ORIGINAL ARTICLE

Autoantibodies to IgE can induce the release of proinflammatory and vasoactive mediators from human cardiac mast cells

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Abstract

Mast cells are multifunctional immune cells with complex roles in tissue homeostasis and disease. Cardiac mast cells (HCMCs) are strategically located within the human myocardium, in atherosclerotic plaques, in proximity to nerves, and in the aortic valve. HCMCs express the high-affinity receptor (FcεRI) for IgE and can be activated by anti-IgE and anti-FcεRI. Autoantibodies to IgE and/or FcεRI have been found in the serum of patients with a variety of immune disorders. We have compared the effects of different preparations of IgG anti-IgE obtained from patients with atopic dermatitis (AD) with rabbit IgG anti-IgE on the release of preformed (histamine and tryptase) and lipid mediators [prostaglandin D_2 (PGD_2) and cysteinyl leukotriene C_4 (LTC_4)] from HCMCs. Functional human IgG anti-IgE from one out of six AD donors and rabbit IgG anti-IgE induced the release of preformed (histamine, tryptase) and de novo synthesized mediators (PGD_2 and LTC_4) from HCMCs. Human IgG anti-IgE was more potent than rabbit IgG anti-IgE in inducing proinflammatory mediators from HCMCs. Human monoclonal IgE was a competitive antagonist of both human and rabbit IgG anti-IgE. Although functional anti-IgE autoantibodies rarely occur in patients with AD, when present, they can powerfully activate the release of proinflammatory and vasoactive mediators from HCMCs.

Keywords Autoantibodies · Heart · Histamine · Leukotriene C_4 · Mast cells · Prostaglandin D_2

Abbreviations

AA	Arachidonic acid
AD	Atopic dermatitis
BSA	Bovine serum albumin
$cPLA_2$	Cytosolic phospholipase A_2
CSU	Chronic spontaneous urticaria
FcεRI	High-affinity receptor for IgE
FCS	Fetal calf serum
H-aIgE	Human IgG anti-IgE
HCMC	Human cardiac mast cell
HR	Histamine release
Ig	Immunoglobulin
IgE	Immunoglobulin E
IL	Interleukin
LTA_4	Cysteinyl leukotriene A_4
LTC_4	Cysteinyl leukotriene C_4
LTD_4	Cysteinyl leukotriene D_4
LTE_4	Cysteinyl leukotriene E_4
MHC	Major histocompatibility complex
PGD_2	Prostaglandin D_2
PLA_2	Phospholipase A_2
r	Coefficient of correlation

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SEM   Standard error of the mean
TCR   T cell receptor
5-LO 5-Lipoxygenase

Introduction

Mast cells are immune cells present in most connective tissues where they play multifunctional roles in tissue homeostasis and disease [1, 2]. Mast cells have been identified in rodent [3–6], canine [7, 8], and human heart [6, 9–12]. Although mast cells are canonically considered primary effectors of allergic responses [13–17], these cells are critical sentinels in immunity [18, 19]. Mast cells have increasingly gained recognition in a variety of pathophysiological processes including infections [19–21], angiogenesis [22–26], lymphangiogenesis [22, 27], cardiometabolic diseases [9, 28–32], vasculitis [33], autoimmune disorders [34–36], and cancer [37–40].

Human mast cells display a complete ($\alpha\beta\gamma2$), high-affinity receptor (FcεRI) for immunoglobulin E (IgE) and cross-linking of the IgE-FcεRI network triggers the release of preformed (e.g., histamine, tryptase, chymase) and de novo synthesized lipid mediators [e.g., prostaglandin D_2 (PGD_2), cysteinyl leukotriene C_4 (LTC_4)] [41–43]. Mast cells form a highly heterogeneous population of immune cells [44]. In fact, there is marked heterogeneity of human mast cells with respect to the membrane receptors [45–47] and the mediators released from cells isolated from different anatomic sites [45–49].

Recent epidemiological studies have reported an increased risk of coronary artery disease and/or heart failure in patients with IgE-mediated allergic disorders [50–52]. Moreover, increased IgE levels are associated with atherosclerosis [53], and the IgE-FcεRI network has been implicated in pathological cardiac remodeling and dysfunction [54].

Several investigators have reported the presence of autoantibodies targeting either IgE [55–60], and FcεRI [61–64], or both in diverse allergic [55–59, 61, 63, 65–67] and autoimmune disorders [62, 68]. The majority of these studies evaluated the ability of autoantibodies to IgE and/or FcεRI from patients with chronic spontaneous urticaria (CSU) to activate human basophils [55, 56, 61–63]. These results do not exclude the possibility that some autoantibodies to IgE/FcεRI can activate human mast cells.

