

Autoimmune Urticaria

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Abstract

That a subset of patients with chronic idiopathic urticaria possesses functional autoantibodies against the high affinity IgE receptor, or less commonly, IgE, is widely accepted. That these same autoantibodies are causative of chronic urticaria is highly probable, but not entirely proven, since no animal model of chronic urticaria has been devised using these autoantibodies. However we know that intradermal injection of anti-FcεR1 IgG in healthy volunteers does cause an urticarial reaction. The concept of chronic urticaria as a disease caused by autoantibodies activating the normal function of target cells (mast cells) by combination with a receptor epitope is intriguing, although not entirely unique, since a similar mechanism appears to underlie some types of autoimmune hyperthyroidism. The detection of patients with these antibodies is usually not possible by clinical assessment alone. Unfortunately no convenient or reliable immunoassays have been developed to detect the autoantibodies, and the gold standard remains the basophil mediator release assay, using normal human donor basophils or a basophil leukemia cell line. The autologous serum skin test has proved to be a useful screening test for autoimmune urticaria. Identification of patients with autoimmune urticaria is of some importance because, apart from obviating the necessity for irrelevant tests, immunotherapy, using cyclosporin, intravenous immunoglobulin or even plasmapheresis can be contemplated in severely affected treatment-resistant patients.

Index Entries: Urticaria; autoantibodies; basophils; histamine; immunotherapy; cyclosporin.

Introduction

We first published evidence that the cause of itching and wheals in some patients with chronic "idiopathic" urticaria (CIU) may be attributable to histamine-releasing autoantibodies in 1993 (1). Subsequently these findings have been confirmed and extended by several indepen-

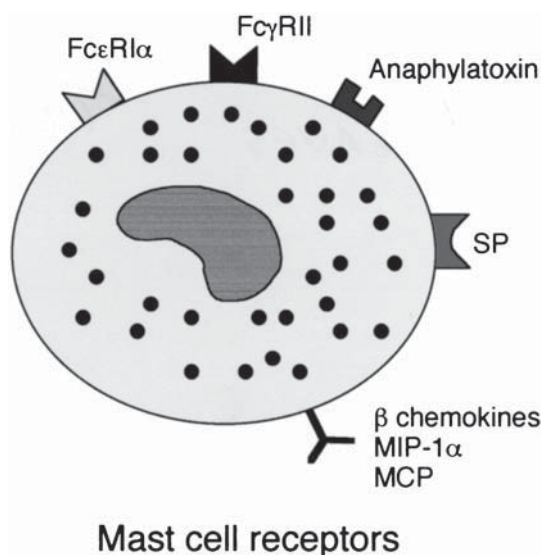


Fig. 1. SP: substance P.

dent groups (2–4) as well as our own (5–8). In particular, the immunopathology and specificity of autoantibodies in CIU has attracted considerable interest over the past few years. After briefly reviewing our early observations, it seems opportune to evaluate critically new information on this common and disabling disease and its impact, if any, on diagnosis and treatment.

Early Clues

In 1974 (9), studies of anti-IgE-evoked histamine release from basophil leukocytes revealed that cells obtained from patients with CIU yielded reduced histamine release as compared to cells from otherwise similar individuals who did not have CIU. The significance of this finding, which suggested desensitization of the basophil leukocytes by a circulating factor in CIU, was not appreciated at the time. Subsequent studies (6,10) have confirmed and extended this observation, and have shown a reduction in basophil numbers as well as reduced anti IgE-evoked histamine release in some patients with CIU. Histamine release evoked from these desensitized basophils by agents which act independently of the high-affinity IgE receptors (FcεRI) or IgE was not reduced, suggesting that the desensitization was specific to this receptor. Some of the receptors expressed on mast cells with ligation leading to activation are shown in Fig. 1. Indirect evidence includes the well-recognized observation of an increased frequency of thyroid autoantibodies and autoimmune thyroid disease in patients with CIU (11)—a fact we have recently confirmed (7,12).

