

Anti-FcεRIα Autoantibodies in Autoimmune-mediated Disorders

Identification of a Structure–Function Relationship

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Abstract

Anti-FcεRIα autoantibodies (autoAbs) occur and may be of pathogenetic relevance in a subset of chronic urticaria (CU) patients. To analyze the prevalence and magnitude of the humoral anti-FcεRIα response in cohorts of CU patients compared with individuals suffering from classic skin-related (auto)immune diseases, we developed an ELISA system for the measurement of anti-FcεRIα autoAbs in non-fractionated serum samples. Results obtained using this assay correlated well with those generated by Western blotting. We found IgG anti-FcεRIα autoreactivity in 38% of CU patients but not in atopic dermatitis patients, psoriatics, or healthy individuals. We frequently detected anti-FcεRIα autoAbs in pemphigus vulgaris (PV, 39%), dermatomyositis (DM, 36%), systemic lupus erythematosus (SLE, 20%), and bullous pemphigoid (BP, 13%). While the autoAb titers in DM, SLE, BP, and PV were similar to those encountered in CU patients, only anti-FcεRIα⁺ CU serum specimens displayed pronounced histamine-releasing activity. The anti-FcεRIα autoAbs in CU patients belong predominantly to the complement-fixing subtypes IgG1 and IgG3, whereas in DM, PV, and BP, they were found to be mainly of the IgG2 or IgG4 subtype. Complement-activating properties of anti-FcεRIα autoAbs can indeed be of pathogenetic relevance, because C5a receptor blockade on basophils as well as de-complementation reduced drastically the histamine-releasing capacity of most anti-FcεRIα-reactive CU sera. As a consequence, therapeutic efforts in CU should aim at altering not only the quantity but also the complement-activating properties of IgG anti-FcεRIα autoAbs. (*J. Clin. Invest.* 1998. 101:243–251.) Key words: autoimmunity • IgE receptors • histamine release • complement activation • chronic urticaria

Introduction

Chronic urticaria (CU)¹ is a distressing affliction of the skin characterized by the frequent and continuous appearance of short-lived wheal reactions (1). IgG autoantibodies (autoAbs)

directed against the α chain of the high-affinity IgE receptor (FcεRIα) occur frequently in CU and may be of pathogenetic significance (2–5). The methodology used for the detection of these autoAbs includes histamine release (HR) assays from basophils (2), skin testing with autologous serum (6), and Western blotting/immunoprecipitation with purified serum IgG (3). While HR and skin tests detect biologically relevant mast cell/basophil-activating serum factors, they provide little, if any, information about the molecular configuration of these moieties, and the need for biologic material as assay substrate renders the standardization of these tests difficult. Western blotting and immunoprecipitation with purified serum IgG have been used successfully to identify FcεRIα-specific IgG autoAbs (3). These tedious procedures are hardly suited for screening purposes and of only limited value for determination of autoAb titers. To overcome these limitations, we attempted to establish a reliable ELISA-based assay for qualitative and quantitative analysis of IgG anti-FcεRIα autoAbs from non-fractionated serum samples. The availability of this test system allowed us to screen sera from large patient cohorts for the presence of IgG anti-FcεRIα autoAbs and, consequently, to determine the disease association of this autoreactivity and glean information concerning the molecular events operative in autoAb-induced activation of FcεRI-bearing effector cells.

Methods

Patients and controls. 281 patients with CU, defined as recurrent wheals lasting < 24 h and occurring at least twice a week for over 2 mo, were recruited (1, 7). Patients with signs of urticarial vasculitis or physical urticaria were excluded. In addition, sera from patients with the established diagnosis of atopic dermatitis (AD, *n* = 32), SLE (*n* = 15), psoriasis (PS, *n* = 30), bullous pemphigoid (BP, *n* = 22), dermatomyositis (DM, *n* = 45), and pemphigus vulgaris (PV, *n* = 28) were collected. Sera from 41 healthy individuals served as controls. All sera were stored at –20°C until investigation.

Human recombinant soluble FcεRIα (rsFcεRIα). The baculovirus-mediated expression and immunoaffinity purification of rsFcεRIα have been described previously (3). Biotin-labeled rsFcεRIα (rsFcεRIα^{biot}) was produced using NHS-LC-Biotin II (Pierce Chemical Co., Rockford, IL) according to the manufacturer's guidelines.

Establishment of an anti-FcεRIα autoAb ELISA.² Depending on the experimental conditions chosen, 1:15, 1:100, or 1:400 serum dilutions were reacted with mAbs directed against the CH3 domain of IgE (mAb BSW17 [8]) for 3 h at room temperature (RT). Aliquots of individual samples were incubated overnight at 4°C in the absence or

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1. Abbreviations used in this paper: Ab, antibody; AD, atopic dermatitis; BP, bullous pemphigoid; CR, complement receptor; CU, chronic urticaria; DM, dermatomyositis; FcεRI, high-affinity receptor for IgE; HR, histamine release; PS, psoriasis; PV, pemphigus vulgaris; rRhPV1, recombinant rhesus papilloma virus L1 protein; rsFcεRIα, recombinant soluble α chain of FcεRI; RT, room temperature; scOD, specific dilution-corrected optical density; Th, T helper.

2. Patent pending (case 900-9854).

presence of nonbiotinylated rsFcεRIα or baculovirus-expressed human papilloma virus L1 protein (rRhPVL1, kindly provided by Dr. R. Kirnbauer [9]), each at a final concentration of 0.05–40 ng/ml. Thereafter, all samples were reacted with rsFcεRIα_{biot} (2 nM) for 3 h at 37°C. 100 μl aliquots of these reaction mixtures were transferred to streptavidin (Bio-Rad Laboratories, Hercules, CA)-coupled ELISA plates (Costar Corp., Cambridge, MA) and incubated for 1 h at RT. After several rounds of washing, plates were reacted with peroxidase-coupled goat F(ab')₂ anti-human IgG (1:10,000), F(ab')₂ anti-human IgM (1:10,000), or F(ab')₂ anti-human IgE Abs (1:2,000; all from Sigma Chemical Co., St. Louis, MO) for 1 h at RT. Plate-bound enzyme was visualized using 2,2'-azino-bis(3-ethylbenzthiazoline-sulfonic acid) (ABTS; Sigma Chemical Co.) as a substrate. The reaction was stopped when the extinction of the ELISA wells exposed to an anti-FcεRIα autoAb-containing control serum and, subsequently, to enzyme-labeled anti-human IgG reached an OD of 1.0 at a wavelength of 405 nm. The ODs of individual samples were multiplied by the serum dilution factor (corrected OD, cOD). The specific cOD (scOD) of individual samples was calculated by subtracting the cOD in the presence of competing nonbiotinylated rsFcεRIα from the cOD in its absence. The mean scOD of healthy individual's sera plus 2.5× SEM was calculated as the threshold level for anti-FcεRIα autoAb reactivity.

