicit (*a*) localized urticarial skin reactions upon intradermal injection and (*b*) histamine release from basophils in vitro (8, 9). IgG anti-IgE autoantibodies have been implicated as one of the factors responsible for this phenomenon. Due to their capacity to bind and, possibly, cross-link IgE molecules bound to FcεRI on tissue MC, these autoantibodies may have the potential to induce histamine release in an IgE-dependent fashion. Indeed,

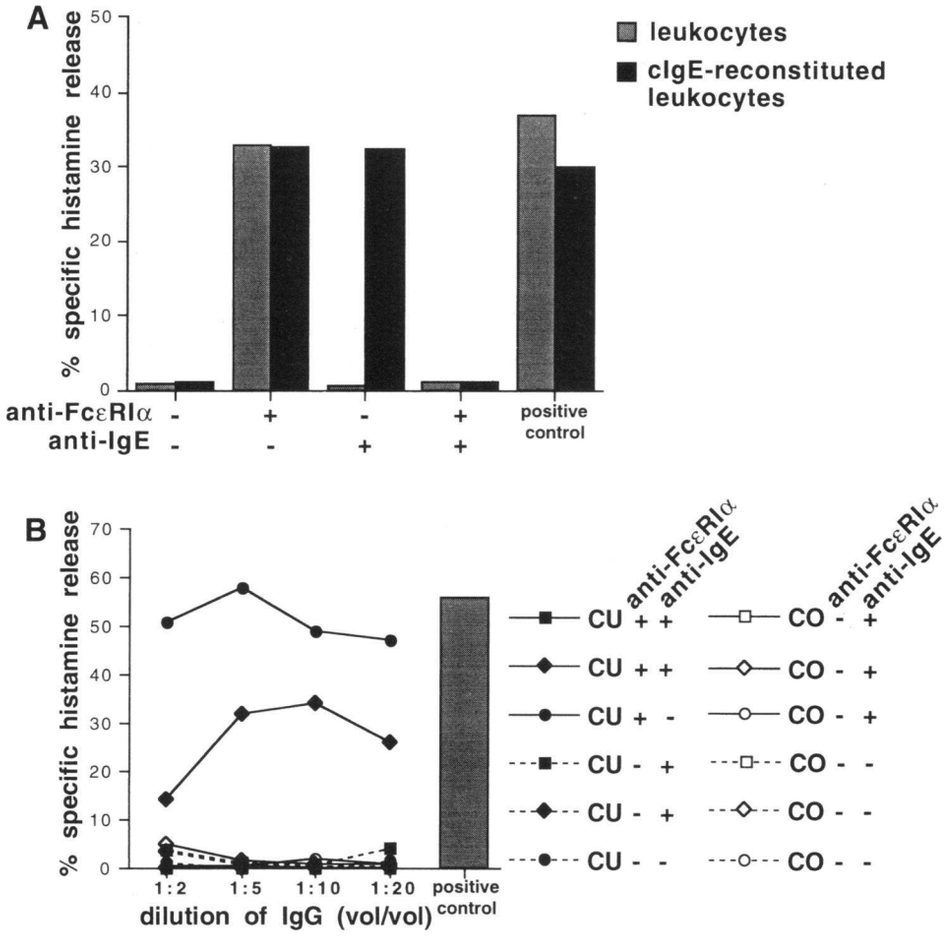

Figure 6. (*A*) Both anti-FcεRIα–reactive and anti-IgE–reactive, IgE-depleted CU-IgG fractions can release histamine from peripheral blood basophils. Anti-FcεRIα–reactive and/or anti-IgE–reactive CU-IgG preparations were incubated for 40 min at 37°C with either untreated or IgE-exposed, basophil-enriched leukocytes before harvesting the supernatants. (*B*) The lack of histamine-releasing capacity of certain FcεRIα-reactive and of all nonreactive IgG samples is not reverted by increasing the concentration of IgG. For this purpose, basophil-enriched leukocytes were stripped and exposed to 1:2, 1:5, 1:10, or 1:20 diluted CU- or CO-IgG. As a positive stimulation control, cells were treated with a mouse anti-IgE mAb; histamine release in the presence of incubation buffer alone (negative control) was always < 5% of total cellular histamine. Specific histamine release (%) is given on the ordinate (total cellular histamine minus spontaneous release = 100%).