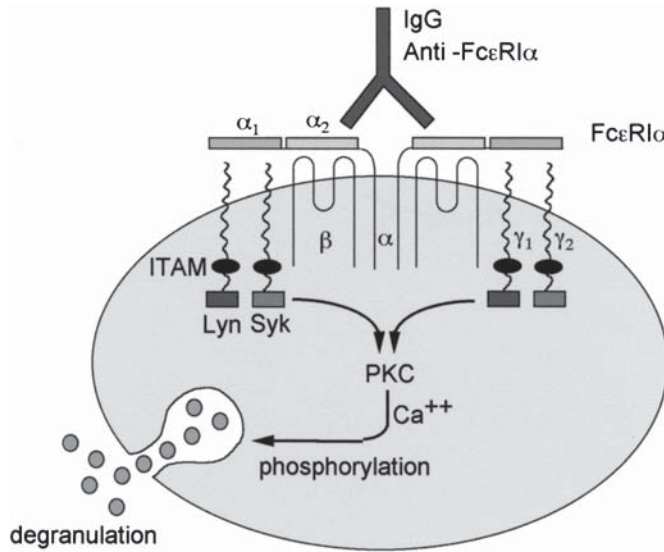


Fig. 2. ITAM: immunoreceptor tyrosine kinase activation motif. PKC: protein kinase C.

An interesting study by Kaplan's group (13) reported finding functional anti-IgE autoantibodies in some patients with physical urticarias, urticarial vasculitis, and CIU. In some cases, the sera of these patients released histamine from normal basophils. The anti-IgE autoantibodies in CIU were of the IgG isotype (13).

We and other authors have approached the problem from a different perspective with the finding that serum from certain CIU patients causes a wheal-and-flare reaction upon autologous intradermal injection (14–16). A positive reaction proved specific to CIU, occurring in about 60% of patients, and was not found in other inflammatory dermatoses such as atopic eczema and physical urticarias, or in healthy individuals (15,16). A number of candidate mediators have been considered to explain positive autologous serum skin test (ASST) reactions in CIU. These include cytokines (17,18) and anti-IgE (19). However further investigation revealed that in most cases in which the circulating wheal-producing factor could be characterized, it proved to be an antibody with specificity against the high-affinity IgE receptor expressed by basophil leukocytes and mast cells (1).

Evidence for an Autoimmune Mechanism in the Pathogenesis of CIU

We studied 26 patients with CIU—all with a positive autologous serum intradermal skin test (ASST) (16), and 10 healthy control sub-

jects (1). The serum of 17 of the 26 CIU patients, but not the serum of controls, released significant amounts of histamine from the basophils of one or both of two healthy donors. The basophils of donor 1 expressed little or no basophil-bound IgE; those of donor 2 expressed substantial amounts of IgE. The serum of 5 of the 26 patients released histamine from the cells of donor 2, but not donor 1, and therefore was considered to contain anti-IgE autoantibodies predominantly. The characteristics of histamine release from the two donors' basophils by the sera of the remaining 12 patients suggested histamine-releasing activity distinct from anti-IgE. The sera from four of these was studied in detail (1). The histamine-releasing activity of these sera was heat-stable, suggesting that it did not involve complement activation. Furthermore, it was enhanced by removal of IgE from the basophils of donor 2 using lactic acid "stripping," and abolished by resensitization of these cells by IgE, further indicating that the four patients' sera released histamine independently of IgE. Evidence that the histamine-releasing activity of the four sera involved reaction with the alpha-chain of the high-affinity IgE receptor ($Fc\epsilon RI\alpha$) was obtained by inhibition experiments utilizing human soluble recombinant alpha-chain ($sFc\epsilon RI\alpha$). Preincubation of the four sera with $sFc\epsilon RI\alpha$ caused concentration-dependent inhibition of histamine release from donor 1 basophils. The previously described histamine-releasing activity was caused by the IgG isotype, as confirmed by protein G chromatography. In all these characteristics, the four patients' sera mimicked the action of 6F7—a monoclonal antibody (MAb) that releases histamine by crosslinking adjacent $Fc\epsilon RI$ via the IgE-binding sites of $Fc\epsilon RI\alpha$ (Fig. 2). Thus, it was concluded that the histamine-releasing activity of the four CIU sera was caused by an IgG autoantibody in competition with IgE, and therefore presumably reacting with the IgE-binding site of $Fc\epsilon RI\alpha$ (1).

Our studies indicated that the sera of other patients would release histamine equally well from the untreated basophils of both donors, suggesting that in these patients the anti- $Fc\epsilon RI\alpha$ autoantibodies are not competitive with IgE, and therefore bind to the non-IgE-binding domain of the alpha chain. We concluded that anti- $Fc\epsilon RI\alpha$ autoantibodies were a major cause of histamine-release whealing and itching in some patients with CIU (1).