The complement-binding capacity of rsFcεRIα_{biot}-bound autoAbs was determined by using the same ELISA procedure with the modification that rabbit anti-human C1q, C3b, C3d, or C5a (all diluted 1:2,500; all from Serotec Ltd., Blackhorn, UK) followed by peroxidase-coupled goat F(ab')₂ anti-rabbit IgG (1:10,000; Amersham International, Buckinghamshire, UK) was used instead of the peroxidase-labeled F(ab')₂ anti-human IgG as the detection reagent.

SDS-PAGE and immunoblotting. The immunoblotting technique used for the detection of anti-FcεRIα autoAbs in serum IgG isolates has been described previously (3). Briefly, rsFcεRIα was electrophoresed on 10–13% SDS gels and blotted onto nitrocellulose membranes (Bio-Rad Laboratories) which were then exposed to 5% dry milk/0.05% Tween 20 (Sigma Chemical Co.)/PBS for at least 6 h. For the detection of FcεRIα-reactive serum Abs, membranes were reacted with 1:10 diluted, mAb BSW17–prereacted serum specimens, and FcεRIα-bound IgG was detected with rabbit anti-human IgG (1:50,000; Jackson ImmunoResearch Laboratories, Inc., Richmond, CA). For analysis of IgG subtype composition of anti-FcεRIα-reactive autoAbs, serum-exposed membranes were reacted with mAbs against human IgG1, IgG2, IgG3, or IgG4 (20 μg/ml, clones G17-1, G18-21, G18-3, and JDC-14; all from PharMingen, San Diego, CA) followed by a goat anti-mouse horseradish peroxidase conjugate (1:40,000; Bio-Rad Laboratories) and ECL developing solution (Amersham International).

HR. HR assays were performed with basophil-enriched peripheral blood cells from three unrelated healthy donors as described previously (3). After dextran sedimentation, basophil-enriched cells were incubated for 40 min at 37°C in IL-3 (50 pg/ml; Endogen, Inc., Cambridge, MA)-supplemented buffer containing 1:10 dilutions of either native or heat-decomplemented (3 h, 56°C) patients' sera or 0.5–1.0 mg/ml protein G (Pierce Chemical Co.)-eluted serum IgG. Before use in the HR assay, all sera/IgG fractions were exposed to mAb BSW17 (2 nM) for at least 3 h at RT. In an additional series of experiments, basophil-enriched cells were either reacted with C5a or C3a receptor-blocking octapeptides (both 10⁻⁷ M; Bachem Feinchemikalien AG, Bubendorf, Switzerland) before use (1 h, 4°C) and during HR assays, and/or exposed to rsFcεRIα (0.05–5 μg/ml)-supplemented sera/IgG fractions. Cell free and total cellular histamine were measured using an RIA (Immunotech International, Marseille, France). The percentage of specific HR was calculated as described previously (3). The spontaneous release always accounted for < 5% of the total cellular histamine. The positive control HR induced by the anti-FcεRIα mAb 15-1 (10) varied from 22 to 56% (mean±SEM: 38±8.7%, n = 12). In all experiments, the serum/serum IgG-induced HR was expressed as percentage of the HR elicited by the anti-FcεRIα mAb.

The modulation of serum-induced HR by either basophil pretreatment with complement receptor–inhibitory peptides or by heat de-complementation of sera was expressed as percent inhibition of the HR induced by native serum on nonmodified basophils.

Statistical analysis. Statistical significance was determined using the Wilcoxon signed rank test, and a *P* value of < 0.05 was considered significant. Correlation coefficients were calculated using Cricket-Graph software.

Results

ELISA-based detection of IgG anti-FcεRIα autoAbs. We aimed at designing an assay system that allows the detection of serum molecules with affinity for the extracellular portion of FcεRIα. The system developed consists of two subsequent reaction steps: (a) a ligand (i.e., serum antibody)–target (i.e., biotinylated rsFcεRIα) interaction in liquid phase and (b) the immobilization of ligand–target complexes onto streptavidin-coated matrices. The latter step allows the solid phase enzyme-based immunodetection of FcεRIα-bound serum components. Substantial IgG binding to rsFcεRIα was seen in the case of certain CU sera but in none of the AD specimens tested (Fig. 1 A, a representative experiment). As expected, we observed IgE binding to rsFcεRIα in IgE-containing CU and AD sera (Fig. 1 B, a representative experiment). Since IgE can inhibit autoAb binding to FcεRIα presumably by masking relevant epitopes on this molecule (3), and serum IgE–IgG complexes performed in vivo (11, 12) may produce false positive results in the rsFcεRIα binding and anti-IgG detection system, we tried to eliminate serum IgE interference by exposing sera to anti-IgE mAbs recognizing the FcεRIα-binding CH3 domain of IgE. Indeed, anti-IgE treatment abolished the serum IgE-derived signals (Fig. 1 B) but, importantly, did not diminish CU-IgG binding to rsFcεRIα (Fig. 1 A). To test whether serum biotin and/or FcεRIα-nonreactive serum IgG can influence ELISA results, we exposed streptavidin-coated plates overnight to various concentrations of an anti-FcεRIα autoAb⁻ serum pool before the assay procedure, or titrated FcεRIα-nonreactive sera to a fixed concentration of autoAb⁺ or autoAb⁻ sera. As demonstrated in Fig. 2, neither the presence of serum biotin (Fig. 2 A) nor the serum IgG concentration (Fig. 2 B) affects the detectability of anti-FcεRIα-reactive IgG autoAbs. To better analyze the specificity of the assay, anti-FcεRIα-reactive sera were exposed to various concentrations of nonbiotinylated rsFcεRIα and, for control purposes, rRhPVL1 before subjecting them to the ELISA procedure. Whereas the irrelevant protein rRhPVL1 did not affect the binding of anti-FcεRIα-reactive IgG (Fig. 2 C), preincubation with rsFcεRIα resulted in a dose-dependent decrease in the amount of plate-bound anti-FcεRIα autoAbs, with a half-maximal inhibition at a molar ratio of nonbiotinylated competitor to biotinylated ligand of ~ 0.5 (Fig. 2, C and D). No change in the anti-IgG signal occurred when Western blot autoAb⁻ control sera were subjected to the same binding-inhibition protocol (Fig. 2, C and D). As a consequence, IgG anti-FcεRIα serum reactivity can be expressed as the absolute OD measured in ELISA or, more specifically, by the reduction in OD achieved by pre-reaction of diluted test sera with defined concentrations of nonbiotinylated rsFcεRIα competitor protein (scOD). In the following experiments, we used a competitor:ligand ratio of 1.0 and calculated the scOD as the quantitative measure for IgG anti-FcεRIα immunoreactivity. The threshold scOD level for

