

Natural anti-FcεRIα autoantibodies may interfere with diagnostic tests for autoimmune urticaria

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Received 27 May 2003; revised 27 August 2003; accepted 15 September 2003

Abstract

IgG autoantibodies against the α-chain of the high affinity IgE receptor are claimed to play a pathogenetic role in autoimmune urticaria. The best methods for detection of functional autoantibodies are currently the autologous serum skin test and the basophil histamine release assay. A simplified and feasible screening test would facilitate the diagnosis of autoimmune urticaria.

Here we offer an explanation for the difficulties in establishing a screening test for autoantibodies directed against the α-chain of the high affinity IgE receptor in autoimmune urticaria. Identical autoantibodies in chronic urticaria patients and healthy donors belonging to the natural autoantibody repertoire were found by sequence analysis of anti-α-chain autoantibodies isolated by repertoire cloning from antibody libraries. These natural autoantibodies bound to the receptor and triggered histamine release but only if IgE was previously removed from the receptor. Diagnostic assays used for detection of antibodies directed against the IgE receptor may require signal comparison with and without the artificial removal of IgE, immune complexes, and complement in order to avoid false positive or negative results. After IgE removal diagnostic tests will detect natural autoantibodies against the high affinity IgE receptor regardless of whether they are pathogenic or not.

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Keywords: Autoimmune urticaria; Diagnostic test; IgE receptor; Natural autoantibodies; Phage display

1. Introduction

Urticaria is characterized by short-lived itchy wheals of the skin due to degranulation of mast cells and release of histamine. The diagnosis of urticaria is primarily clinical and a thorough patient history is essential [1–3]. Angioedema should be separated from other pres-

entations of urticaria since etiology and treatment may differ [4]. Chronic urticaria is defined by the occurrence of short-lived wheals (lasting less than 24 h), appearing at least twice a week, over a period of more than 6 weeks. Diseases that can be associated with chronic urticaria include systemic diseases such as thyroid dysfunction [5–7], lymphoma [8], Still's disease, as well as IgM monoclonal gammopathy in Schnitzler syndrome [9]. Protracted or relapsing diseases usually regarded as causes for acute urticaria such as allergies and adverse reaction to drugs (e.g. NSAID) should receive differential diagnostic consideration. Furthermore, urticaria may be a symptom of infections, e.g.

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Abbreviations: Ab, antibody; CDR, complementary determining region; CU, chronic urticaria; FcεRIα, α-chain of the high affinity IgE receptor; FR, framework

parasitic infestation, common cold especially in children, post-streptococcal condition [10], *Helicobacter Pylori* infection [11], hepatitis B and C, infectious mononucleosis, and Lyme borreliosis [12] and urticaria can occur after vaccination [13,14]. Rare manifestations of chronic urticaria are syndromes linked with chromosome 1q44 (familial cold urticaria and Muckle–Wells syndrome [15,16]. Additionally, it has been postulated that reactions due to food rich in histamine in patients with deficiency of diamine oxidase [17] could manifest themselves as chronic urticaria.

Chronic urticaria is classified as autoimmune if histamine releasing autoantibodies (autoAbs) can be detected. IgG autoAbs either against IgE or against the α -chain of the high affinity IgE receptor (Fc ϵ RI α) were claimed to cause histamine release in a subset of chronic urticaria (CU) patients [18–22]. IgG autoAbs against Fc ϵ RI α were detected in patients with autoimmune diseases, *Helicobacter* infection, food and drug intolerance [5,23]. It has been shown that autoimmune urticaria tends to be more severe than other forms of urticaria and is poorly responsive to conventional therapy [24]. An early diagnosis of autoimmune urticaria is therefore essential to optimize the care of these patients and improve the outcome of their disease. Blood basophil numbers are reduced in patients with active chronic urticaria and are related to serum histamine releasing activity [25]. A reliable method has to be established for counting the low numbers of basophils in blood before it can be used as a parameter in the accurate diagnostics of urticaria [26]. The best methods for detection of functional autoAbs in serum of patients with urticaria are currently the autologous serum skin test and the basophil histamine release assay. The latter is difficult to standardize because it depends on freshly isolated basophils from healthy donors which have variations in histamine releasing capacity amongst different donors and even amongst different blood donations from the same donor, furthermore it is impractical, as it requires relatively large amounts of blood from the basophil donor. To date this test has remained confined to research or reference laboratories. Mediator release by Fc ϵ RI α transfectomas might be easier to standardize but maintenance of a transfectoma cell line may not be suitable for routine use. The autologous serum skin test implies injection of human serum that is not pre-tested for infectious agents, and due to the danger of sample mislabelling and confusion of identity is not allowed in certain countries for ethical reasons. In addition, this test does not distinguish between a reaction caused by anti-IgE, anti-Fc ϵ RI α autoAbs or complement components. Thus, a screening test for autoAbs, e.g. based on an enzyme linked immunoassay would simplify the diagnosis of autoimmune urticaria.

