

Conditional autoimmunity mediated by human natural anti-FcεRIα autoantibodies?

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ABSTRACT Natural antibodies provide an early defense mechanism against pathogens, show a frequent self-reactivity, and are present throughout life. Two questions concern the physiological control of self-reactivity and the pathogenetic link to autoimmune disease. Here we propose a concept of conditional autoimmunity involving natural antibodies against the α chain of the high-affinity receptor for IgE (FcεRIα). Like other natural antibodies, anti-FcεRIα antibodies are found in sera of healthy donors. We now report the first human recombinant anti-FcεRIα autoantibodies isolated by repertoire cloning from a human tonsillar IgM library. These high-affinity antibodies recognize FcεRIα on cells and trigger histamine release from freshly isolated blood basophils. However, the latter effect requires IgE removal from the FcεRI. The same conditional histamine release is seen when using sera from individual normal donors and affinity-purified anti-FcεRIα antibodies isolated from multidonor therapeutic IgG preparations. We propose that such anti-FcεRIα antibodies can become pathogenic and that this is dependent on the state of occupancy of the FcεRIα by its natural ligand IgE. We suggest that an imbalance between FcεRIα occupancy and natural anti-FcεRIα antibodies may be implicated in the pathogenesis of autoimmune urticaria.—Horn, M. P., Pachlopnik, J. M., Vogel, M., Dahinden, M., Wurm, F., Stadler, B. M., Miescher, S. M. Conditional autoimmunity mediated by human natural anti-FcεRIα autoantibodies? *FASEB J.* 15, 2268–2274 (2001)

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THE PATHOGENIC ROLE of natural antibodies in autoimmune disease is controversial. These antibodies are often polyreactive with a variety of both self and foreign antigens. However, the presence of such physiological autoantibodies does not generally precipitate autoimmune disease. Our recent investigations of autoantibodies associated with autoimmune urticaria suggest they are indistinguishable from natural antibodies found in healthy subjects.

Autoantibodies reacting with the α-subunit of the

human high-affinity immunoglobulin E (IgE) receptor (FcεRIα) have been described in autoimmune urticaria (1, 2). However, we previously reported the presence of anti-FcεRIα autoantibodies in the serum of healthy donors as well as in multidonor intravenous IgG (IVIg) preparations (3). Thus, these anti-FcεRIα autoantibodies may belong to the natural antibody repertoire reacting with a restricted set of self-antigens (4, 5). Functional studies of anti-FcεRIα autoantibodies using serum or IVIg preparations are difficult to interpret due to the polyclonal nature of the antibody preparations, which probably contain mixtures of different types (specificities) of anti-FcεRIα autoantibodies. Therefore, to characterize these natural anti-FcεRIα autoantibodies and investigate their functional activity in vitro and in vivo, we generated human recombinant anti-FcεRIα antibodies by repertoire cloning from a nonimmune IgM library displayed on the filamentous phage M13.

Here we show that two recombinant human natural anti-FcεRIα autoantibodies can react with the FcεRIα displayed on human blood basophils but only in the absence of IgE. This ‘conditional autoreactivity’ to an autoantigen, which under physiological conditions is sequestered, could be demonstrated in vitro by histamine release. Based on these results, we have formulated a hypothesis termed ‘conditional autoimmunity’ that could be relevant to other autoimmune diseases as well.

MATERIALS AND METHODS

Antibodies

Human monoclonal hybridoma IgE-SUS11, mouse monoclonal anti-human IgE antibody Le27, and peroxidase-conjugated rabbit anti-phage antibody were produced in our laboratory as described previously (6–8). Mouse anti-FcεRIα

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monoclonal antibody 15–1 was kindly provided by Prof. J. P. Kinet (Boston, MA). Human anti-Tetanus toxoid monoclonal antibody ST-18 was donated by Dr. A. Lang (Swiss Serum and Vaccine Institute, Bern, Switzerland). Human multidonor IgG, IVIg was donated by ZLB Bioplasma, Bern, Switzerland.