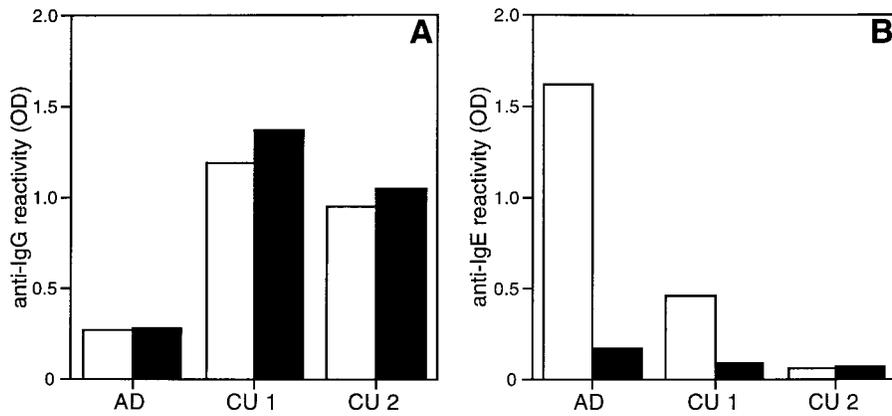


Figure 1. mAbs directed against CHε3 (IgE heavy chain) inhibit serum IgE but not IgG binding to rsFcεRIα. mAb BSW17-pretreated (black bars) or non-pretreated (white bars) sera (diluted 1:15) from an AD and two chronic urticaria patients (CU 1 and CU 2) were exposed to biotinylated rsFcεRIα, and the ELISA procedure was carried out as described in Methods. OD of rsFcεRIα-bound anti-IgG (A) and anti-IgE (B) reactivity is given on the y axis.

IgG anti-FcεRIα reactivity was estimated as mean scOD plus $2.5 \times$ SEM produced by sera from 41 healthy individuals (Fig. 3). Based on this calculation, a given serum specimen contains anti-FcεRIα autoAbs if scOD > 39. To control the validity of this approach, we performed comparative Western blot and ELISA analyses. We found that immunoblot-reactive sera exhibited pronounced ELISA reactivity in all five cases tested (mean scOD ± SEM: 120 ± 76), and all four immunoblot non-reactive samples yielded an ELISA reactivity below the thresh-

old level (mean scOD ± SEM: 25 ± 6). Due to high background staining, certain sera ($n = 3$) could not be judged either autoAb⁺ or autoAb⁻ by Western blot. By ELISA, the same sera were found to contain either low anti-FcεRIα autoAb levels or were autoAb⁻ (mean scOD ± SEM: 33 ± 52). These findings indicate that the results achieved by the ELISA-based detection system not only correlate with Western blot analysis but may be even more reliable when a given serum contains low autoAb titers.

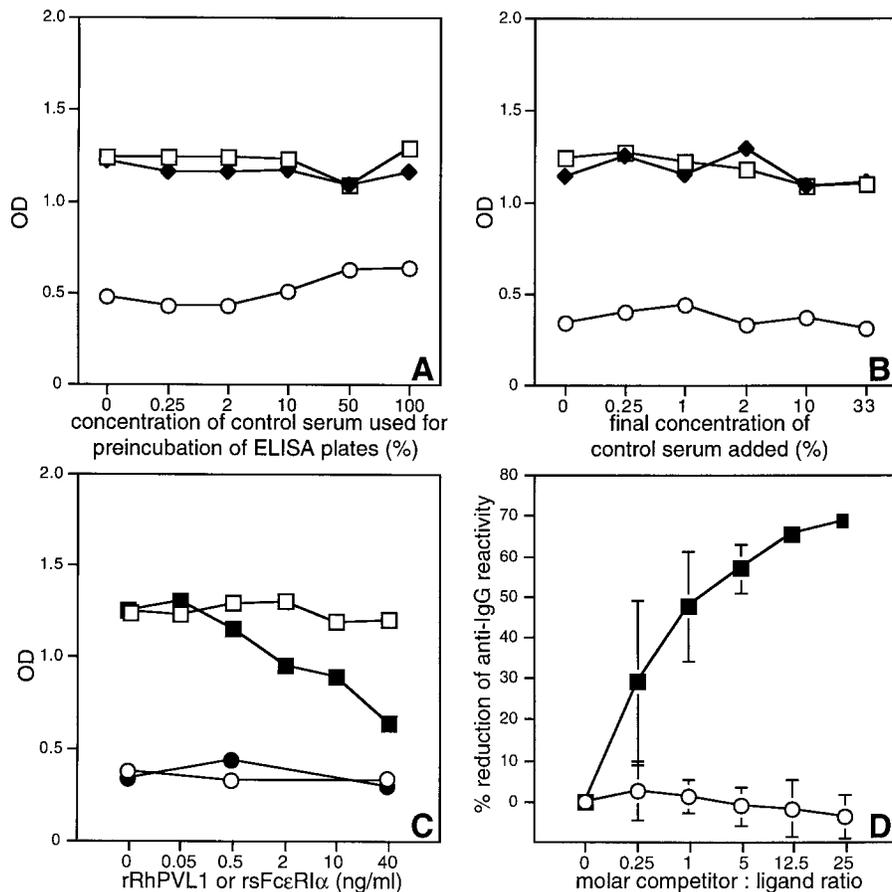


Figure 2. Specificity and reliability of rsFcεRIα-based ELISA. (A) Serum biotin does not interfere with detection of IgG anti-FcεRIα autoAbs. Streptavidin-coated ELISA plates were incubated overnight at the indicated concentrations with a pool of anti-FcεRIα-nonreactive sera. After three washes, ELISA was performed with one anti-FcεRIα-nonreactive (○) and two anti-FcεRIα-reactive (□ and ◆) sera (all diluted 1:400). The OD produced by these samples is depicted as a function of the serum concentration added to the plates before the assay. (B) Serum components of FcεRIα-nonreactive sera do not interfere with detectability of IgG anti-FcεRIα autoAbs. Anti-FcεRIα-reactive (□ and ◆) and -nonreactive (○) specimens (all sera diluted 1:400) were mixed with an FcεRIα-nonreactive serum pool at the final concentrations indicated and then subjected to ELISA. The same sera as in A were used. (C) Competitive cold inhibition of IgG anti-FcεRIα autoAb binding to biotinylated rsFcεRIα. Anti-FcεRIα-reactive (squares) and -nonreactive (circles) sera were reacted with the indicated concentration of nonbiotinylated rsFcεRIα (filled symbols) or rRhPVL1 (open symbols) before the assay procedure. (D) Effect of the molar competitor (nonlabeled rsFcεRIα) to ligand (biotinylated rsFcεRIα) ratio on the detectability of anti-FcεRIα autoAbs. The mean competitor-induced inhibition (±SEM) of anti-IgG immunoreactivity

(x axis) is depicted as the function of the competitor to ligand ratio used (y axis). Filled squares and open circles, Mean inhibition values obtained with anti-FcεRIα-reactive ($n = 2$) and -nonreactive ($n = 3$) serum specimens, respectively.