That these autoantibodies were pathogenic rather than irrelevant epiphenomena is suggested by: (1) their high potency in releasing histamine from human basophils and mast cells; (2) the absence of similar autoantibodies in the sera of healthy controls; (3) evidence of reduced basophil numbers and reduced anti-IgE-evoked histamine releasability in CIU; (4) (6,9,10), clinical improvement in parallel with reduced autoantibody levels, after plasmapheresis (20), and intravenous immunoglobulin (IVIG) infusions (21).

These observations were extended in further studies (5) involving an increased number of 165 patients with CIU. In these sera from 43 (26%) were demonstrated to contain anti-Fc ϵ RI autoantibodies, and 8 others (5%) showed predominantly anti-IgE autoantibodies. In these studies, we also confirmed the ability of anti Fc ϵ RI autoantibodies to release histamine from human dermal mast cells. Using sera from 12 CIU patients, we demonstrated net releases ranging from 4% to 34%, utilizing mast cells in incubated human skin slices. Protein G chromatography and inhibition experiments utilizing human recombinant sFc ϵ RI α confirmed that this mast-cell-directed histamine-releasing activity was indeed caused by IgG autoantibodies directed against the alpha chain of Fc ϵ RI. These findings further supported the view that, at least in some patients, CIU is an autoimmune disease dependent upon the actions of functional anti Fc ϵ RI α , or, less often, anti-IgE antibodies.

Methodology for Assay of Anti-Fc ϵ RI and Anti-IgE Autoantibodies

A functional indirect method—the assay of histamine released from donor basophils by the CIU patients' sera or purified IgG—represents the “gold standard” for detection of anti-Fc ϵ RI or anti-IgE autoantibodies. This assay is reproducible when basophils from the same donors are consistently used. We used well-characterized donor basophils from two healthy individuals with a low and a higher serum IgE, respectively. The washed basophils of the latter but not the former released substantial amounts of histamine upon challenge by anti-IgE. The MAb 6F7, which competes with IgE for its alpha-chain-binding site, released from the low—but not the high—IgE basophils. Another MAb, 29C6, which binds to the non-IgE binding domain of the alpha chain, released equally well from basophils of both donors. Human mast cells can also be used as an alternative to basophils (5), but are less freely available, less reliable, and yield lower histamine releases. Rat basophil leukemic cell lines, transfected with the genes encoding for the alpha and gamma chains of Fc ϵ RI, do express functional receptors, and can be used in a functional assay for anti-Fc ϵ RI autoantibodies (4). In this assay, the release of beta-hexosaminidase represents the indicator for a positive test.

Direct immunoassays have also been widely used. These assays detect both non-functioning and functional anti Fc ϵ RI autoantibodies. Methods used include semi-quantitative immunoblotting (4,22), immunoprecipitation, and ELISA (22). The latter has not been fully characterized by demonstration of dose-response relationships, and is prone to poor specificity because of nonspecific binding. Currently, work is in progress in our laboratories to develop and refine this immunoassay.

Incidence of Anti-Fc ϵ RI and Anti-IgE Autoantibodies in CIU

Using a basophil histamine-release assay, we (1,5,7) demonstrated that 24% of CIU patients possessed anti-Fc ϵ RI α autoantibodies, and that 31% of these were competitive with IgE. About 5% of CIU patients had anti-IgE autoantibodies. Subsequently, other investigators also used the basophil histamine-release assay to detect histamine-releasing autoantibodies in CIU. Zweiman (3) detected histamine releasing activity against basophils from a panel of normal donors in the sera of 30% of 70 CIU patients. It was also observed that the response of basophils to these sera was in broad proportion to disease activity. This activity was shown to be located in the IgG fraction, and was demonstrable using basophils from which IgE had been removed by lactic-acid stripping. Although no confirmatory inhibition experiments were done using the human recombinant soluble alpha chain, it seems likely that these authors were observing histamine release evoked by anti-Fc ϵ RI α autoantibodies. Kaplan's group (4) studied the sera of 50 patients with CIU and reported that 52% of these manifested histamine-releasing activity against donor basophils. They further investigated the effects of their sera on histamine release from rat basophil leukemia (RBL) cells which were transfected with genes encoding for Fc ϵ RI α , and found positive beta hexosaminidase release in 76% of these sera. This activity proved to be located in the IgG fraction. Since RBL cells not transfected with Fc ϵ RI α showed no release in response to CIU sera or IgG, the authors concluded that these sera contained IgG autoantibodies directed against Fc ϵ RI α . This conclusion was further confirmed by Western blotting, using purified sFc ϵ RI α . A subsequent report from the same group (23) compared functional and binding assays for anti-Fc ϵ RI in 68 patients with CIU. They tested these sera for histamine-releasing activity against both basophils and isolated human mast cells, and obtained significant histamine release in 48% and 46% of sera, respectively. These same sera showed positive evidence of anti Fc ϵ RI α autoantibodies in 64% by immunoblotting, suggesting the presence of non-functional anti Fc ϵ RI autoantibodies in some patients.