Construction of IgM Fab library

An IgM Fab library was constructed from children's tonsil B cells and displayed on pIII of the filamentous bacteriophage M13. Messenger RNA was isolated from CD19-positive B cells from the tonsils of four children (age: 5.5 ± 1.6 years; 3–6.6 years), and cDNA and PCR reactions were performed as described (9). For generation of the Fd fragments, six upstream primers were used that hybridize to each of the six V_H families (8). The downstream IgM primer was 5'-GCT CAC ACT AGT AGG CAG CTC AGC AAT CAC-3'. The Fd fragments were cloned into the pMVS vector. The pMVS vector (M. Vogel, unpublished results) has been reconstructed based on pComb3H (10); an oligonucleotide containing the myc and 6His tags has been inserted at the *Xba*I site.

Isolation of human recombinant anti-Fc ϵ RI α Fab clones

The original library was amplified in *Escherichia coli* XL-1 cells (Stratagene, San Diego, CA), and phages were produced and precipitated as described (11). Anti-Fc ϵ RI α Fab phages were selected on immobilized human recombinant Fc ϵ RI α (3) in polystyrene immunotubes (Becton Dickinson, Rutherford, NJ). After six rounds of panning, 50 clones were analyzed by nitrocellulose filter lift technique (8); the variable regions of the heavy and light chains of Fc ϵ RI α -positive clones were sequenced at Microsynth GmbH (Balgach, Switzerland) and compared with the V Base Sequence Directory (12).

Generation of full-length IgG

Full-length IgG was produced using the integrated vector system (kindly provided by Dr. A. Bradbury, Trieste, Italy). The V_H and λ light chain regions were recloned using the primers as recommended by Persic et al. (13).

HEK-293T cells were grown in DMEM:F12 medium (Gibco BRL, Grand Island, NY) supplemented with 2% FCS at 37°C in 5% CO₂. Plasmid-DNA (heavy chain : light chain, 7:3, total 10 μ g/ 2×10^6 cells) was transfected with lipofectamine 2000 reagent (Gibco BRL) according to the manufacturer's instructions and the cells were cultured for 5 days. Antibodies in the cell supernatant were purified on protein G Sepharose columns (Pharmacia, Piscataway, NJ) and purity was controlled on a 9% SDS-acrylamide gel. The concentration of the purified IgG was determined by sandwich ELISA using two goat anti-human IgG antibodies (TAGO, Burlingame, CA).

IgE inhibition assay

RIA/EIA plates (Costar, Cambridge, MA; Integra Biosciences, Fernwald, Germany) were coated with Fc ϵ RI α (5 μ g/ml) and blocked with PBS containing 0.15% casein (PBS-C). Due to limited amounts of purified anti-Fc ϵ RI α antibodies, the assay was performed under nonsaturating conditions using constant concentrations of anti-Fc ϵ RI α antibodies that resulted in an O.D. signal of 1.0 in the ELISA assay. Thus, concentrations of LTM α 15 or LTM α 35 (11 ng/ml and 6 ng/ml, respectively) were incubated with different amounts of IgE-SUS11 (diluted in twofold dilution steps starting at a concentration of 24 μ g/ml) on the Fc ϵ RI α for 4 h at 37°C. IgG binding was detected with peroxidase-conjugated sheep anti-human IgG

(The Binding Site, Birmingham, UK) and visualized with TMB (3,3',5,5'-tetramethylbenzidine; Fluka, St. Louis, MO) and the reaction was stopped with 1 volume 1M H₂SO₄. Plates were read at 450 nm with a $v_{\max}$ kinetic microplate reader (Molecular Devices, Palo Alto, CA).

FACS analysis

CHO cells transfected with the human Fc ϵ RI α and Fc ϵ RI γ chains (provided by Prof. J. P. Kinet) were maintained in RPMI containing 10% FCS and 1 mg/ml geneticin G-418 (Gibco BRL). FACS staining was performed by incubating 10^4 cells with 5 μ g/ml of the antibodies LTM α 15, LTM α 35, or ST-18 in PBS containing 0.5% BSA and 0.02% NaN₃ (PBSA-Az) in 96-well V-bottomed polystyrene plates (Dynatech, Vienna, VA) for 30 min at 4°C. Subsequently, cells were washed twice with 150 μ l PBSA-A and antibodies binding to the cells were detected by FITC-conjugated sheep anti-human IgG antibody (The Binding Site), and analyzed by Epics Coulter FACS.