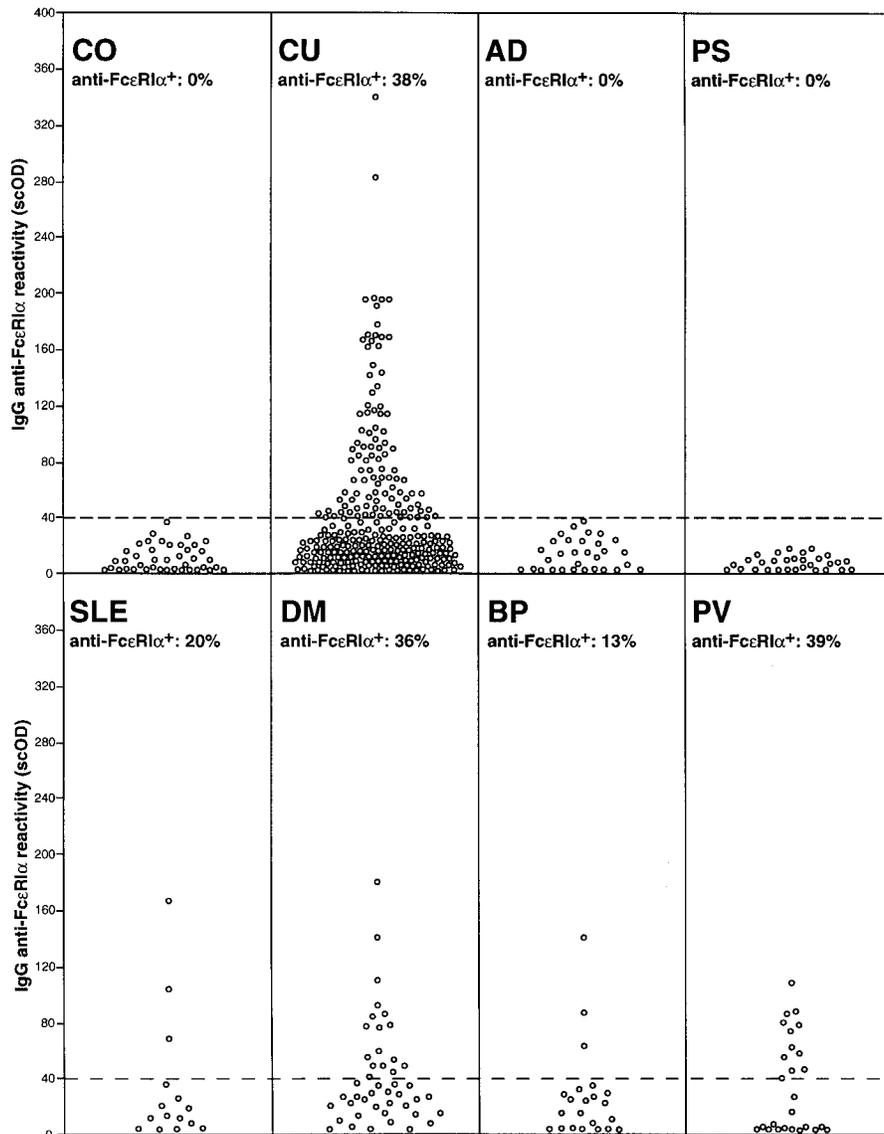


Figure 3. Prevalence and titer of anti-FcεRIα autoAbs in various inflammatory and autoimmune-mediated disorders. Sera (diluted 1:400) from 41 healthy controls (CO) as well as from patients suffering from CU (n = 281), AD (n = 32), PS (n = 30), SLE (n = 15), DM (n = 45), BP (n = 22), or PV (n = 28) were analyzed for IgG anti-FcεRIα reactivity. The IgG anti-FcεRIα reactivities are expressed as scOD. Broken lines, Cut-off level for IgG anti-FcεRIα autoAb positivity.

Prevalence of anti-FcεRIα autoAbs in various (auto)immune-mediated diseases. We next evaluated the prevalence of IgG anti-FcεRIα autoAbs in sera from patients suffering from CU or AD. Results obtained confirmed and extended those of our previous Western blot studies (3): 38% of the CU patients (106/281) exhibited anti-FcεRIα reactivity, whereas none of the 32 AD serum specimens contained anti-FcεRIα autoAbs (Fig. 3). Furthermore, 30 sera from patients with PS were uniformly devoid of anti-FcεRIα autoAbs (Fig. 3). These data suggest that cutaneous inflammation per se does not suffice for autoimmune sensitization to FcεRIα. However, the possibility existed that tissue-specific or systemic autoimmunity might lower the threshold of immunocompetent cells to recognize FcεRIα. To test this assumption, we screened sera from patients with cutaneous autoimmune manifestations only (BP or PV) or with skin involvement in the context of a systemic autoimmune disease (SLE or DM). Interestingly, we identified FcεRIα autoAb-containing sera in all of these patient groups regardless of whether the disease entity was of systemic or skin-restricted nature (Fig. 3). The specificity of the ELISA results in these patient groups was confirmed by our finding that

sera from DM (n = 9), BP (n = 2), and PV (n = 2) patients, which were unequivocally autoAb⁺ by ELISA, always displayed pronounced IgG reactivity against Western-blotted rsFcεRIα. It is noteworthy that the anti-FcεRIα-sensitization rates and autoAb titers found in patients with these autoimmune diseases are roughly equal to those of CU patients.