Fiebiger et al. (2) used recombinant sFc ϵ RI α as a target for Western blot studies in 32 patients with CIU. They found that 37% of sera contained IgG immunoreactivity against Fc ϵ RI α , and these results were confirmed by immunoprecipitation. They also noted that not all of the CIU sera that exhibited anti Fc ϵ RI α immunoreactivity also showed histamine-releasing (i.e., functional) activity. Thus figures for the frequency of anti-Fc ϵ RI α autoantibodies in CIU show reasonable agreement between different centers using different assays. These range from 25% to as many as 70%.

Table 1
Percentage Incidence of Anti-FcεR1 and IgE Autoantibodies in CIU

Authors	n	Anti-FcεR1	Anti-IgE	Assay
Greaves London	107	24	5	BHR
Zweiman Philadelphia	70	30	—	BHR
Kaplan S. Carolina	50	76	—	β-hex. rel. from RBL cell line
Stingl Vienna	32	37	69	Immuno-blotting

BHR: basophil histamine release. RBL: rat basophil leukemia.

Anti-IgE autoantibodies have also been reported in CIU by other groups. Fiebiger (2) found immunoreactive anti-IgE autoantibodies in 69% of CIU patients, but Kaplan’s group found this actively in only 6 of 50 patients (12%) (4). These figures compare with a 5% incidence in our own patients using a basophil histamine-release assay (5). The results of these studies are summarized in Table 1.

Disease Specificity of Anti-FcεRIα and Anti-IgE Autoantibodies

Sera of Healthy Individuals

Using the functional basophil histamine-release assay, we (24) found no evidence of anti-FcεRIα autoantibodies in the sera of any of 59 healthy individuals (24). Furthermore, sera from 22 healthy individuals did not release histamine from donor dermal mast cells (5). Using Western blotting, Fiebiger (2) found no evidence of immunoreactive anti-FcεRIα autoantibodies in the sera of 15 healthy controls, and these negative results were subsequently confirmed by ELISA in the sera of 41 healthy controls (22). Only two of 20 sera from healthy control subjects studied by Tong (4) released β-hexosaminidase from FcεRIα-transfected rat basophil leukemia cells, or from human donor basophil leukocytes. Similar negative results were later obtained by the same group in sera of 245 healthy controls (23). Recently immunoreactive, but non-histamine-releasing FcεRIα autoantibodies were reported in the sera of healthy donors as well as CIU patients using ELISA (25). Surprisingly, these antibodies crossreacted with tetanus toxoid antigen with greater affinity than with FcεRIα. Their significance awaits further characterization and study. These results are summarized in

Table 2
Disease Specificity of Anti-FcεR1 Autoantibodies

Authors	Incidence in healthy people	Assay
Greaves	0/59	BHR
London	0/22	DMC
Stingl	0/15	WB
Vienna	0/41	ELISA
Kaplan	2/20	β-Hexosaminidase: RBL cell line
S. Carolina	2/20	

BHR: basophil histamine release. RBL: rat basophil leukemia. WB: Western blotting. DMC: dermal mast cells.

Table 2. Clearly, anti-FcεRIα autoantibodies that are also functional (i.e., release histamine or show other signs of basophil or dermal mast-cell activation) are specific to CIU, and do not occur to a significant extent in the sera of healthy persons.

Wedi et al. (26) examined the effects of healthy control sera on donor atopic and non-atopic basophils using histamine-release, basophil CD63 expression and sulphidoleukotriene secretion as indicators of activation. Sera from 10 atopic and 10 non-atopic controls were studied. These sera triggered a mean histamine release of approximately 5% using atopic donor basophils, and no attempt was made to characterize the nature of this activity as immunoglobulin-related or reactive with alpha-chain or IgE. For these reasons and in view of the very low histamine releases involved, these data are difficult to interpret.