Activation of human cardiac mast cells (HCMCs) induces the release of preformed (e.g., histamine, tryptase) and de novo synthesized proinflammatory mediators (LTC_4, PGD_2) involved in several cardiovascular and metabolic disorders [1, 69]. For instance, histamine exerts profound cardiovascular effects in humans [70, 71], while tryptase mediates cardiac fibrosis [72, 73]. LTC_4, produced by HCMCs [10, 74],

is detrimental for atherosclerosis and myocardial infarction [75] and dilated cardiomyopathies [10]. In addition, PGD_2, the main cyclo-oxygenase product of HCMCs, negatively affects the cardiovascular and respiratory systems [69, 76] and modulates fibrosis [77].

In this study, we first examined the effects of functional and non-functional human IgG anti-IgE obtained from patients with atopic dermatitis (AD) on the release of preformed and de novo synthesized mediators from HCMCs. Second, we compared the effects of functional human IgG anti-IgE and rabbit IgG anti-IgE on the release of histamine and lipid mediators from cardiac mast cells. Finally, we evaluated whether human monoclonal IgE can antagonize the activating properties of human and rabbit IgG anti-IgE.

Materials and methods

Reagents and buffers

Antibiotic–antimycotic solution (10,000 IU penicillin, 10 mg/mL streptomycin, and 25 µg/mL amphotericin B), collagenase (Worthington Biochemical Co., Lakewood, NJ, USA), bovine serum albumin, human serum albumin, [piperazine-N,N'-bis (2-ethanesulfonic acid) (Pipes)], Hanks' balanced salt solution, fetal calf serum (FCS) (Thermo-Fisher, Grand Island, NY, USA), pronase, deoxyribonuclease I (Calbiochem, La Jolla, CA, USA), Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden), and CD117 MicroBead kit (Miltenyi Biotech, Bologna, Italy), $HClO_4$ (Baker Chemical Co., Deventer, Netherlands), hyaluronidase, chymopapain, elastase type I, cysteinyl leukotriene C_4 (LTC_4), and prostaglandin D_2 (PGD_2) (Sigma Chemical Co., St. Louis, MO), (^{3}H)-LCT_4 and (^{3}H)-PGD_2 (New England Nuclear, Boston, MA) were commercially purchased. Rabbit IgG anti-IgE antibody, produced by rabbit immunization with the Fc fragment of a human IgE myeloma (patient PS) and then absorbed with the IgE Fab, was kindly donated by Kimishige and Teruko Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA) [78]. Rabbit anti-LTC_4 and anti-PGD_2 antibodies were kindly donated by Lawrence M. Lichtenstein (The Johns Hopkins University, Baltimore, MD). The Pipes (P) buffer was made by 25 mM Pipes, 110 mM NaCl, 5 mM KCl, pH 7.37, and referred to as P. P2CG, contains, in addition to P, 2 mM $CaCl_2$ and 1 g/L dextrose [9].

Atopic dermatitis patients

This study was approved by the Ethics Committee of the University of Naples Federico II, School of Medicine (Protocol N. 198/18), and informed consent was obtained from participants prior to the collection of blood specimens

according to recommendations from the Declaration of Helsinki. Peripheral blood was obtained from six AD patients (aged 5–17 years) with similar clinical pictures (e.g., chronic pruritic skin erythema, papules, or lichenification of flexural areas of the extremities, face and neck) [79]. Blood samples were obtained from these patients not taking any drug for at least 1 week.

Purification of human IgG anti-IgE antibody

Serum from six AD patients and comparable high levels of IgG antibodies to anti-IgE were passed through the immunosorbent Sepharose column coated with purified IgE (ADZ). Immunosorbent-bound IgG with anti-IgE activity were collected. IgE content was less than 0.05 U/ml [59].

Purification of human monoclonal IgE and polyclonal IgG

IgE myeloma protein was purified from a myeloma patient (ADZ) as previously described [80–82]. No IgG, IgM, or IgA contamination was detected by immunoassays [83]. Human polyclonal IgG were purified from the serum of five healthy donors and two patients with AD as previously described [81, 84].