IgG anti-FcεRIα-reactive CU sera can release histamine from basophils. We next tested whether diluted sera from the various patient groups can activate basophils from healthy donors. As demonstrated in Fig. 4 A, IgG autoAb-containing CU sera exhibited pronounced histamine-releasing activity. CU sera that lacked IgG anti-FcεRIα autoAbs had significantly lower histamine-releasing activity than those containing autoAbs (P < 0.001) and, in this respect, did not differ from sera of healthy individuals. The percentage of sera with basophil-activating properties (i.e., samples that induced an HR > 15% of the positive control) was 66% in the autoAb⁺ and 17% in the autoAb⁻ CU subgroups. Interestingly, the autoAb titers in the histamine-releasing and the nonreleasing autoAb⁺ CU sera were not significantly different (P > 0.05). Irrespective of autoAb status, sera from non-CU autoimmune patient groups

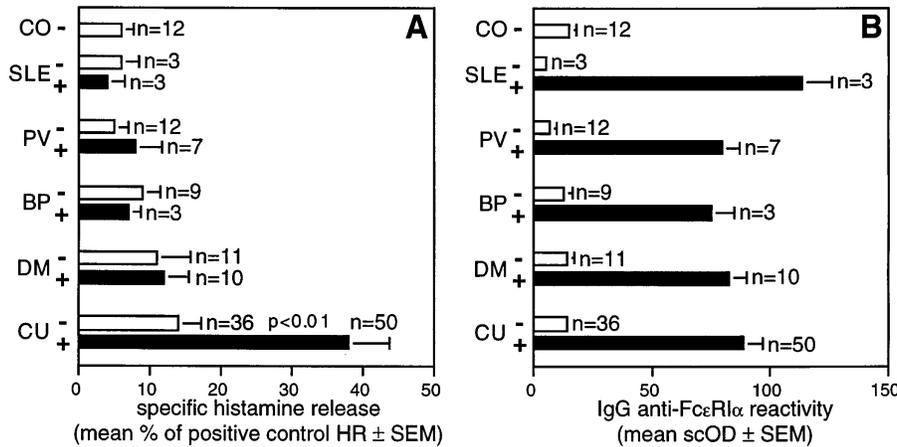


Figure 4. Anti-FcεRIα⁺ sera from patients with CU but not from patients with DM, BP, PV, or SLE exhibit pronounced histamine-releasing activity. (A) IgG anti-FcεRIα-reactive (black bars) and -nonreactive (white bars) patient sera as well as serum specimens from healthy individuals (CO) were analyzed for their ability to degranulate peripheral blood basophils. Numbers of serum specimens investigated are given next to the bars. Magnitude of serum-induced HR is given as mean percentage of positive control release ± SEM for the various groups investigated (x axis). (B) IgG anti-FcεRIα autoAbs in the various patient groups have comparable titers. Mean scOD ± SEM produced by the same serum specimens as in A is given on the x axis.

did not contain more histamine-releasing activity than sera from healthy controls (Fig. 4 A). It is unlikely that differences in autoAb concentrations are responsible for the observed phenomenon, since CU sera and sera from the other disease entities contained similar amounts of IgG autoAbs (Fig. 4 B). To test the possibility that IgM anti-FcεRIα autoAbs occur in the IgG autoAb⁺ CU sera and may contribute critically to the basophil-activating properties of these specimens, we modified our ELISA protocol by substituting the anti-IgG for an anti-IgM immunodetection step. Comparatively weak IgM anti-FcεRIα reactivity was detected in CU sera as well as sera from the other groups investigated, and was not linked to IgG autoAb status (data not shown). Thus, it appears that IgG rather than IgM anti-FcεRIα autoAbs are responsible for basophil activation. This finding suggests further that these IgG autoAbs in CU exhibit structural and/or functional properties that are not shared by the autoAbs occurring in the other diseases.

Disease-restricted IgG subtypes of anti-FcεRIα autoAbs. Analysis of the IgG subtypes of anti-FcεRIα autoAbs in various disease groups revealed striking differences. In CU sera, we found a predominance of IgG1 (55%, $n = 22$) and IgG3 autoAbs (77%; Table I). AutoAbs of either IgG1 or IgG3 subtype were observed in 91% of these serum specimens. IgG2 occurred in only 3 of 22 samples (14%) and FcεRIα-specific IgG4 was evident in 3 of 22 specimens (14%). In a single CU patient, we detected IgG4 but no IgG1 or IgG3 autoAbs. In contrast, IgG2 or IgG4 autoAbs accounted for the anti-FcεRIα reactivity in the majority of patients suffering from DM (89%, $n = 9$; Table I), PV (100%, $n = 2$; data not shown), or BP (100%, $n = 2$; data not shown). Seven DM serum specimens contained IgG2 (78%), and five (56%) displayed IgG4 anti-FcεRIα reactivity, whereas FcεRIα-specific IgG1 and IgG3 were found in only two (22%) and three (33%) samples, respectively. A single DM patient displayed IgG3 but no IgG2 or IgG4 autoAbs. Taken together, the predominance of IgG1 and IgG3 autoAbs in histamine-releasing CU sera and the low prevalence of this immunoreactivity in sera from the other disease groups suggests that IgG subtype-dependent functions may be of pathogenetic relevance for the occurrence of autoimmune mast cell/basophil activation.

Anti-FcεRIα autoAbs from CU sera exhibit complement-activating properties in vitro. To analyze the complement-acti-

vation properties of anti-FcεRIα autoAbs after ligand binding, we modified the ELISA protocol by exchanging the anti-IgG detection step for an incubation with enzyme-labeled Abs specific for various complement components. In this assay, we detected substantial quantities of complement factor C3d-binding autoAbs in the anti-FcεRIα-reactive CU but not DM, SLE, BP, or PV sera (Fig. 5 A). A short period of heat treatment, which leads to serum decaplementation but, reportedly, does not affect the autoAb-target interaction (3), abolished the occurrence of autoAb-associated anti-C3d immunoreactivity (Fig. 5 A). In none of the disease groups could autoAb-bound C1q, C3b, or C5a be detected (data not shown). This result is not surprising, as these moieties, unlike C3d, undergo rapid degradation unless stabilized artificially (13). To test the possibility that the complement-binding activity of anti-FcεRIα autoAbs may be of importance for their basophil-activation properties, 11 autoAb⁺ CU serum samples were selected on the basis of their histamine-releasing activity (HR > 15%) and subjected to HR assays in both native and heat-depleted form. Decaplementation resulted in a substantial reduction of the HR induced by 9 of 11 samples, which correlated well in magnitude ($r^2 = 0.49$, $P < 0.05$) with the scOD values obtained in the anti-C3d ELISA (Fig. 5 B). In three further experiments, a total of 21 anti-FcεRIα autoAb-containing CU serum samples were analyzed for the impact of basophil complement receptor (CR) triggering on the autoAb-elicited HR. Similar to the results shown in Fig. 5 B, a substan-

Table I. IgG Subtype Composition of Anti-FcεRIα AutoAbs from CU and DM Patients

Subtype	CU	DM
	$n = 22$	$n = 9$
IgG1	55% (12/22)	22% (2/9)
IgG2	14% (3/22)	78% (7/9)
IgG3	77% (17/22)	33% (3/9)
IgG4	14% (3/22)	56% (5/9)
IgG1 or IgG3	91% (20/22)	44% (4/9)
IgG2 or IgG4	27% (6/22)	89% (8/9)
IgG1/3 but not IgG2/4	73% (16/22)	11% (1/9)
IgG2/4 but not IgG1/3	5% (1/22)	56% (5/9)