Sera of Patients with Diseases Other Than CIU

Further evidence that immunoreactive (non-histamine-releasing) anti-FcεRIα autoantibodies are not specific to CIU has emerged from the studies of Fiebiger et al. (22). Fiebiger used ELISA to screen sera from patients with various autoimmune diseases and obtained positive results in small numbers of patients with systemic lupus, dermatomyositis, pemphigus vulgaris, and bullous pemphigoid. Yet it is important to note that none of these patients had functional anti-FcεRIα autoantibodies, in contrast with sera from CIU patients.

We recently submitted 78 CIU, 15 physical urticaria, and 39 healthy control sera for analysis on a “blind” basis to autologous serum skin testing, histamine-release assays using healthy basophils and mast cells and Western blotting, in collaboration with the group of D. Maurer (Vienna). Results with these various methods showed excellent agreement in the CIU and healthy control groups, although there were a few weak false-positives in the physical urticaria group.

Specificity of Anti-IgE Autoantibodies

Immunoreactive anti-IgE autoantibodies have been detected in a number of clinical contexts other than CIU. These include urticarial vasculitis and cold urticaria (13), atopic dermatitis (27,28), and normal individuals (29). More recently, Fiebiger detected immunoreactive anti-IgE autoantibodies in 26% of healthy control sera, but these were not histamine-releasing (2). However, the histamine-releasing (i.e., functional) anti-IgE autoantibody has been reported only rarely (one serum from a cold urticaria patient (13), one of six patients with atopic dermatitis (30)).

As with anti-Fc ϵ RI α autoantibodies, it appears that when functional histamine-releasing anti-IgE autoantibodies are found in CIU, it is reasonable to suspect their involvement in the pathophysiology of the disease.

The Role of Complement in Autoimmune Urticaria

The role of complement remains controversial. Our early results (1) indicated that complement activation was not involved. Heating for 1 h at 56°C had no effect on the ability of four CIU sera to release histamine from donor basophils, and similar results were obtained with purified IgG. A subsequent study (5) of sera from 12 patients yielded less definitive results, and heat inactivation caused a reduction of about 20% in basophil histamine-releasing activity. However, heat-treated CIU sera still retained the ability to release histamine from donor basophils in our experience. A similar conclusion was reached by Zweiman following 1-h heat-inactivation experiments using sera from eight CIU patients. However, he found that heating the same sera for 4 h at 56°C did cause a 50% reduction in histamine-releasing capacity. These results, which suggest crosslinking of Fc ϵ RI by anti-Fc ϵ RI α autoantibodies, are sufficient to cause basophil or mast-cell activation, in contrast to those of other investigators. Fiebiger (22) found that 20 of 22 CIU sera were of the IgG1, or IgG3 subtypes, and these subtypes were associated characteristically with complement C5a activation. They also showed that inactivation of complement by heating (3 h at 56°C) greatly improved the capacity of those sera to release histamine, that histamine-releasing IgG from CIU patients reduced basophil anti-C5a-receptor immunoreactivity, and blockade of C5a receptor by a C5a-inhibitory peptide also resulted in reduced histamine release. Since the C5a receptor is expressed by dermal, but not lung mast cells (31), this conveniently explains the otherwise puzzling clinical observation that patients with clinically extensive autoimmune (anti-Fc ϵ RI α) urticaria show little or no clinical evidence of inflammatory or bronchoconstrictive lung disease. However, we have clearly shown that heat-inactivated anti-Fc ϵ RI α -

positive sera will release histamine from human lung as well as dermal mast cells (5). It seems probable that complement activation occurs in some but not all cases, determining factors possible including the population density of FcεRI expressed on the surface of target mast cells and basophils, and differences between the *in vivo* and *in vitro* situations.