Isolation of human cardiac mast cells

This study was approved by the Ethics Committee of the University of Naples Federico II, School of Medicine (Protocol N. 7/19). Heart tissue was obtained from patients undergoing heart transplantation as previously described [31, 74]. The explanted heart was finely minced into 2–5 mm fragments and subjected to enzymatic dispersion [31]. HCMCs were partially purified by flotation through a discontinuous Percoll gradient yielding a population of mast cell purity ranging from 0.3% to 26%. HCMCs were further purified using a CD117 MicroBead kit sorting system (Miltenyi Biotec, Bologna, Italy). Mast cell purity using these techniques ranged from 29 to 61% as assessed by Alcian blue staining [85].

Mediator release from human cardiac mast cells

HCMCs ($\approx 3 \times 10^4$ mast cells per tube) resuspended in P2CG buffer were placed in 12×75 mm polyethylene tubes. Then, 0.2 ml of each prewarmed releasing stimulus was added and incubation was continued at 37° C for 45 min [86, 87]. At the end of incubation, cells were centrifuged ($1000 \times g$, 4 °C, 5 min) and supernatants were stored at $-20°$ C for subsequent assay of histamine, tryptase, LTC_4, and PGD_2 content [87, 88].

Immunoassay of tryptase

Tryptase concentration was measured by fluoroenzyme immunoassay (FEIA) using Uni-CAP100 (Phadia Diagnostics AB, Uppsala, Sweden) as previously described [89]

Immunoassay of LTC_4 and PGD_2

LTC_4 and PGD_2 were measured by radioimmunoassay [86, 90]. The anti-LTC_4 and anti-PGD_2 antibodies are highly selective, with less than 1% cross-reactivity to other eicosanoids [90, 91].

Statistical analysis

Data were analyzed with the GraphPad Prism 8 software package (GraphPad Software, La Jolla, CA, USA). Values are expressed as mean $\pm$ SEM (standard error of the mean). Statistical analysis was performed using Student's t-test or one-way analysis of variance [92]. Correlations between two variables were assessed by Spearman's rank correlation analysis and reported as coefficient of correlation (r). Values of $p \leq 0.05$ were considered significant.

Results

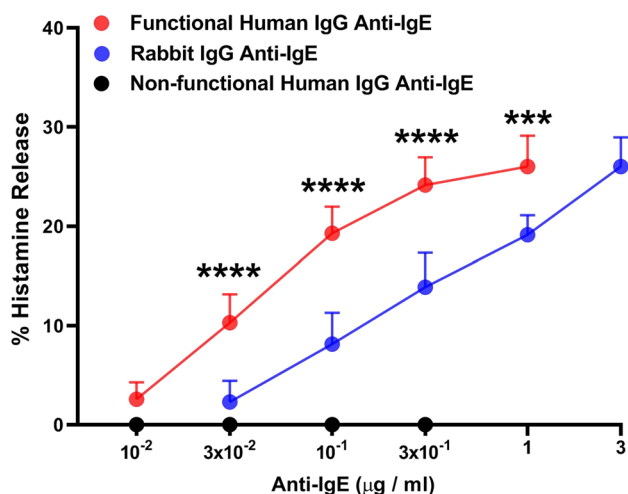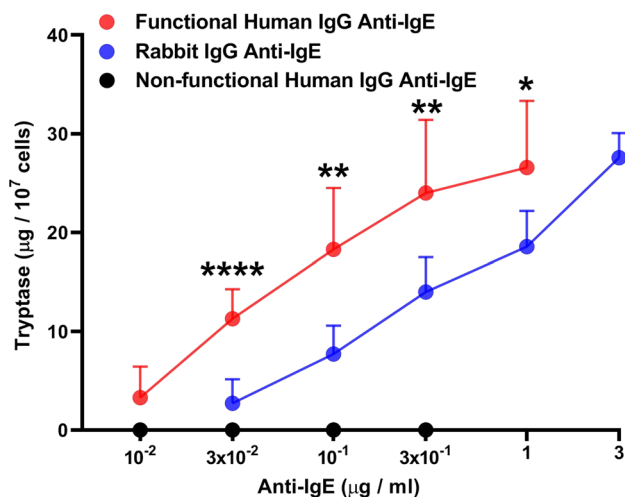## Effects of human and rabbit IgG anti-IgE on histamine release from HCMCs