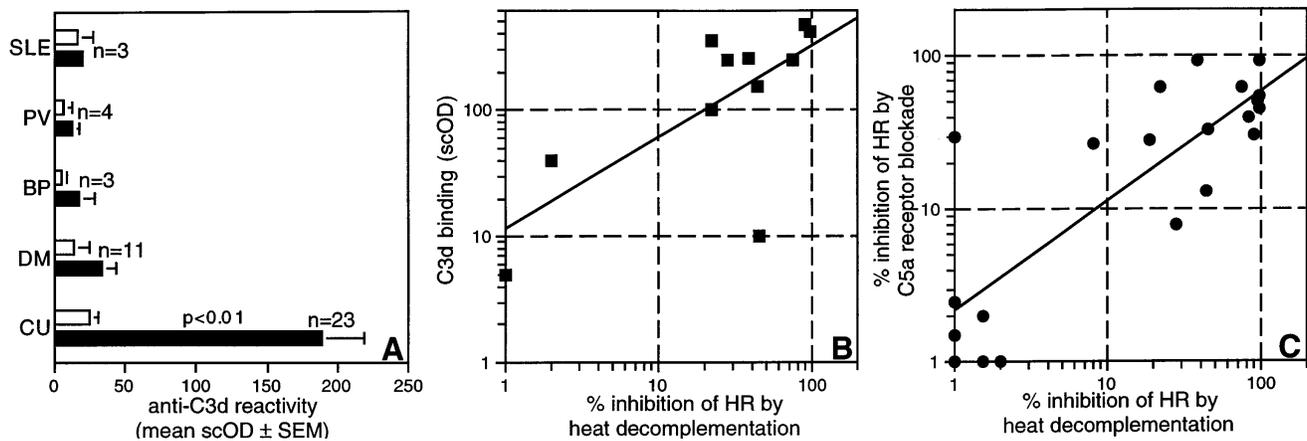


Figure 5. The histamine-releasing activity of IgG anti-FcεRIα autoAbs is linked to their complement-fixing properties. (A) Selectively, anti-FcεRIα autoAbs from CU patients fix substantial amounts of the complement factor C3d upon target binding. Native (black bars) or heat-decomplemented (3 h, 56°C; white bars) serum specimens were analyzed by ELISA for autoAb-mediated C3d binding, and data were expressed as mean scOD ± SEM for each group investigated. (B) Correlation of C3d binding and decompensation-sensitive HR by IgG anti-FcεRIα autoAbs. 11 histamine-releasing (HR > 15%) autoAb⁺ CU sera (■) were incubated in heat-decomplemented and native form with basophils. Results obtained with individual samples are given as decompensation-induced reduction in HR relative to release induced by native serum versus C3d-binding capacity of serum-derived autoAbs. (C) Decomplementation of CU sera as well as C5aR blockade on basophils result in substantial inhibition of serum-induced HR. 21 decomplemented and native sera (HR > 10%) were incubated with basophils either nonmodified or (pre)exposed to C5aR-blocking peptides. Results obtained with individual sera (●) are given as decompensation- and C5aR blockade-sensitive HR. Inhibition values < 1 were set to the 1% level on the logarithmic scale.

tial proportion (67%) of the test sera showed reduced basophil-activation capacity after heat decompensation, with a mean HR inhibition of $60 \pm 12\%$ (mean ± SEM; Fig. 5 C). Pretreatment of the basophils with a synthetic C5a peptide which prevents C5a from binding to its receptor resulted in an inhibition that was of similar magnitude ($46 \pm 9\%$) and correlated well with that achieved by serum decompensation ($r^2 = 0.68$, $P < 0.01$; Fig. 5 C). The failure of C3aR-inhibitory peptides to block autoAb-induced HR (data not shown) may be explained by the rather low histamine-releasing capacity of C3a and the fact that the receptor-triggering competence of C3a but not of C5a is lost upon proteolytic cleavage of a carboxy-terminal arginin residue (13, 14). The HR elicited by the remaining 33% of the sera could not be inhibited by either serum decompensation or—with the exception of a single serum specimen—by C5aR blockade (Fig. 5 C). The mean histamine-releasing activity in the decompensation-sensitive and -insensitive groups was of comparable magnitude (50 ± 3 vs. $51 \pm 6\%$ of the positive control HR). In a preliminary series of experiments, we analyzed the modulation of basophil C5aR expression upon exposure to autoAb⁺ CU sera as a direct measure of C5a-induced receptor occupation/internalization (15, 16). Three of seven autoAb⁺ CU serum specimens as well as synthetic C5a were found to decrease the anti-C5aR immunoreactivity of basophils (data not shown). Whereas IgG fractions of the same three sera diluted in C5-deficient control serum could not reduce basophil C5aR expression, reconstitution of these samples with purified C5 led to a down-regulation of basophil C5aR that was comparable in magnitude to that observed with the nonfractionated sera (data not shown).

Complexation with soluble recombinant FcεRIα renders autoAbs from certain CU patients capable of eliciting basophil HR in a complement/C5aR-dependent manner. The administration of rsFcεRIα has been implicated as an effective means of

inhibiting IgE-mediated allergic tissue inflammation *in vitro* and *in vivo* (17–19). To test whether rsFcεRIα could be of similar benefit for the inhibition of autoAb-dependent effector cell activation, anti-FcεRIα-reactive and -nonreactive CU as well as sera from healthy controls were exposed to rsFcεRIα before basophil challenge. AutoAb⁺ CU sera were selected on the basis of their high (HR > 30%, HR⁺) and low or absent (HR < 15%, HR⁻) HR capacity. Preadsorption of diluted HR⁺ CU sera with $0.5 \mu\text{g/ml}$ rsFcεRIα led to a substantial reduction in their basophil-activating capacity ($P < 0.05$; Fig. 6 A). In striking contrast, the anti-autoAb⁺ CU sera, which had displayed only limited HR activity in the absence of rsFcεRIα, induced robust basophil activation in its presence (Fig. 6 A). This phenomenon appears to depend critically on IgG anti-FcεRIα autoAbs, since the autoAb⁻ CU as well as the autoAb⁻ sera from healthy controls were not able to mount a similar response. To address experimentally the mechanism(s) leading to the rsFcεRIα/anti-FcεRIα autoAb-induced response, we analyzed the rsFcεRIα-dependent HR capacity of sera and IgG fractions from CU patients and controls. As exemplified in Fig. 6 B, the occurrence of rsFcεRIα-induced HR critically requires not only anti-FcεRIα autoAbs but also non-IgG serum components, since IgG anti-FcεRIα-reactive HR⁻ CU sera but not IgG fractions therefrom activated basophils in the presence of soluble FcεRIα. As expected, the HR induced by an HR⁺ CU serum and its IgG fraction were reduced in the presence of rsFcεRIα (Fig. 6 B). To investigate whether the *in vitro*-formed rsFcεRIα/anti-FcεRIα autoAb complexes in HR⁻ sera may activate FcεRI-bearing effector cells via CR triggering, basophils were exposed to HR⁻ autoAb⁺ ($n = 4$) and autoAb⁻ ($n = 1$) CU sera in the presence or absence of inhibitory C5a peptides and/or rsFcεRIα. As demonstrated in Fig. 6 C, C5aR blockade abolishes rsFcεRIα-induced HR, indicating that anti-FcεRIα autoAbs upon binding to their recombinant target can initiate complement breakdown and subsequent