Clinical Diagnosis

There are no distinctive clinical or histopathological features that enable autoimmune chronic urticaria to be distinguished from non-autoimmune types. We have found the autologous serum skin test (16,32) to be a useful screening test for autoimmune chronic urticaria. This test has recently been reviewed in detail (16). Fifty microlitres of undiluted autologous serum is injected intradermally into clinically uninvolved skin; simultaneously injected controls include saline and histamine 0.5 µg. The test is positive at 30 min if the serum-injected site manifests a red wheal with a diameter at least 1.5 mm greater than that of the saline wheal. Interestingly, the test is positive in 60% of CIU patients tested—but in our laboratory, only about half of these can be proven to have histamine-releasing autoantibodies against FcεRI or IgE. However, the test is consistently negative in healthy persons or patients with physical urticarias. Its positivity is also related to disease activity (32). That a positive autologous serum skin test can also be reproduced by passive transfer of serum by intradermal injection into a healthy human recipient has also been demonstrated (24). Histological examination of serial skin biopsies of a positive test reveals an initial invasion of injected skin by neutrophils and eosinophils, followed by mononuclear cells, resembling a classical “late phase” response to antigen-induced immediate hypersensitivity. No vasculitis occurred.

What is the significance of the small number of autologous serum skin-test-positive CIU patients in whom no autoantibodies can be detected by *in vitro* tests? Only speculative answers can be offered. We know that approximately 8–10% of CIU patients possess a circulating wheal-producing factor, which we have called a “mast-cell-specific factor” occurring in autologous serum skin-test-positive CIU patients. This factor, which releases histamine from human dermal or lung mast cells, but not from human basophils, possesses a mol wt of more than 30 kd, and was shown not to be an immunoglobulin (33). This subgroup of CIU patients has also been subsequently observed independently (23). Its identity and significance await full characterization. For the remainder, there is indirect evidence to suggest that many of these autologous serum skin-test-positive patients with negative *in vitro* evidence for an autoimmune basis may actually possess anti-FcεRI or anti-IgE autoantibodies. However, failure to detect them may be an expression of the

limitations of our in vitro basophil histamine-release assay. Sensitivity has been sacrificed in order to retain specificity. Our cut-off point is arbitrarily set at a net histamine release (after subtracting basal release) of 5%. Thus, a net histamine release of 4% would be deemed negative. Accordingly there are a number of CIU patients with positive autologous serum skin tests, with net releases of approximately 4%. That these may actually have autoimmune urticaria is suggested by the following indirect evidence:

- (i) the histological (34) and clinical (7) features of these patients are indistinguishable from patients with proven autoimmune urticaria;
- (ii) the peripheral-blood basophil count and histamine content of semi-purified basophil suspensions are reduced in both proven autoimmune and non-autoimmune groups;
- (iii) both categories may respond to treatment by plasmapheresis (20) and intravenous (iv) immunoglobulin (35).

At present, autologous serum skin testing, followed by confirmation of positive results by in vitro basophil histamine-release assay, remains the "gold standard" for diagnosis, but a considerable number of false-negative results may occur.

Therapeutic Implications

Non-specific immunotherapy using plasmapheresis (20), iv immunoglobulin (35), and cyclosporin (36) have proven of value in the treatment of severe antihistamine-resistant CIU. In the future, epitope mapping could lead to development of structure-based peptides which, if humanized, could be used for specific immunotherapy. Alternative approaches include use of monoclonal anti-IgE which has been demonstrated by MacGlashan to downregulate the population density of FcεRI on basophils and induction of tolerance by administration of the humanized α-chain of FcεRI.

Future Priorities

Improved cheap diagnostic laboratory tests are needed to enable rapid and accurate diagnosis of autoimmune urticaria. An improved ELISA at present being developed in collaboration with Pharmacia Inc, would be valuable although inherently prone to false-positive results. Flow cytometry, utilizing rat basophil leukemia or Chinese hamster ovary cell lines transfected with FcεRIα could be designed to identify functional as well as immunoreactive FcεRI. Such tests would also enable more accurate definition of the prevalence of anti-FcεRI autoantibodies in the population.

The aetiology of autoimmune urticaria is a challenging problem. We know that autoimmune urticaria is associated with increased frequency of certain HLA DR and DQ alleles (38). Additional triggering factors such as virus infections, adverse drug reactions, or other perturbations of the immune system may also be crucial. It is of interest that HLA class II antigens are expressed, although weakly, on the surface of mast cells (39). The close proximity of HLA class II antigens and the α -chain of Fc ϵ RI presents a scenario in which α -chain epitopes could be presented to the immune system. Alternatively, molecular mimicry—for example, by *Helicobacter pylori*, as recently proposed (40) may be important. Clearly, more work needs to be done in this fascinating area.

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