We first compared the effects of increasing concentrations of IgG anti-IgE purified from the sera of six patients with AD, and rabbit IgG anti-IgE on the release of histamine from seven different preparations of HCMCs. Figure 1 shows that one preparation of human IgG anti-IgE (10^{-2} to 1 µg/ml) isolated from AD patient [58] triggered histamine release from HCMCs from seven different donors. By contrast, five preparations of IgG anti-IgE isolated from different AD patients did not induce histamine release from HCMCs. In the same experiments, we also evaluated the effects of rabbit IgG anti-IgE (3×10^{-2} to 3 µg/ml), which also induced a concentration-dependent release of histamine (Fig. 1). The maximal percent histamine release (HR$_{MAX}$) of HCMC response to functional human anti-IgE ($26.6\% \pm 1.15\%$) was similar to mast cell reactivity to rabbit anti-IgE ($26.0\% \pm 1.11\%$) (Table 1). By contrast, the threshold sensitivity (HR$_{SENS}$) [i.e., the secretagogue concentration inducing half-maximal histamine release (EC$_{50}$)] induced by functional human anti-IgE ($4.2 \times 10^{-2} \pm 5 \times 10^{-3}$ µg/ml) was lower than HR$_{SENS}$ caused by rabbit anti-IgE ($4.6 \times 10^{-1} \pm 5 \times 10^{-2}$ µg/ml) ($p < 0.005$) (Table 2). These results indicate that a

Table 1 Maximal mediator release induced by human IgG anti-IgE and rabbit IgG anti-IgE from human cardiac mast cells

	Human IgG anti-IgE	Rabbit IgG anti-IgE	p value
Maximal percent histamine release	26.57 ± 1.15	26.0 ± 1.11	$p > 0.05$
Maximal tryptase release ($\mu g/10^7$ cells)	28.14 ± 2.24	27.57 ± 0.94	$p > 0.05$
Maximal LTC_4 release ($ng/10^6$ cells)	61.43 ± 2.97	59.4 ± 3.67	$p > 0.05$
Maximal PGD_2 release ($ng/10^6$ cells)	58.4 ± 2.48	62.8 ± 3.10	$p > 0.05$

Table 2 Concentrations of human IgG anti-IgE and rabbit IgG anti-IgE inducing half-maximal (EC_{50}) mediator release from human cardiac mast cells

	Human IgG anti-IgE	Rabbit IgG anti-IgE	p value
EC_{50} histamine release ($\mu g/ml$)	$4.2 \times 10^{-2} \pm 5 \times 10^{-3}$	$4.6 \times 10^{-1} \pm 5.2 \times 10^{-2}$	$p = 0.0013$
EC_{50} tryptase release ($\mu g/ml$)	$5.8 \times 10^{-2} \pm 1.2 \times 10^{-2}$	$3 \times 10^{-1} \pm 3.3 \times 10^{-2}$	$p < 0.0001$
EC_{50} LTC_4 release ($\mu g/ml$)	$6.7 \times 10^{-2} \pm 1.4 \times 10^{-2}$	$4.7 \times 10^{-1} \pm 3.6 \times 10^{-2}$	$p = 0.0002$
EC_{50} PGD_2 release ($\mu g/ml$)	$5.2 \times 10^{-2} \pm 6.8 \times 10^{-3}$	$2.7 \times 10^{-1} \pm 5.6 \times 10^{-2}$	$p = 0.002$

Fig. 1 Effects of increasing concentrations of functional human IgG anti-IgE (red), rabbit IgG anti-IgE (blue), and non-functional human IgG anti-IgE (black) on histamine release from human cardiac mast cells (HCMCs) obtained from seven different donors. HCMCs were incubated (45 min at 37 °C) in the presence of the indicated concentrations of human or rabbit IgG anti-IgE. Each point represents the mean ± SEM obtained from different preparations of HCMCs. Error bars are not shown when graphically too small. ****$p < 0.001$; ****$p < 0.0001$ when compared to the corresponding value of rabbit IgG anti-IgE

Fig. 2 Effects of increasing concentrations of functional human IgG anti-IgE (red), rabbit IgG anti-IgE (blue) and non-functional IgG anti-IgE (black) on tryptase release from human cardiac mast cells (HCMCs) obtained from seven different donors. HCMCs were incubated (45 min at 37 °C) in the presence of the indicated concentrations of human or rabbit IgG anti-IgE. Each point represents the mean ± SEM obtained from different preparations of HCMCs. Error bars are not shown when graphically too small. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$ when compared to the corresponding value of rabbit IgG anti-IgE

preparation of human IgG anti-IgE (hereafter referred to as "human anti-IgE") is more potent than rabbit IgG anti-IgE in inducing histamine release from HCMCs.