We have recently reported anti-Fc ϵ RI α autoAbs in urticaria patients and healthy donors that belong to the natural autoantibody repertoire [27]. The sequence analysis reported showed identical IgM anti-Fc ϵ RI α autoAbs in urticaria patients and healthy donors. Furthermore, IgM clones from both healthy donors and CU patients induced histamine release from basophilic granulocytes but this effect was restricted to basophils that were pretreated for removal of IgE from Fc ϵ RI α .

Both, IgE and anti-IgE Abs in patients serum can interfere with detection of anti-Fc ϵ RI α autoAbs. For detection of anti-Fc ϵ RI α autoAbs current tests may require denaturation of the tested serum and IgE removal from Fc ϵ RI α in order to avoid false positive and false negative results caused by IgE, by IgE-anti-IgE immune complexes, or by complement. However, under these conditions anti-Fc ϵ RI α autoAbs that belong to the natural autoAb repertoire will gain access to the Fc ϵ RI α and may interfere with diagnostic methods used to detect pathogenic autoAbs in autoimmune urticaria.

2. Materials and methods

2.1. Serum donors and autologous serum skin test

Patients with chronic urticaria, defined as recurrent wheals lasting less than 24 h and occurring at least twice a week for more than 6 weeks (mean 3.4 ± 3.2 years, 10 weeks to 12 years) were screened by autologous serum skin test. The control group consisted of healthy members of the laboratory staff. Skin tests in patients were performed by injecting 50 μ l of autologous serum into the dermis of the volar forearm [18]. The wheal volume was determined at 60 min and corrected for the value obtained after control injection of phosphate-buffered saline (PBS). Wheal volumes $>9 \text{ mm}^3$ were considered as a positive response.

For in vitro tests, IgE in serum samples was subjected to heat denaturation in a water bath at 56°C for 3 h. Destruction of IgE was controlled in a sandwich enzyme-linked immunosorbent assay (ELISA) based on recombinant Fc ϵ RI α and horseradish peroxidase (POX) labeled Le27, an anti-IgE monoclonal Ab (data not shown).

2.2. Reagents

Mouse monoclonal anti-Fc ϵ RI α Ab 15-1, directed against the IgE-binding epitope of the high-affinity IgE receptor α -chain, was kindly provided by J.P. Kinet (Boston, MA, USA) [28]. POX labeled mouse anti-human IgG Ab was purchased from Nordic (Tilburg, the Netherlands). Mouse monoclonal anti-human IgE Ab Le27 was produced in our laboratory [29]. Recombinant Fc ϵ RI α , a fusion protein of human serum

albumin as carrier, flanked by two extracellular parts of the α chain of the high affinity IgE receptor was kindly provided by Novartis AG, Switzerland.

2.3. ELISA analysis of sera

Total IgE concentrations in sera were determined by FEIA Caps System (Pharmacia & Upjohn AG, Switzerland).

Sandwich ELISA was performed with Immulon ELISA plates (Dynex Technologies Inc., Chantilly, VA, USA) coated with recombinant Fc ϵ RI α (10 μ g/ml), HSA (10 μ g/ml) or IgE-SUS11 [30] (5 μ g/ml) at 4 °C overnight, blocked with phosphate-buffered saline containing 1.5 mg/ml casein (PBSC). Sera were diluted 1:20 in PBSC, bound Abs were detected using POX labeled mouse anti-human IgG (The Binding Site, Sodiag AG, Switzerland), 3,3',5,5'-Tetramethylbenzidine (Fluka, Switzerland), and 1 M H₂SO₄. Absorbance was read at 450 nm on a Molecular Devices ELISA reader (Paul Bucher, Switzerland).