Affinity purification of anti-Fc ϵ RI α antibodies from pooled IgG

Anti-Fc ϵ RI α antibodies were purified by affinity chromatography on immobilized Fc ϵ RI α as described (3). To exclude anti-IgE antibodies from the eluted fraction (as a result of IgE/anti-IgE complex formation), the enriched anti-Fc ϵ RI α antibody fraction was further purified on immobilized human IgE-Sav (kindly provided by Dr. V. Savazal, Pilsen, Czech Republic).

Histamine release from basophils

This assay was performed using primary cells (i.e., basophil-enriched peripheral blood leukocytes from three different donors) purified by dextran sedimentation and Percoll gradient (Pharmacia). Each individual sample was divided into treated and untreated samples. The treated samples were incubated with lactic acid buffer (0.13 M NaCl, 0.005 M KCl, 0.01 M lactic acid, pH 3.9) for 10 min on ice. After washing once with HEPES buffer containing 0.25 mg/ml BSA, cells were resuspended in a cell buffer (wash buffer containing 1 mM MgCl₂ and 1 mM CaCl₂) supplemented with or without 50 μ g/ml IgE-SUS11. Both treated and untreated basophils were first stimulated with 10 ng IL-3 (kindly provided by Novartis AG, Basel, Switzerland) for 10 min, followed by the addition of different antibodies for 20 min at 37°C. Triggering of basophils was stopped by incubating the cells on ice for 20 min. Samples were analyzed using an automated fluorometric method (14) and calculated as a percentage of total histamine. Anaphylactic and nonanaphylactic controls were always included and results were calculated relating back to the spontaneous release after each manipulation of the cells. To exclude binding of anti-Fc ϵ RI α autoantibodies to Fc γ Rs through their Fc region, an isotype-matched control antibody, ST-18, was included. No triggering activity on human basophils under any conditions was seen with this antibody.

EXPERIMENTAL RESULTS

Generation of recombinant antibodies against the Fc ϵ RI α

We isolated two human monoclonal anti-Fc ϵ RI α autoantibodies—LTM α 15 (4/6 clones) and LTM α 35 (2/6

clones)—by repertoire cloning from an IgM Fab phage library constructed from children's tonsil B cells. Comparison with the known germline sequences of human V_H and V_L segments (V Base Sequence Directory) (12) indicated that both clones had unmutated germline V_H sequences, whereas λ light chains were slightly mutated (Table 1). Both recombinant anti-Fc ϵ RI α autoantibodies were produced as full-length IgG molecules to allow functional assays (e.g., histamine release), which requires bivalent recognition and signaling via the Fc ϵ RI α expressed on basophils. Fab fragments were recloned into the integrated vector system described by Persic et al. (13), followed by transient transfection and expression in HEK293T, human endothelial kidney cells. The harvested cell culture supernatant was purified on protein G, followed by SDS-PAGE and Western blot analysis. The resulting purified IgG antibodies bound specifically to immobilized Fc ϵ RI α in an ELISA (data not shown). The affinity of both recombinant anti-Fc ϵ RI α autoantibodies was assessed by online monitoring of the binding kinetics using the IAsys cuvette system (3). The affinities were 7.2×10^{-9} M for antibody LTM α 15 and 1.4×10^{-8} M for antibody LTM α 35.

In vitro analysis of recombinant full-length IgG₁ anti-Fc ϵ RI α autoantibodies

Anti-Fc ϵ RI α autoantibodies may only be functional in vivo when they are able to react with the Fc ϵ RI α displayed on the cell surface of effector cells. FACS analysis (Fig. 1A) demonstrated that both anti-Fc ϵ RI α autoantibodies recognized cell surface-expressed Fc ϵ RI α on transfected CHO cells compared with the IgG isotype control ST18, a Tetanus toxoid-specific human IgG monoclonal antibody.