Effects of human and rabbit IgG anti-IgE on tryptase release from HCMCs

HCMCs contain and immunologically release tryptase [31, 74], which is involved in cardiac fibrosis [72, 73] and myocardial infarction [30]. Figure 2 shows that human anti-IgE (10^{-2} to 1 $\mu g/ml$) concentration-dependently induced tryptase release from HCMCs. In the same experiments, higher concentrations (3×10^{-2} to 3 $\mu g/ml$) of rabbit IgG anti-IgE also caused tryptase secretion. By contrast, five preparations of non-functional IgG anti-IgE did not induce the release of tryptase. The maximal tryptase release induced by human anti-IgE from HCMCs was similar to that caused by rabbit IgG anti-IgE (Table 1). By contrast, the EC_{50} caused by human anti-IgE ($5.8 \times 10^{-2} \pm 1.2 \times 10^{-2}$ $\mu g/ml$) was significantly lower than of rabbit IgG anti-IgE ($3 \times 10^{-1} \pm 3.3 \times 10^{-2}$ $\mu g/ml$) ($p < 0.0001$) (Table 2).

Effects of human and rabbit IgG on lipid mediators from HCMCs

Cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4) are derived from arachidonic acid (AA) through the 5-lipoxygenase (5-LO) pathway [41]. Upon cell activation, cytosolic phospholipase A_2 ($cPLA_2$) cleaves phospholipids at the outer nuclear membrane to generate free AA. 5-LO then oxidizes AA in the presence of 5-LO activating protein to generate leukotriene A_4 (LTA_4), which is subsequently converted to LTC_4 by LTC_4 synthase [41, 93]. Activated HCMCs metabolize AA through the 5-LO to form LTC_4 and through the cyclo-oxygenase to form PGD_2 [31, 74]. In seven experiments, we compared the effects of human anti-IgE (3×10^{-2} to 1 µg/ml) and rabbit anti-IgE (3×10^{-2} to 3 µg/ml) on the de novo synthesis of LTC_4 and PGD_2 from HCMCs. Figure 3A shows that both human and rabbit anti-IgE caused a concentration-dependent release of LTC_4 from HCMCs. The maximal LTC_4 release induced by human anti-IgE was similar to that caused by rabbit anti-IgE (Table 1). However, the LTC_4 release induced by each concentration of human anti-IgE tested was significantly higher than that caused by rabbit anti-IgE (Fig. 3A). Accordingly, the EC_{50} for LTC_4 release was significantly lower for human anti-IgE ($6.7 \times 10^{-2} \pm 1.4 \times 10^{-2}$ µg/ml) compared to rabbit anti-IgE ($4.7 \times 10^{-1} \pm 3.6 \times 10^{-2}$ µg/ml) ($p < 0.0002$) (Table 2). Similar results were obtained when we compared the effects of increasing concentrations of human and rabbit anti-IgE on PGD_2 release from HCMCs (Fig. 3B, Tables 1 and 2).

Effects of human polyclonal IgG on mediator release from HCMCs

In these experiments, we evaluated the effects of increasing concentrations of human polyclonal IgG purified from five healthy donors on the release of preformed (histamine, tryptase) and de novo synthesized mediators (LTC_4, PGD_2) from HCMCs. Figure 4 shows the results of these experiments indicating that a wide spectrum of concentrations (10^{-2} to 10 µg/ml) of human polyclonal IgG failed to induce the release of proinflammatory and vasoactive mediators from HCMCs. Similar results were obtained when human polyclonal IgG purified from AD patients were incubated with HCMCs (data not shown).

Correlations between mediator release induced by human and rabbit IgG anti-IgE from HCMCs

Figure 5A shows that there was a positive correlation between the release of two mediators (histamine and tryptase), which specifically reside in the secretory granules of human mast cells, induced by human anti-IgE from HCMCs ($r = 0.79$; $p < 0.0001$). These results suggest that
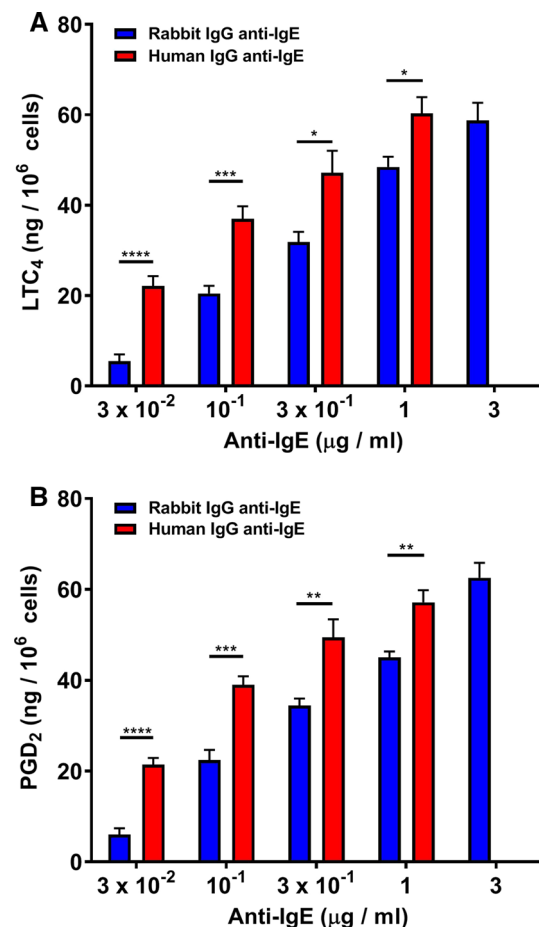