Statistical significance was determined using the Wilcoxon rank sum test, unpaired. A *P* value of <0.05 was considered significant.

2.4. Histamine release from basophils

Heterologous basophils were purified from a blood sample by dextran sedimentation and Percoll gradient (Pharmacia & Upjohn AG, Switzerland) [31] and resuspended in Hepes buffer containing 0.25 mg/ml BSA, 1 mM MgCl₂, and 1 mM CaCl₂. Cells were prestimulated with 0.1 μ g/ml IL-3 (kindly provided by Novartis AG, Switzerland) [32,33] at 37 °C for 10 min, followed by addition of plasma preparations diluted 1:10 in PBSC or control Ab 15-1 used at a concentration of 5 μ g/ml at 37 °C for 20 min. Cell stimulation was stopped by incubating the cells on ice for 20 min. Histamine in the supernatants was measured using an automated fluorimetric method [34,35].

2.5. Cell donors, preparation of cells, and Fab library construction

Three libraries (LTM, UM, and UG) were constructed that displayed Fab on the pIII of the filamentous bacteriophage M13. Construction of IgM Fab library LTM from CD19-positive B cells from the tonsils of 3 children (aged 5.5 $\pm$ 1.6 years, 3–6.6 years) and selection of clones is described elsewhere [36,37]. IgM Fab library UM and IgG Fab library UG were constructed from blood B cells of two CU patients. Patient no. 18, a male donor, aged 21 years, was suffering from CU since 4 months and had occasionally suffered from swelling of the hands. Patient no. 19, a female donor, aged 75 years, was suffering from CU since more than

4 years. There was no identifiable cause for the urticaria, e.g. drug reaction, food or contact allergy in these patients, the localization of the wheals did not imply physical irritation of the skin to be the main trigger and patients had no clinical signs of another autoimmune disease, neither at the moment of blood sample collection nor during 3 years of follow up. Patients had stopped corticosteroid and antihistaminic therapy 2 weeks prior to a dermatological routine control and were not taking any further immunosuppressive drugs. Without therapy the patients were suffering from generalized urticaria. Blood was taken during this active urticarial phase. Informed consent was obtained from the donors. Histamine release from basophil enriched human leukocytes by heat denatured serum samples of these two patients was increased after removal of IgE from the basophils [27].

From a 100 ml blood sample of each donor mononuclear cells were isolated by Ficoll gradient centrifugation (Lymphoprep, Pharmacia & Upjohn AG, Switzerland) and cultured for 3 days in RPMI-1640 medium (Seromed, Switzerland) containing 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml Penicillin, 10 U/ml IL-2, and 10 μ g/ml pokeweed mitogen. Total RNA was then extracted from these cells by acid guanidinium thiocyanate–phenol–chloroform procedure [38].

Reverse transcription of RNA into cDNA and PCR were performed as described [39]. Subsequently, cloning of light chains and heavy chain fragments into the pMVS vector, transformation into XL1-Blue cells, and production of phages were carried out [40,41]. The pMVS vector (M.Vogel, unpublished results) has been reconstructed based on pComb3H [42]. An oligonucleotide containing the myc and 6His tags was inserted at the *Xba*I site. The *Escherichia coli* strain XL-1 Blue and helper phage VCSM 13 were purchased from Stratagene (La Jolla, CA, USA).

2.6. Biopanning and selection of human recombinant anti-Fc ϵ RI α Fab clones

Biopanning was performed on Fc ϵ RI α coated to polystyrene immunotubes (Becton Dickinson, Switzerland) at 4°C overnight. PBS containing 1.5 mg/ml Casein (Fluka Chemie AG, Switzerland) was used as blocking agent alternating from one panning round to the other with PBS containing 5% bovine serum albumin. Tubes were incubated with 4 ml of phage preparation at room temperature for 90 min. Washing, elution steps and amplification of phages were done basically as described [43]. The titers of eluted phages were <10⁵, 10⁶, and >10⁷ phages/ml after the 3rd, 7th, and 8th/9th panning round (with the UM/UG library) respectively. From each of these populations 100 clones were screened and specific clones identified by

Colony Screening in a filter lift assay [43]. All FcεRIα-specific clones were sequenced between frameworks 1 and 4 containing the three hypervariable regions CDR 1–3.