we (this study) and others (8) were able to show that exposure of IgE-bound basophils to IgG anti-IgE antibody containing IgG fractions from CU patients can, but does not necessarily, induce histamine release from these cells. Although detected in the serum of most CU patients (reference 7 and this study), IgG anti-IgE autoantibodies have also been found in sera of patients suffering from atopic dermatitis (references 21–23 and this study) and, even, of healthy individuals (reference 24 and this study). In atopic individuals, the analysis of epitopes recognized by IgG anti-IgE autoantibodies revealed that these antibodies recognize mainly idiotypic Fab rather than framework IgE heavy chain determinants (25). Therefore, they should have the potential to cross-link receptor-bound IgE rather than prevent Fc-IgE from binding to IgE receptors. Despite the presence of such autoantibodies, atopic patients—in contrast to CU patients—very rarely suffer from recurrent wheal-and-flare reactions in the absence of allergen. It is therefore quite unlikely that IgG anti-IgE autoantibodies play a major pathogenetic role in CU.

Recent evidence points to the possibility that IgG autoreactivity directed against the α chain of the high affinity receptor for IgE can elicit clinical symptoms in CU (9). Although this study convincingly demonstrated IgG-mediated, FcεRIα-dependent histamine release from basophils, the experimental strategies used did not exclude the possibility that in vivo formed, protein G–purified IgG/anti-IgE immune complexes were responsible for the observed phenomenon. Biochemical results obtained in our study definitively prove the existence of true IgG anti-FcεRIα autoantibodies in CU sera by the demonstration that (*a*) anti-FcεRIα–reactive serum antibodies were detected with anti-IgG but not anti-IgE reagents and that (*b*) strategies resulting in complete elimination of IgE from CU-IgG samples (anti-IgE immunoabsorbance and selective heat denaturation of IgE) did not decrease IgG-mediated anti-FcεRIα immunoreactivity. The evaluation of the prevalence of anti-FcεRIα autoantibodies in CU and AD versus CO revealed that—in contrast to the anti-IgE reactivity—IgE-independent FcεRIα-specific autoreactivity resides selectively in the IgG fraction of 37% of CU patients but was not detected in either the AD or the CO group. The preferential, if not selective, occurrence of IgG anti-FcεRIα autoantibodies in CU may not only constitute a discriminating disease marker but, further, may be of central pathophysiological relevance for the disease itself. To address this issue, we investigated the capacity of IgE-depleted anti-FcεRIα autoantibodies containing IgG fractions to release histamine from peripheral blood basophils. 50% of these CU-IgG preparations were able to elicit responses that were shown to be IgE-independent since a similar histamine release was observed when IgE-saturated and IgE-eluted basophils were used. The fact that not all of the anti-FcεRIα–positive CU-IgG samples were able to release histamine in vitro suggests that factors other than the mere presence of the Abs, such as the affinity, epitope specificity, and/or IgG subtype composition of autoantibodies, may determine the outcome of the individual biological response. In particular, the subtype composition of autoantibodies is important for the quality and/or quantity of

biological responses since IgG subtypes exhibit different capacities for complement fixation (26) and for binding to FcγRs (5). Putative complement fixing properties of these autoantibodies would explain why MC degranulation in CU is largely restricted to skin MC which—in contrast to lung MC—can be activated via the C5a receptor CD88 (27). It is tempting to speculate that autoantibodies incapable of inducing histamine release in vitro have MC-activating properties in the presence of complement in vivo. Finally, activation of MC in vivo may also be modulated by cross-linking of FcεRIα-bound IgG by anti-IgG autoantibodies, e.g., rheumatoid factors, by physical interaction with adjacent FcγR-bearing tissue-bound cells, or even by FcγRs expressed on the MC surface itself.

Our data demonstrate that anti-FcεRIα autoantibodies occur preferentially, or perhaps even selectively, in CU patients and that, at least in certain incidences, these autoantibodies can induce histamine releases. The observation that anti-FcεRIα antibodies apparently occur in a subset of CU patients only emphasizes the need for a reliable diagnostic screening system for this autoreactivity. The biochemical test system described in this study offers a routine diagnostic procedure for the detection of autoreactivity in CU sera and, therefore, allows the definition of an autoimmune-mediated subentity of this disease. Furthermore, these findings may form a basis for the development of new strategies for the treatment of CU. In this regard, the definition of non-IgE but autoantibody-binding peptides and their application in immunoaffinity-based selective elimination of FcεRIα-reactive autoantibodies may be a be a promising approach to the treatment of severe recalcitrant cases of CU.

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