The binding of these antibodies to recombinant Fc ϵ RI α could be inhibited in a dose-dependent manner by human IgE, the natural ligand of the receptor (Fig. 1B), indicating that both antibodies share with IgE an overlapping epitope on the Fc ϵ RI α . Due to the limiting and nonsaturating amounts of anti-Fc ϵ RI α autoantibodies available, a relatively large excess of IgE was required to inhibit 50% of the binding of the anti-Fc ϵ RI α autoantibodies to the solid-phase Fc ϵ RI α . However, the point of this inhibition assay was to show that the recombinant anti-Fc ϵ RI α autoantibodies would be able to bind in vivo to their antigen, but only in the absence of IgE.

Biological activity of the recombinant anti-Fc ϵ RI α autoantibodies

We assessed the anaphylactogenic potential of the two human recombinant anti-Fc ϵ RI α autoantibodies in a histamine release assay using freshly isolated peripheral blood lymphocytes enriched for basophils (Fig. 2A). Under physiological conditions represented by the untreated basophils, neither the two anti-Fc ϵ RI α autoantibodies LTM α 15 and LTM α 35 nor a mouse mono-

clonal anti-Fc ϵ RI α antibody, 15–1, triggered the cells for histamine release. In contrast, an anaphylactogenic mouse monoclonal anti-human IgE antibody, Le27, triggered high amounts of histamine release compared with the spontaneous release in the presence of IL-3 alone.

Fc ϵ RI α expressed on freshly isolated basophils is usually occupied by endogenous IgE (15). In Fig. 1B, we show the inhibition of antibody binding by IgE. Thus, both findings suggest that Fc ϵ RI α is normally not accessible for anti-Fc ϵ RI α autoantibodies such as LTM α 15 or LTM α 35. Upon stripping the cells with lactic acid in order to remove cell-bound IgE from the Fc ϵ RI α (15), there was a clear increase in histamine release induced by the two human recombinant anti-Fc ϵ RI α autoantibodies. The control mouse monoclonal anti-Fc ϵ RI α antibody 15–1, whose binding is also inhibited by IgE, showed the same result. The histamine-releasing activity of anti-human IgE antibody Le27 was reduced after removal of IgE but still measurable, indicating that lactic acid treatment results in only a partial removal of surface-bound IgE (15). Notably, resensitization of lactic acid-treated cells with an excess of IgE (50 μ g/ml) resulted in a complete block of the anaphylactogenic activity of anti-Fc ϵ RI α autoantibodies LTM α 15 and LTM α 35, as well as the mouse anti-Fc ϵ RI α antibody 15–1 when compared with the relevant spontaneous histamine release levels (Fig. 2A). To preserve the basophils in a good condition and prevent spontaneous triggering from excess handling, the basophils were not washed after the lactic acid treatment and before addition of IgE. Thus, the observed reduction of the triggering activity of anti-human IgE antibody Le27 was most likely due to the excess of IgE in the cell buffer, such that all available receptors were occupied with IgE and the remaining IgE blocked Le27 in solution. Overall, these results indicate that the anaphylactogenic potential of the recombinant anti-Fc ϵ RI α autoantibodies was dependent on the degree of occupancy of the Fc ϵ RI α by IgE.

The same antibody activity exists in human IgG preparations

The same phenomena could be observed by anti-Fc ϵ RI α autoantibodies that had been affinity-purified from a human multidonor IgG preparation (Fig. 2B) and by using serum from individual nonatopic donors (Fig. 2C). Again, histamine release was induced only after removal of IgE from the Fc ϵ RI α expressed on basophils. This suggests that similar conditionally anaphylactogenic anti-Fc ϵ RI α autoantibodies like LTM α 15 or LTM α 35 may exist in vivo.