2.7. Sequence analysis

Plasmid DNA was purified by Wizard Plus SV Minipreps (Promega, Catalys AG, Switzerland). Primers used for sequencing of the heavy chains were: CHIgM (5' GCT GCT CGT ATC CGA CGG 3'), pCH3H (5' GG AGG AAT TTA AAA TGA AAT AC 3'), and CHγ1 (5' CGC TGT GCC CCC AGA GGT 3'). Primer used for sequencing of the light chains was: pCL3H (5' GTG GAA TTG TGA GCG GAT AAC 3'). Sequencing was performed by Microsynth, Switzerland. Amino acid sequences were deduced using DNASIS software, compared with the Immunogenetics (IMGT) database (www.dnablot.de/input/human_v.html) and aligned to the germline V_H and V_λ families. UMa16, a clone from the urticaria patient library showed an identical sequence to LTMα15 from the children tonsil library and was therefore called LTMα15, even though deriving from preparations made with phages from the urticaria patient library.

3. Results

AutoAbs either against IgE or against FcεRIα have been proposed to cause histamine release in CU patients with a positive autologous serum skin test. We measured levels of IgE, anti-IgE and anti-FcεRIα autoAbs in sera from CU patients that had been tested for the autologous serum skin reaction. Before measurement of anti-IgE and anti-FcεRIα autoAbs, serum samples were heat denatured in order to destroy endogenous IgE. As shown in Fig. 1A, most of the CU patients had IgE levels above the reference range of 100 kU/l IgE in serum although some individuals had normal or low IgE concentrations, regardless of reaction to the autologous serum skin test. The patient with the highest IgE level had a negative autologous serum skin test. Anti-IgE levels although seemingly higher in patients showing a positive autologous serum skin test, in fact showed no statistically significant difference ($P=0.06$). Therefore no diagnostic correlation could be made due to the lack of a clear cut off between patients with positive and negative skin reactions (Fig. 1B). As shown in Fig. 1C, all blood samples revealed low levels of anti-FcεRIα autoAbs. Measurements were based on recombinant FcεRIα (a fusion protein of HSA as carrier, flanked by two recombinant FcεRIα). Background binding of serum IgG Abs to HSA on the solid phase was minimized by dilution of sera in 2% HSA. The remaining background signal of IgG binding to HSA on the solid phase was