Fig. 3 Effects of increasing concentrations of human IgG anti-IgE (■) and rabbit IgG anti-IgE (■) on the de novo synthesis of cysteinyl leukotriene C_4 (LTC_4) (**A**) and prostaglandin D_2 (PGD_2) (**B**) from human cardiac mast cells (HCMCs) obtained from seven different donors. HCMCs were incubated (45 min at 37 °C) in the presence of the indicated concentrations of human or rabbit IgG anti-IgE. Each bar shows the mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ when compared to the corresponding value

these cells are a source of both mediators in the supernatants of anti-IgE-activated HCMCs. Similarly, there was a positive correlation between the release of histamine and LTC_4 ($r = 0.89$; $p < 0.0001$) (Fig. 5B), histamine and PGD_2 ($r = 0.83$; $p < 0.0001$) (Fig. 5C), and LTC_4 and PGD_2 ($r = 0.83$; $p < 0.0001$) (Fig. 5D). Comparable results were obtained when we examined the correlations between the release of different mediators induced by rabbit anti-IgE from HCMCs (Fig. 6).

Effects of human monoclonal IgE on human or rabbit anti-IgE-induced histamine release from HCMCs

The previous results are compatible with the hypothesis that human and rabbit anti-IgE induce the release of
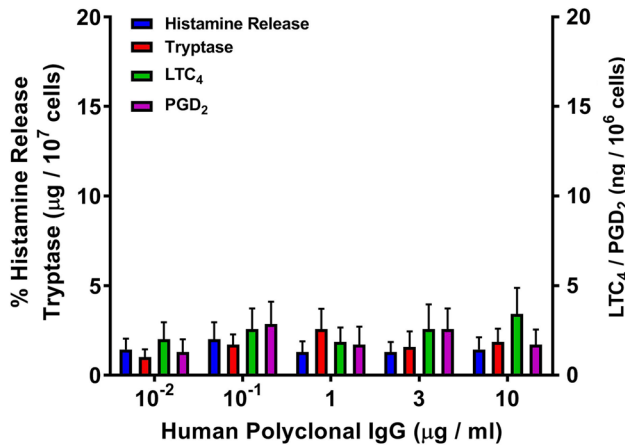
Fig. 4 Effects of increasing concentrations of human polyclonal IgG obtained from five healthy donors on the release of histamine, tryptase, LTC_4, and PGD_2 from human cardiac mast cells (HCMCs). HCMCs were incubated (45 min at 37 °C) in the presence of the indicated concentrations of different preparations of human polyclonal IgG. Each bar shows the mean $\pm$ SEM. Similar results were obtained in two additional experiments

mediators through the interactions with IgE-bound to FcεRI on HCMCs. To support this hypothesis, we examined whether human monoclonal IgE purified from a myeloma patient (ADZ) [81] interfere with the activating properties of human and rabbit IgG anti-IgE. HCMCs were preincubated

with increasing concentrations of monoclonal IgE and then incubated in the presence of graded concentrations of human or rabbit IgG anti-IgE. Figure 7 shows the results of a typical experiment showing that increasing concentrations of human monoclonal IgE shifted to the right the activating properties of both human (Fig. 7A) and rabbit anti-IgE (Fig. 7B) in a concentration-dependent manner without affecting the maximal release. The parallel shift to the right of the concentration–response curve induced by increasing concentrations of IgE on both human and rabbit anti-IgE was compatible with the hypothesis that human monoclonal IgE acted as a competitive inhibitor of both stimuli. Preincubation of HCMCs with higher concentrations (10^{-1} and 1 μg/ml) of human polyclonal IgG did not modify the activating capacity of either human or rabbit anti-IgE to induce histamine release from mast cells (data not shown).