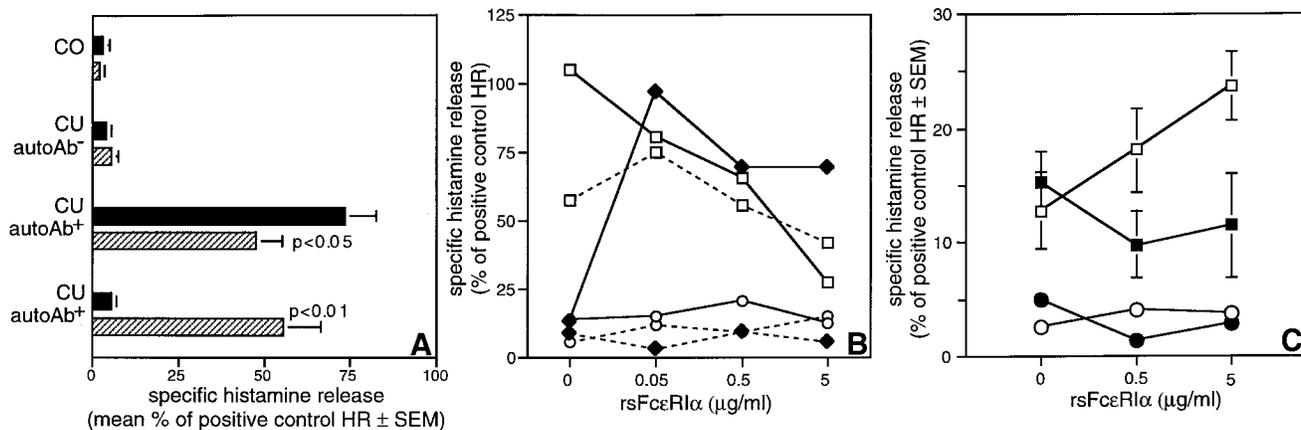


Figure 6. Binding to soluble FcεRIα renders poorly stimulatory autoAbs capable of eliciting basophil HR in a complement/C5aR-dependent manner. (A) rsFceRIα-induced basophil HR by autoAb⁺ CU sera with low to absent basal basophil stimulatory activity. AutoAb⁺ CU sera with either low to absent (HR < 15%, HR⁻; n = 9) or pronounced HR activity (HR > 30%, HR⁺; n = 9), autoAb⁻ CU sera (n = 3), and autoAb⁻ sera from healthy controls (CO, n = 2) were exposed to rsFceRIα (hatched bars) or medium (black bars) before the HR assay. Data are given as mean ± SEM of the specific HR induced by individual samples in the presence or absence of 0.5 μg/ml rsFceRIα. (B) Elicitation of rsFceRIα-induced basophil HR depends on the presence of non-IgG serum components. HR⁻ (◆, solid line) and HR⁺ (□, solid line) autoAb⁺ CU sera and an autoAb⁻ serum from a healthy control individual (○, solid line), as well as IgG fractions from all of these serum samples (broken lines) were exposed to the indicated concentrations of rsFceRIα and assayed for their HR activity. (C) C5aR-mediated HR by CU autoAbs complexed to rsFceRIα. Specific HR elicited by four autoAb⁺ HR⁻ CU sera (mean HR ± SEM, squares) and one autoAb⁻ CU serum (circles) in the absence or presence of rsFceRIα on nontreated (open symbols) or C5aR inhibitory peptide-(pre)treated basophils (filled symbols) is depicted.

C5a-dependent basophil activation. In contrast, C5a-inhibitory peptides neither affected the HR capacity of autoAb⁻ CU sera (Fig. 6 C) nor reduced the magnitude of the HR induced by mouse mAbs directed against FcεRIα (data not shown). In contrast to the substantial contribution of CR triggering for autoAb-mediated HR, a putative role of autoAb Fc part–FcγR interactions on basophils appears to be of minor relevance, as the functional blockade of the low affinity FcγR, FcγRII, by Fab fragments of the mAb IV-3 did not affect significantly the autoAb⁻ or autoAb–rsFceRIα complex–induced HR (data not shown).

Discussion

In this study, we demonstrate that IgG anti-FcεRIα autoAbs occur not only in CU patients but can also be detected in sera of patients suffering from other systemic or skin-restricted autoimmune diseases. Anti-FcεRIα autoAbs in CU but not in the other autoimmune-mediated disorders are of apparent functional relevance, as they can activate FcεRI-bearing effector cells. An explanation for this discrepancy can be derived from the observation that the autoAb-induced pathogenetic events in CU patients can include complement activation and, thus, involve mechanisms other than mere ligation of FcεRI.

FcεRIα-specific IgG autoAbs with basophil-activating properties occur in a considerable proportion of CU patients (2, 3), and the reduction of serum IgG and, probably, autoAb levels can ameliorate disease activity temporarily in these individuals (20). These findings emphasize the need for a reliable and fast screening system that allows identification of autoAb-bearing hosts and monitoring of autoAb serum levels during the course of the disease. Here we present an ELISA system that uses the recombinant extracellular portion of FcεRIα as the reaction target for serum autoAbs. Unlike a previously described biochemical test (3), the new protocol circumvents the

necessity of serum IgE depletion by the introduction of an incubation step with anti-human CHE3 (IgE heavy chain) mAbs that prevent interference of IgE with the test substrate rsFceRIα. Furthermore, detection of autoAbs recognizing conformational epitopes on FcεRIα is favored by the use of native target protein rather than SDS-denatured material in the biochemical analysis. As opposed to the commonly used functional assays, i.e., basophil HR and skin testing with autologous serum (2, 6), the ELISA-based assay allows identification and quantification of anti-FcεRIα autoAbs irrespective of their in vitro basophil/mast cell-activating properties. This seems of particular importance, since the occurrence of autoAb-induced HR in vitro does not always correlate with that of serum IgG anti-FcεRIα autoreactivity (reference 3, and this study). As a further advantage, the liquid phase autoAb binding and detection system offers the possibility not only of searching for the presence of anti-FcεRIα autoAbs but also of analyzing their isotype(s) and some of their functional (e.g., complement-fixing) properties.