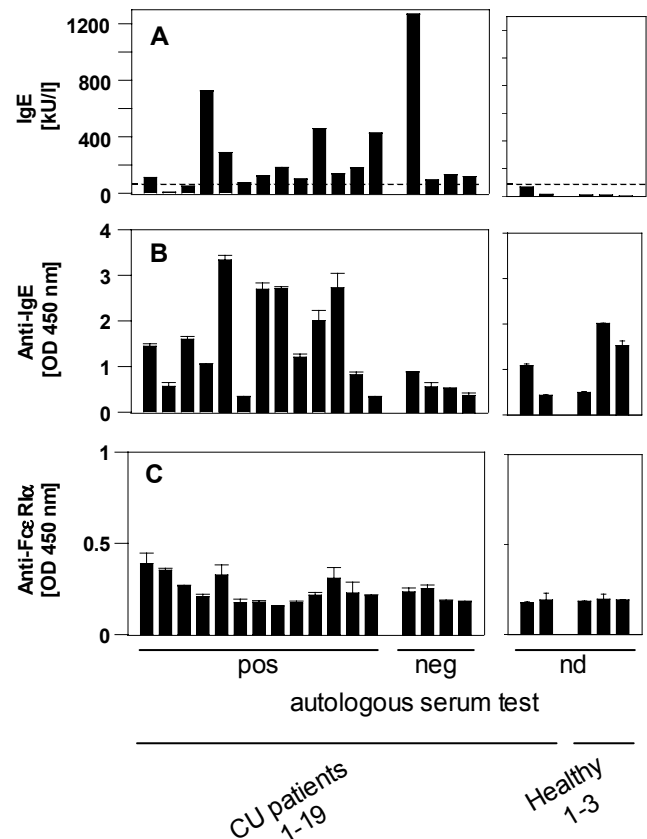


Fig. 1. Ab levels in blood samples from urticaria patients and healthy donors determined by commercial assay and sandwich ELISA. IgE levels were measured in native sera based on a commercial assay (A), anti-IgE Abs were measured in IgE-depleted sera based on IgE-SUS11 and POX labeled anti-human IgG (B), and anti-FcεRIα Abs were measured in IgE-depleted sera based on recombinant FcεRIα and POX labeled anti-human IgG (C). IgE depletion was performed by heat denaturation at 56 °C for 3 h. Nineteen samples derived from CU patients and 3 from healthy donors. Serum samples of urticaria patients were divided into a positive and a negative group regarding the reaction in vivo to intradermal injection of autologous serum and a third group where the autologous serum skin test was not performed (nd). Mean+SD of duplicate measurements in sandwich ELISA is shown.

subtracted from the signal of anti-FcεRIα autoAbs. In summary our data show no correlation between positive autologous serum skin tests or urticaria and increased serum levels of anti-IgE or anti-FcεRIα Abs by binding assay since all tested sera contained low levels of anti-FcεRIα autoAbs.

Ab levels as measured in Fig. 1 are the sum of serum Abs binding with different affinities to all the different epitopes of the IgE- or the FcεRIα-molecule and might therefore not be an accurate parameter to reflect the anaphylactogenic potential of the corresponding serum sample. A more relevant method to evaluate the anaphylactogenic potential of a serum sample may be the autologous serum skin test or the measurement of the serum capacity to induce mediator release from cells

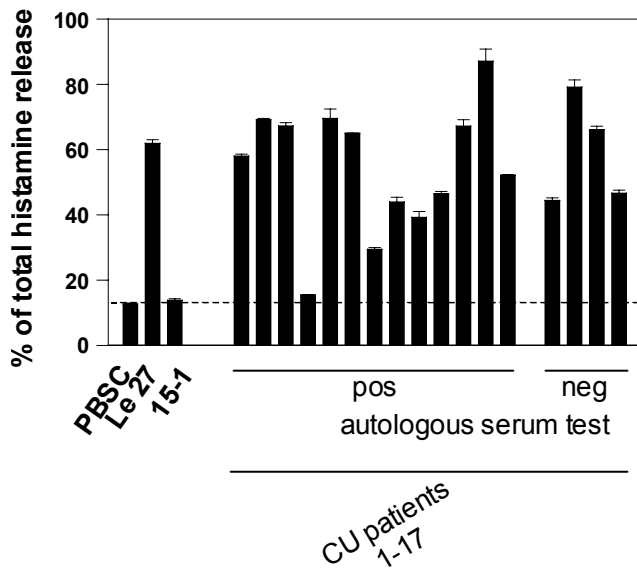


Fig. 2. In vitro histamine release induced by sera of urticaria patients. Sera of CU patients that were previously tested for autologous serum skin test were diluted 1:10 in PBSC and incubated with basophil-enriched human leukocytes. The cells were primed with IL-3. Mouse mAbs 15-1 and Le27 were used as controls for desensibilization. Mean+SD of histamine measurement in cell supernatants of duplicate cultures is shown.

bearing FcεRIα. Serum samples of CU patients that had been tested for the autologous serum skin reaction were added to basophilic granulocytes. Sera were not heat denatured and IgE was not removed from the basophils. Fig. 2 shows the subsequently measured histamine in basophil supernatants. Basophils incubated with PBSC alone, with the anaphylactogenic mouse mAb anti-IgE Le27, and with the mouse mAb anti-FcεRIα 15-1 that triggers histamine release only after removal of IgE from the FcεRIα were used as controls. Histamine release pattern by the control Abs implies that the FcεRIα are occupied by IgE. Almost all serum samples were able to release histamine. Histamine release by serum samples may therefore be triggered by anaphylactogenic anti-IgE autoAbs, anti-FcεRIα autoAbs that do not compete with IgE for binding to FcεRIα or other serum components, e.g. complement, but probably not by anti-FcεRIα autoAbs that compete with IgE for binding to FcεRIα. There was no correlation between histamine release in this experiment and positive or negative autologous serum skin reaction. Thus, in order to enable serum anti-FcεRIα autoAbs that compete with IgE for binding to FcεRIα to release histamine from basophils, serum samples have to be heat denatured to inactivate IgE within the sera [44].