Discussion

Mast cells are strategically located in different sections of murine [3, 6, 94] and human heart [6, 9, 10, 12, 31]. These cells are involved in cardiometabolic diseases [1, 32], myocardial infarction [30] and remodeling [95], atrial fibrillation [96], and myocarditis [28, 97, 98]. Moreover, mast cells are present in atherosclerotic lesions [11, 99] and promote atherogenesis [100]. Understanding how HCMCs are

Fig. 5 **A** Correlation between the percent histamine release and tryptase secretion induced by functional human anti-IgE from human cardiac mast cells (HCMCs). **B** Correlation between the percent histamine and LTC_4 release induced by human anti-IgE from HCMCs. **C** Correlation between histamine and PGD_2 release induced by human anti-IgE from HCMCs. **D** Correlation between LTC_4 and PGD_2 release induced by human anti-IgE from HCMCs
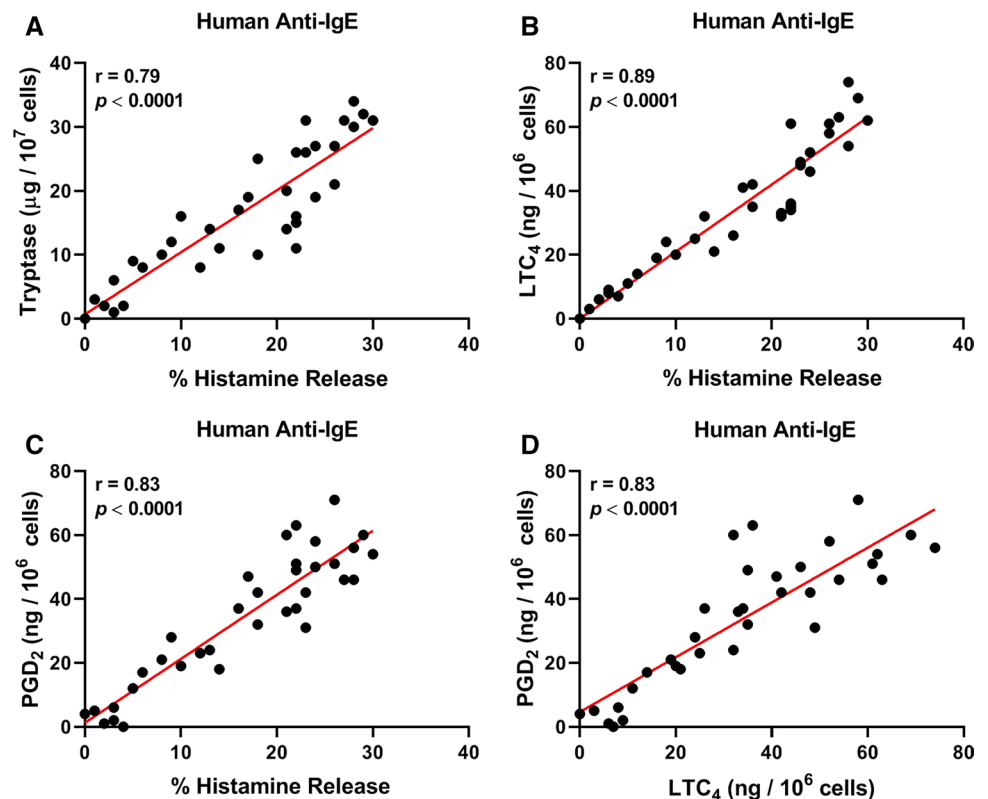
Fig. 6 **A** Correlation between the percent histamine release and tryptase secretion induced by rabbit anti-IgE from human cardiac mast cells (HCMCs). **B** Correlation between the percent histamine and LTC$_4$ release induced by rabbit anti-IgE from HCMCs. **C** Correlation between histamine and PGD$_2$ release induced by rabbit anti-IgE from HCMCs. **D** Correlation between LTC$_4$ and PGD$_2$ release induced by rabbit anti-IgE from HCMCs
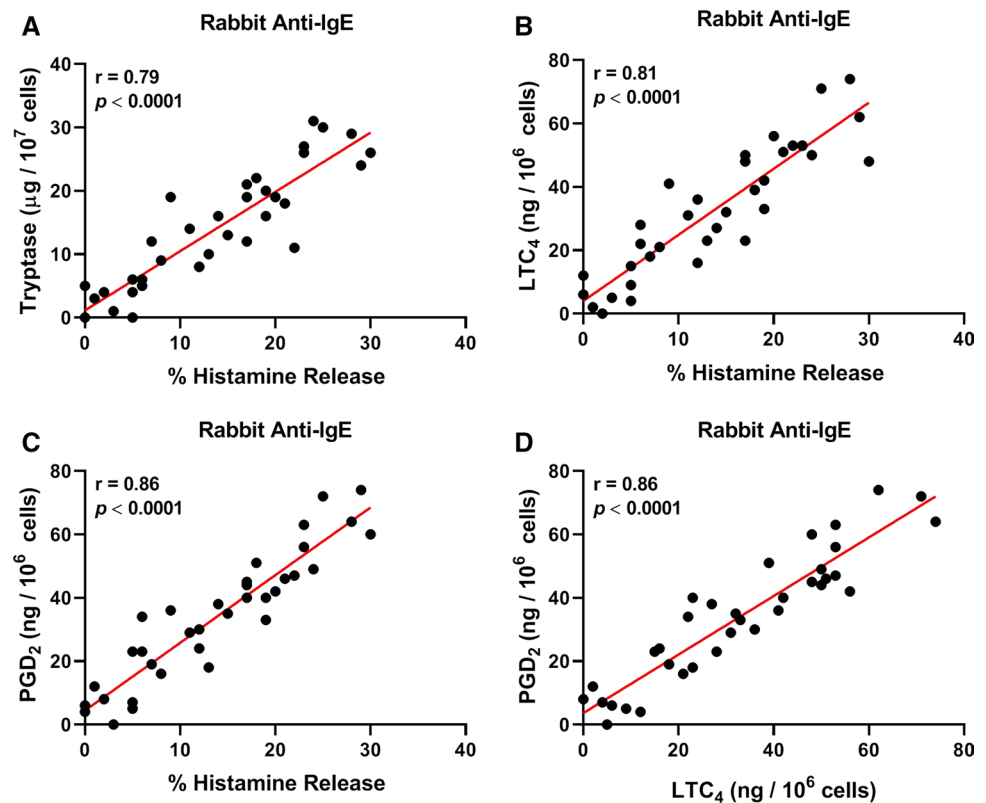
immunologically activated can help in the comprehension of how these cells participate in these different cardiovascular disorders [1, 6, 11, 28, 30, 32, 53, 54].