Large scale screening of sera revealed the presence of IgG anti-FcεRIα autoAbs not only in 38% of CU patients but also in a proportion of sera from patients with organ-specific (BP [13%] and PV [39%]) or systemic autoimmune diseases (SLE [20%] and DM [36%]). Sera from healthy individuals as well as from patients with atopic or psoriatic skin inflammation were devoid of IgG anti-FcεRIα immunoreactivity. The occurrence of anti-FcεRIα autoAbs seems to parallel the distribution pattern of FcγR-specific autoAbs detected in sera from autoimmune (e.g., SLE or RA) patients but not in sera from healthy individuals (21). Importantly, most of these FcγR-reactive autoAbs are of the IgM isotype, indicating low substrate specificity and affinity as well as absence of FcγR-specific T helper (Th) cells. Concerning anti-FcεRIα autoAbs, antigen-specific IgM appears to be of minor importance, since this immunoreactivity (a) was found only in low titers in the

various patient groups, (b) did not correlate in magnitude with the titer of IgG autoAbs of the same specificity, and (c) could be detected even in some of the sera from healthy controls (data not shown). Thus, it is conceivable that in CU as well as in other autoimmune diseases, affinity-matured IgG autoAbs account for most of the observed humoral anti-FcεRIα reactivity. Although FcεRIα-specific T cells capable of providing IgG isotype switch and maturation signals for B cells have not yet been isolated, the occurrence of IgG1 and IgG3 autoAbs in CU as opposed to IgG2 and IgG4 autoAbs in the other autoimmune diseases suggests that FcεRI-specific Th cells exist in vivo, and that these cells display a disease-specific polarized secretory potential. In particular, isotype switching to IgG4 in humans (and to IgG1 in mice) depends on the presence of IL-4-producing Th2 cells, whereas the production of the murine homologues of the human complement-binding IgG1 and IgG3 isoforms (22) is IFN-γ- and, thus, Th1 cell-dependent (23).

In this study, we found a high prevalence of complement-fixing and basophil-activating anti-FcεRIα autoAbs in CU, whereas apparently nonfunctional autoAbs without complement-activating properties occur preferentially in non-CU autoimmune diseases. Our contention that complement activation by autoAbs in CU is of functional significance is supported by the finding that decompensation of most autoAb-containing sera reduces significantly their HR capacity (Fig. 5, B and C), and that the C3d-binding activity of anti-FcεRIα autoAbs correlates with the magnitude of the decompensation-sensitive portion of the serum-induced HR (Fig. 5 B). The complement receptor most likely involved is C5aR, since CU-IgG can reduce basophil anti-C5aR immunoreactivity in a C5-dependent manner (data not shown), and the blockade of C5aR (Fig. 5 C) but not of C3aR (data not shown) results in a substantial inhibition of the HR induced by autoAb⁺ CU sera. An augmentative functional interplay of signal transduction via the tyrosine kinase-activating FcεRI and the G protein-coupling C5aR (16, 24–26) is suggested by our preliminary observations that the exposure of basophils to anti-FcεRIα-reactive CU-IgG fractions can lower the cells' threshold to respond to recombinant C5a (data not shown). The further observations that a subset of anti-FcεRIα autoAb⁺ sera activated basophils in an entirely complement/C5aR-independent manner, and that many sera displayed a complement/C5aR-independent component of HR activity (Fig. 5, B and C), suggest that the relative contribution of autoAb-induced FcεRI cross-linking and CR triggering varies among individual specimens.

A surprising observation in this study is that certain anti-FcεRIα autoAb-containing CU sera failed to trigger basophil degranulation. Low autoAb levels in these sera are apparently not responsible for the lack of effector cell stimulation, since, by ELISA, IgG anti-FcεRIα reactivity was of comparable magnitude in histamine-releasing and nonreleasing autoAb⁺ CU serum samples. A likely explanation is that autoAbs in the latter sera recognize preferentially epitopes on soluble FcεRIα that are hidden on the membrane-bound receptor form expressed on basophils from healthy donors. This argument gains support from our finding that the HR⁻ anti-FcεRIα autoAbs can upon complexation with their soluble ligand convert into stimulatory agents which release histamine from basophils in a complement/C5aR-dependent manner (Fig. 6). But how can it be that such autoAbs are pathogenetic in vivo? A particularly tempting speculation is that certain cell types (e.g., mast cells, antigen-presenting cells of the skin) not only express cell

surface-bound FcεRIα, but can also release the α chain as a soluble molecule. This appears not too unlikely, since many Fc receptor types, i.e., FcγRII/CD32, FcγRIII/CD16, and FcεRII/CD23, have been shown to exist in soluble forms (27–30). Thus, in certain cases of CU, tissue-bound soluble FcεRIα may allow the localized formation of anti-FcεRIα autoAb-containing immune complexes capable of complement activation and C5a-dependent mast cell stimulation.

In summary, our observations ascribe to the complement system an augmentative and perhaps, in certain cases, critical pathogenetic role in autoimmune-mediated CU. This concept gains support from the in vivo observations that the treatment of CU patients with pharmacological inhibitors of complement activation can reduce disease activity (31), and that, unlike in normal skin, mast cells within lesional skin of CU patients lack anti-C5aR immunoreactivity, possibly due to C5a-mediated occupation, downregulation, and/or shedding of this receptor (32). If the proposed pathophysiological scenario is of relevance in vivo, the skin-restricted distribution of symptoms in CU could be explained by the fact that mast cells of the skin but not of either the lung or intestinal mucosa express C5aR (33, 34).

The treatment modalities currently used for CU serve mainly to ameliorate symptoms rather than to cure, and, unfortunately, only incomplete remission frequently results. The rsFcεRIα-based extracorporeal adsorption of serum autoAbs and the pharmacologic modulation of their target binding properties are strategies certainly worth pursuing. Systemic administration of rsFcεRIα, which may be of benefit in IgE-mediated allergic tissue inflammation, appears to be a treatment modality less well-suited for autoimmune CU, as this compound may exaggerate the disease activity in a complement-dependent manner and, thus, result in anaphylaxis and/or immune complex disease. A new attractive prospect would be to interfere in a concerted fashion with autoAb binding to IgE receptors and anti-FcεRIα autoAb-induced complement activation, thus blocking the initiation of the pathological cascade that results in this distressing affliction.

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