DISCUSSION AND HYPOTHESIS

Many reports show that natural antibodies react with self-antigens and can be detected in the serum of normal donors (16). Our data confirm and extend

TABLE 1. *Deduced amino acid sequences of variable regions of recombinant anti-FcεR1α autoantibodies*

Heavy chains									
	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4		
DP-47	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYAMS	WVRQAPGKGLEWVS	AISGGSGTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK				
LTMα15	-----	-----	-----	-----	-----	GERWLPSYMDV	WGKGTITVTYSS		
LBα6	-----	-----M-----	-----	G--H-ANTFF	-----S-----	WVRGVIPLGF	YWGQTLVTVSS		
UMα16	-----	-----	-----	-----	-----	GERWLPSYMDV	WGKGTITVTYSS		
UGα8	-----	-----	-----	-----	-----	GERWLPSYMDV	WGQGTILVSVSS		
VHVCW	EVQLVQSGAEVKKPGESLKISCKGSGYSFT	SYWIG	WVRQMPGKGLEWVG	IIPGDSDTRYSPSFQG	QVTISADKSISTAYLQWSSSLKASDTAMYYCAR				
LTMα35*	---LE-----	-----	-----	-----	-----	LGYDFWSGSDYYIMDV	WGKGTITVTYSS		
Light chains (all λ)									
	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4		
V1-22	NEMLTQPHSVSESPGKTVTISC	TRSSGSIASNYVQ	WYQQRPGSSPTTVIY	EDNQRPS	GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC	QSYDSSN			
LTMα15	ELVV-----	-C-----I-----	-----	G-----	-----	-----N-HVV	FGGTKLTVLGQP		
UMα16	ELVV-----	-C-----I-----	-----	G-----	-----	-----N-HVV	FGGTKLTVLGQP		
UGα8	EL-----	---D-----	---R---A-----F	A-DR---	-----A--Y-----R-----	-----N-LWV	FGGTKLTVLGQP		
DPL-23	SYELTQPPSVSVSPGQTASITC	SGDKLGDKYAC	WYQKPGQSPVLVIY	QDSKRPS	GIPERFSGNSGNTATLTISGTQAMDEADYYC	QAWDSSTA			
LBα6	ELVV-----	---R-E--IS	---RR-----L-	---I---	---T--I-----T-A-TL-----	-V-----DDHPHV	FGGTKVTVLGQP		
DPL-8	QSVLTQPPSVSGAPGQRTVISC	TGSSSNIAGAGYDVH	WYQLPGTAPKLLIY	GNSNRPS	GVPDRFSGSKSGTSASLAITGLQAEDEADYYC	QSYDSSLSG			
LTMα35	-----V--	-----N	-----	---N---	-----	-----AVV	FGGTKLTVLGQP		

* FR = framework; CDR = complementary determining region. The sequence data are available from the EMBL Gen bank under the numbers AJ276097 and AJ276098 for LTMα15 heavy and light chains, respectively; also AJ276099 and AJ276100 for LTMα35 heavy and light chains, respectively.