Functional characterization of anti-FcεRIα Abs and interpretation of histamine release assays using serum samples is difficult due to the polyclonal nature of the Abs present, which might contain a mixture of anti-FcεRIα Abs with different biological functions including

different isotypes. In order to dissect the Ab specificities in polyclonal preparations, we investigated autoAbs against FcεRIα on a monoclonal level. Therefore, Fab phage display libraries were constructed. Blood leukocytes from the two CU patients no. 18 and no. 19 (cf. Fig. 1 and [27]) were used to construct the phage display libraries UM and UG (an urticaria IgM and an urticaria IgG library, respectively). These two patients were selected as donors for library construction due to the presence of anti-FcεRIα Abs in their serum and due to the property of denatured sera to induce increased histamine release from basophils that were treated with lactic acid compared to untreated, i.e. IgE bearing basophils. In the panning procedure titers of eluted phages had reached a plateau after the 8th (UM library) and the 9th (UG library) round of panning on human recombinant FcεRIα and 100 clones from each phage population were tested for FcεRIα-specific Fabs expressed by bacteriophages. Each library contained specific clones that were identified by filter lift assays and further tested for binding in immunodot assays and ELISA (data not shown). Sequence comparison of all FcεRIα-specific Fabs (12 of 100 from each urticaria library, IgM and IgG) revealed one almost identical Ab heavy chain, differing in only 3 amino acids in FR 4 (Table 1). Furthermore, all the light chains within one urticaria library showed identical sequences. Thus, in the urticaria libraries selection on FcεRIα resulted in one FcεRIα-specific IgM- and one FcεRIα-specific IgG-clone (named UMa16 and UGa8, respectively) that have almost identical heavy and similar light chains. Specific clones from the 3rd round of panning were sequenced and revealed again the same clones. A broader sequence diversity than the clones obtained from the 8th and 9th round respectively was not found indicating that other possible clones were not lost during the panning procedure.

Sequences of the two clones UMa16 and UGa8 were compared to the previously isolated FcεRIα-specific clones from the LTM library ([37], Table 1). Sequence comparison revealed one clone within the LTM library, namely clone LTMa15, with an identical sequence of heavy and light chain as UMa16. It is remarkable that the same anti-FcεRIα Ab was isolated from IgM libraries of normal donors (LTM library) and urticaria patients (UM library). To avoid confusion, UMa16 is hereinafter known as LTMa15.

Sequence comparison to germline V gene segments revealed that the Ab heavy chains showed nonmutated V_H-germline DP-47 sequence. This heavy chain exclusively paired with λ-light chains derived from the same light chain family, namely V_L germline V1-22. The V_L sequence of LTMa15 (i.e. UMa16) was slightly mutated compared to the germline and the V_L sequence of UGa8 showed a few more point mutations in CDR 1, 2, 3, FR 2 and 3 (Table 1).

Table 1
Deduced amino acid sequences of variable regions of recombinant anti-FcεRIα autoantibodies

Heavy chains:

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
DP-47	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYAMS	WVRQAPGKLEWVS	AISGSGGTTYADSVKG	RFTISRDNKNTLYLQMNSLRAEDTAVYYCAK		
LTMα15	-----	----	-----	-----	-----	GERWLPSYYMDV	WGKGTITVTVSS
UMα16	-----	----	-----	-----	-----	GERWLPSYYMDV	WGKGTITVTVSS
UGα8	-----	----	-----	-----	-----	GERWLPSYYMDV	WGQGTILVSVSS

Light chains (all λ):