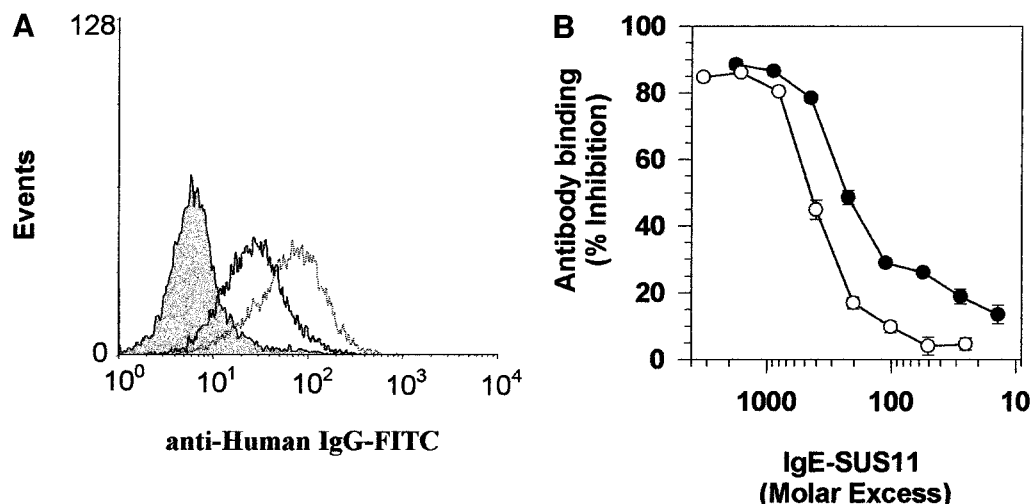


Figure 1. A) FACS staining of Fc ϵ RI α -transfected CHO cells with full-length IgG of LTM α 15 (solid line) and LTM α 35 (dashed line). Human monoclonal anti-Tetanus toxoid antibody ST-18 was used as an isotype control (gray shaded). B) Dose-dependent inhibition of binding of anti-Fc ϵ RI α antibodies LTM α 15 (filled symbols) and LTM α 35 (open symbols) to the Fc ϵ RI α with different amounts of IgE-SUS11. Antibody binding to Fc ϵ RI α was detected with FITC-conjugated sheep anti-human IgG.

these findings by the isolation from an IgM library of the first recombinant human natural anti-Fc ϵ RI α autoantibodies, LTM α 15 and LTM α 35. Moreover, functional studies with these antibodies have uncovered a novel mechanism of histamine release.

These autoantibodies were isolated from a nonimmune library and sequence analysis revealed germline VH paired with a slightly mutated VL, thus supporting their classification as natural antibodies (17). Until now, it has been assumed that natural autoantibodies

are nonpathogenic under physiological conditions for a variety of reasons, including low-affinity reactions and their role in establishing V region-mediated networks (18). However, our results indicate that natural autoantibodies can have a high affinity. Removal of IgE from freshly isolated human basophils resulted in their activation by anti-Fc ϵ RI α autoantibodies, followed by mediator release. Readdition of IgE prevented the autoantibody-induced histamine release. The same activity pattern was also demonstrated with affinity-puri-

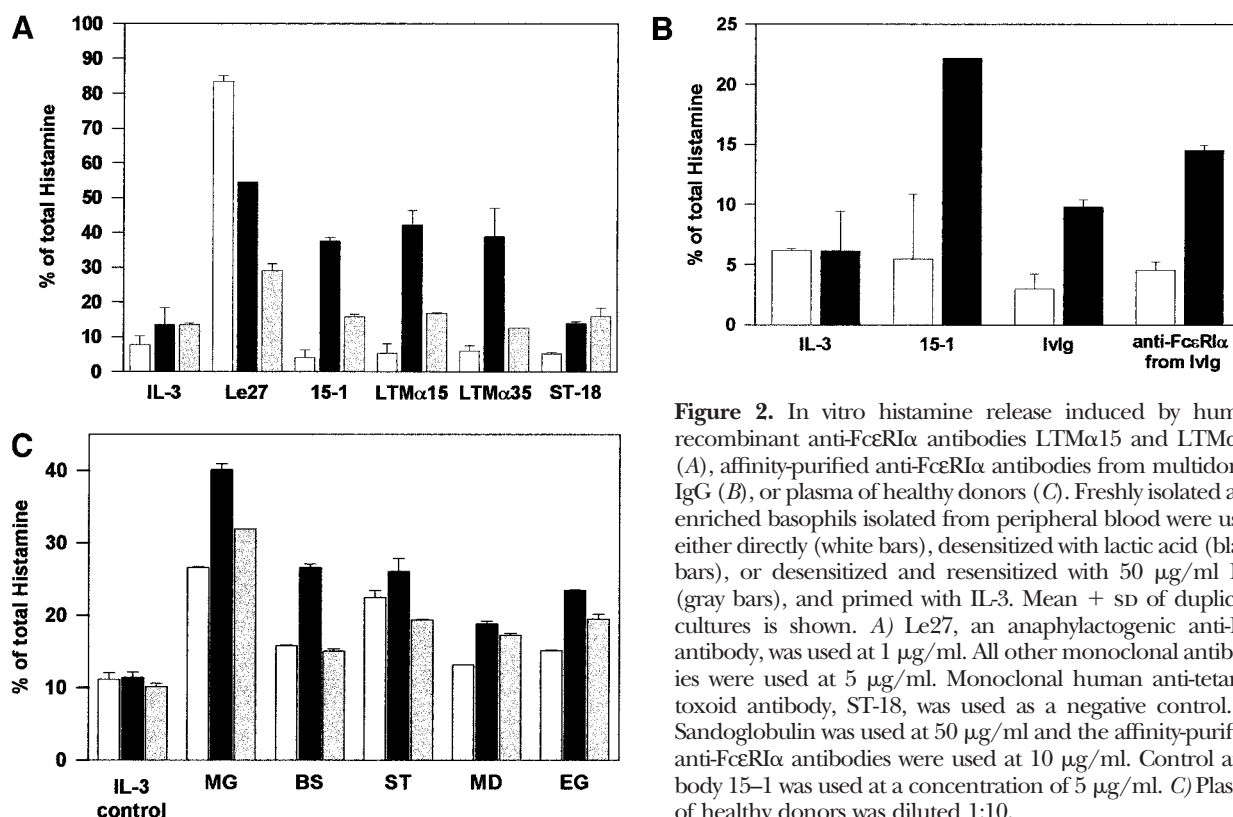
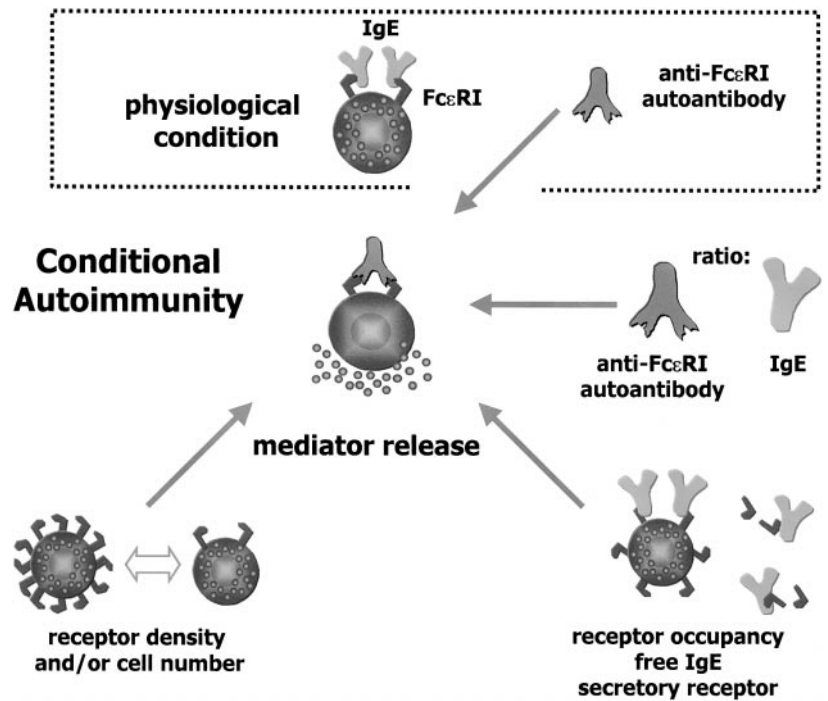


Figure 2. In vitro histamine release induced by human recombinant anti-Fc ϵ RI α antibodies LTM α 15 and LTM α 35 (A), affinity-purified anti-Fc ϵ RI α antibodies from multidonor IgG (B), or plasma of healthy donors (C). Freshly isolated and enriched basophils isolated from peripheral blood were used either directly (white bars), desensitized with lactic acid (black bars), or desensitized and resensitized with 50 μ g/ml IgE (gray bars), and primed with IL-3. Mean + sd of duplicate cultures is shown. A) Le27, an anaphylactogenic anti-IgE antibody, was used at 1 μ g/ml. All other monoclonal antibodies were used at 5 μ g/ml. Monoclonal human anti-tetanus toxoid antibody, ST-18, was used as a negative control. B) Sandoglobulin was used at 50 μ g/ml and the affinity-purified anti-Fc ϵ RI α antibodies were used at 10 μ g/ml. Control antibody 15-1 was used at a concentration of 5 μ g/ml. C) Plasma of healthy donors was diluted 1:10.