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
V1-22	NFMLTQPHSVSESFGKTVTTISC	TRSSGSIASNVVQ	WYQQRPGSSPTTVIY	EDNQRP	GVPDRFSGSIDSSNSASLTISGLKTEADYYC	QSYDSSN	
LTMα15 ^a	EL-----	-C-----I----	-----	G-----	-----	----N-HVV	FGGGTKLTVLGQP
UMα16 ^a	ELVV-----	-C-----I----	-----	G-----	-----	----N-HVV	FGGGTKLTVLGQP
UGα8	EL-----	---D-----	--R---A---F	A-DR---	-----A---Y-----R-----	----N-LWV	FGGGTKLTVLGQP

The sequence data are available from the EMBL Gen bank under the numbers AJ276097 and AJ276098 for LTMα15 heavy and light chains and under the numbers AJ496297 and AJ496298 for UGα8 heavy and light chains, respectively.

^a The difference in the first amino acids in FR1 of the light chains (a substitution from glutamate to glutamine) results from the restriction sites for cloning the Fabs into the vector pMVS.

Selection of the two clones LTMα15 and UGα8 was based on their binding capacity to recombinant FcεRIα. To allow functional assays, the anti-FcεRIα clones were produced as full length IgG molecules. It has been shown that binding of LTMα15 to FcεRIα was inhibited by IgE and induced histamine release from freshly isolated basophils upon removal of IgE [37]. Histamine release from basophils by UGα8 as full length IgG molecule required significantly higher Ab concentrations implying a lower affinity to FcεRIα than LTMα15 (data not shown).

4. Discussion

There is increasing evidence that a subset of patients with chronic urticaria suffer from an autoimmune disorder caused by autoAbs against IgE or FcεRIα on mast cells. Two different types of anaphylactogenic anti-FcεRIα autoAbs were described in autoimmune urticaria: autoAbs that compete with IgE for the binding to FcεRIα and autoAbs that are not competitive and therefore bind to non-IgE-binding domains of FcεRIα [45]. Here we show that the basophil mediator release assay corresponds neither to the autologous serum skin test nor can it distinguish between the different serum components that could potentially cause histamine release. This discrepancy could partly be caused by bradykinin since it gives a positive skin test but has no effect on basophils [46]. The components that could potentially cause histamine release include complement, anti-IgE Abs, anti-FcεRIα autoAbs that compete with IgE for the binding to FcεRIα or autoAbs that are not competitive. It has been reported that purified IgG from patient sera were inactive or poorly reactive with cutaneous mast cells or human basophils

[47]. Basophil histamine release was markedly augmented by complement if release by IgG alone was low [48]. Further work demonstrated that histamine release from basophils occurred with IgG alone and could be augmented by complement component C5a [49]. To distinguish anti-IgE from anti-FcεRIα autoAbs functionally, comparison of histamine release before and after IgE removal is necessary. Thus, detection of anti-FcεRIα autoAbs that compete with IgE for binding may require lactic acid treatment of the basophils and heat denaturation of sera. Heat denatured IgE is no longer capable of binding to FcεRIα and most monoclonal anti-IgE antibodies seem to recognize epitopes that disappear during heat denaturation [50]. For the measurement of serum anti-FcεRIα autoAbs that compete with IgE for binding to the receptor it can be useful to denature the serum samples even though it will not fully inactivate complement. Furthermore standard basophil histamine release assays use basophils from heterologous donors which adds another variable to the assay. Both, lactic acid treatment of basophils and the heterologous source of basophils may not correspond to the situation in vivo in an individual urticaria patient, e.g. an epitope on the FcεRIα which was hidden in vivo may be exposed in vitro.

We describe phage display antibody libraries constructed from blood leukocytes of urticaria patients and we previously described those from tonsil lymphocytes of healthy donors [37]. Sequence analysis showed one major anti-FcεRIα autoAb in urticaria patients. This anti-FcεRIα autoAb was identical with a clone found in healthy donors. Furthermore, this anti-FcεRIα autoAb competed with IgE for binding to FcεRIα. Thus it bound to an epitope that may be hidden in vivo. Additionally this anti-FcεRIα autoAb was able to

trigger histamine release only after removal of IgE from its receptor. Since this recombinant anti-FcεRIα Ab was only slightly mutated in comparison to the germline sequence, it can be considered as a natural Ab [51,52]. It is known that sera of healthy individuals contain natural Abs of the IgG, IgM and IgA isotype. They are termed “natural” as they are generated in the absence of immunization and independently of exposure to foreign antigens [53].

We found low levels of anti-FcεRIα autoAbs in all tested blood samples, regardless of whether the samples were derived from healthy donors or urticaria patients in contrast to previously published data by Fiebiger et al. where anti-FcεRIα autoAbs were selectively found in patients suffering from urticaria or certain other autoimmune diseases [19,20]. A possible explanation for this finding may be that all sera tested in our experiments would not have had anti-FcεRIα autoAbs levels above the cut off level used in the publications of Fiebiger et al. But a further explanation may be that these anti-FcεRIα autoAbs represent natural autoAbs and may therefore be present in all individuals. The anti-FcεRIα autoAbs selected from the phage display libraries of urticaria patients showed a very restrictive repertoire. Sequence analysis of early panning rounds indicated that no other clones were lost during panning procedures. Since the libraries were constructed using leukocytes from only two urticaria patients, it cannot be excluded that other anti-FcεRIα autoAbs reacting with different epitopes may be found in libraries constructed from a range of different urticaria patients. Nevertheless, our data indicate the presence of natural anti-FcεRIα autoAbs in urticaria patients which independent of their pathogenic potential may influence histamine release assays if IgE is removed from the system.

We have shown that autoAbs that compete with IgE for the binding to FcεRIα are present in both urticaria patients and healthy donors. Therefore these anti-FcεRIα autoAbs have been termed conditional autoAbs. The affinity of IgE to FcεRIα is very high (10^{-12} M) and it has been shown that by decreasing serum IgE levels, IgE receptor density was down regulated on basophils and mast cells [54,55]. Thus, in physiological conditions the FcεRIα is covered by its natural ligand IgE and the IgE binding site of FcεRIα can be regarded as an immune privileged site. Whether breakdown of this so called immune privileged site (i.e. any disturbance of the balance between occupied versus free FcεRIα) contributes to the pathogenesis of autoimmune urticaria is not known. Detection of anti-FcεRIα autoAbs in histamine release assays as well as in diagnostic tests based on recombinant FcεRIα includes measurement after removal of IgE from the system, either by lactic acid (removal of IgE from cell surface) [56] or by heat denaturation (removal of IgE from sera) [50]. Such assays do not account for the fact that urticaria most

often appears on the skin as a local phenomenon and thus a serological profile may not be a true indicator of the in vivo pathology. Thus, local disturbances in the homeostatic balance between receptor and ligand might allow natural anti-FcεRIα autoAbs access to the FcεRIα resulting in a pathogenic interaction accompanied by a local release of mediators [57]. It is not known how many receptors need to be crosslinked to induce mediator release, maybe because the amount depends also on the affinity of the ligand [58]. Whilst natural anti-FcεRIα autoAbs may only become pathogenic in vivo in situations of dysequilibrium between occupied and free IgE receptors they will in contrast interfere with every diagnostic test that requires the artificial removal of IgE before measurement.

Urticaria patients with anti-FcεRIα autoAbs seem to have more severe disease and more often suffer from urticaria resistant to antihistaminic therapy [24]. Therefore the measurement of functional anti-FcεRIα autoAbs is very important for the estimation of prognosis, determining treatment protocols, and better surveillance of these patients and will remain an important parameter in the diagnostics of urticaria. One has to take into account that a positive signal in the currently used diagnostic tests for anti-FcεRIα autoAbs could be due to natural anti-FcεRIα autoAbs which still have an undefined pathogenetic significance. A negative result in an assay using patients sera does not exclude the presence of pathogenic anti-FcεRIα autoAbs in the skin of the patients. For the future it will be important to design tests that can define and distinguish between the different anti-FcεRIα autoAbs so far reported in the literature and specific immunotherapies can be developed based on epitope mapping.

Acknowledgements

The excellent technical assistance of Santa Eglite, Sonja Kuhn, Jana Tilch, Andreas Hofmann, and Michael Stadler is acknowledged. This work was supported by grant Nr. 435 of the Federal Office of Education and Science and grant Nr. 31-55955.98 of the Swiss National Foundation to S.M.M.

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