Figure 3. Possible mechanisms contributing to 'conditional autoimmunity'. The dotted box represents the physiological condition: the FcεRIα is occupied by IgE, thus blocking access of the natural anti-FcεRIα antibodies. Conditional autoimmunity results from a change in the receptor occupancy by IgE. When the FcεRIα is not covered by IgE, the anti-FcεRIα antibodies are free to bind and cause mediator release (arrow from top right). Clockwise, different situations could influence receptor occupancy: an imbalance between the level of anti-FcεRIα antibodies and IgE could result in insufficient cell-bound IgE (arrow from middle right); receptor occupancy could be influenced by the amounts of free IgE bound to secreted FcεRIα chain (arrow from bottom right); or receptor density may be up- or down-regulated by levels of serum IgE and/or actual numbers of cells, and therefore receptors may be increased—for example, during an ongoing allergic inflammation (arrow from bottom left).



fied anti-FcεRIα autoantibodies isolated from multidonor IgG preparations and serum from individual nonatopic donors, indicating that the antibodies isolated by phage display are representative of those present in the serum of healthy donors. We recently isolated, from a chronic idiopathic urticaria library, an IgM anti-FcεRIα autoantibody with 100% sequence homology to the LTMα15 antibody described in this paper (J. M. Pachlopnik et al., unpublished results). We also reported the presence of IgM anti-FcεRIα autoantibodies in human cord blood (3). Other types of anti-FcεRIα autoantibodies may exist with respect to either isotype or function.

Based on our results, we propose a hypothesis for the pathogenic role of anti-FcεRIα autoantibodies in urticaria whereby the critical and central event is the state of occupancy of the FcεRI by its natural ligand IgE. The interplay between numbers of FcεRI, levels of free vs. receptor-bound IgE, levels of free vs. bound anti-FcεRIα autoantibodies, and interference by the secreted soluble FcεRIα (19) may culminate in a disturbance of this receptor–ligand equilibrium and be responsible for the manifestation of urticaria. **Figure 3** shows some of the variables responsible for this receptor–ligand equilibrium.

This hypothesis may offer a unifying concept for other forms of 'nonimmune' urticaria where a triggering event is known, such as cold, heat, sun, pressure, and emotional stress, but the pathogenic mechanism is unknown. Any trigger resulting in an altered local blood supply or change in local concentrations of chemokines may upset the homeostasis between receptor and ligand either temporarily or for longer periods, resulting in some form of urticaria.

It has been shown that FcεRIα receptor up-regula-

tion occurs upon allergen challenge, which may leave the naked, newly expressed FcεRI temporarily unoccupied and allow access to the conditional anti-FcεRIα autoantibodies, which may then play a role in the allergic late phase reaction (20, 21).

The local appearance of hives (typical urticaria symptom) in some patients at the injection site of the therapeutic anti-IgE antibody (known as E25, Xolair®, and Omalizumab Novartis, currently in clinical studies for atopic conditions) may be another example of a temporary imbalance between the FcεRI and its occupancy by IgE. Recent clinical evaluation of Xolair® has shown that it binds soluble IgE (thus reducing the amount of IgE available to occupy the FcεRI) and down-regulates the FcεRI density on basophils and mast cells (22, 23).

In summary, we show that anti-FcεRIα autoantibodies are part of the natural antibody repertoire as they can be cloned from normal donors and are essentially germline. In vitro, we have demonstrated a conditional autoreactivity of these antibodies dependent on their ability to gain access to the FcεRI. We propose that such antibodies may become pathogenic because of a change in the FcεRI occupancy by IgE. This may be temporary or chronic, local or systemic, and initiated by a variety of different stimuli. Further, we suggest that the concept of conditional autoimmunity could be of more general relevance in similar situations where antigens are effectively masked by saturating amounts of ligands. **[F]**

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