Serum IgE levels are elevated in patients with myocardial infarction [101, 102], coronary artery disease [100], and heart failure [54]. These findings suggest that mast cells and perhaps other immune cells expressing IgE bound to FcεRI (e.g., dendritic cells, macrophages, basophils) [100, 103, 104] could play a role in pathological cardiac remodeling and dysfunction.

Autoantibodies to IgE and/or FcεRI can occur in patients with different inflammatory disorders such as CSU [56, 61–63, 65, 105–107] and AD [57–60, 67]. In the vast majority of patients with CSU [56, 65, 105] and AD [58, 62, 108] autoantibodies to IgE and/or FcεRI lacked the capacity to activate mediator release from human basophils. To the best of our knowledge, we provide the first evidence that a functional preparation of human IgG anti-IgE can induce the release of preformed and de novo synthesized mediators from HCMCs.

Although the role of naturally occurring autoantibodies to IgE and/or FcεRI in inflammatory disorders is still a fascinating and unsettled issue [109], several investigators have documented their presence in CSU [55, 61–64, 105–107, 110], asthma [57, 65, 111], and in AD patients [57–60, 67]. The vast majority of these studies have evaluated the effects of autoantibodies to IgE/FcεRI only on histamine release

from human basophils [55, 56, 61–63, 105, 107, 110]. In most cases these autoantibodies lack the capacity to activate human basophils [56, 58, 62, 65, 105]. The above results did not rule out the hypothesis that naturally occurring autoantibodies to IgE and/or FcεRI can activate human mast cells to produce proinflammatory arachidonic acid metabolites.

Our results provide preliminary information on the prevalence of anti-IgE autoantibodies in AD patients. In this study, only one preparation of human IgG anti-IgE out of six patients with AD triggered the release of mediators from mast cells isolated from human cardiac tissue. The apparent low frequency of functional anti-IgE autoantibodies might explain the controversial results on the presence of functional autoantibodies in different types of AD patients [57–59, 62, 67]. Further studies with larger cohorts of AD patients will be necessary to estimate the prevalence of functional and non-functional anti-IgE and anti-FcεRI autoantibodies in this heterogeneous immunologic disorder.

Our results also provide some insight into the potency of naturally occurring IgG autoantibodies anti-IgE. Although the HCMC reactivity to human IgG anti-IgE was similar to that of rabbit IgG anti-IgE, the potency of functional human anti-IgE was consistently higher than that of rabbit anti-IgE in inducing the release of preformed and de novo synthesized mediators from HCMCs.

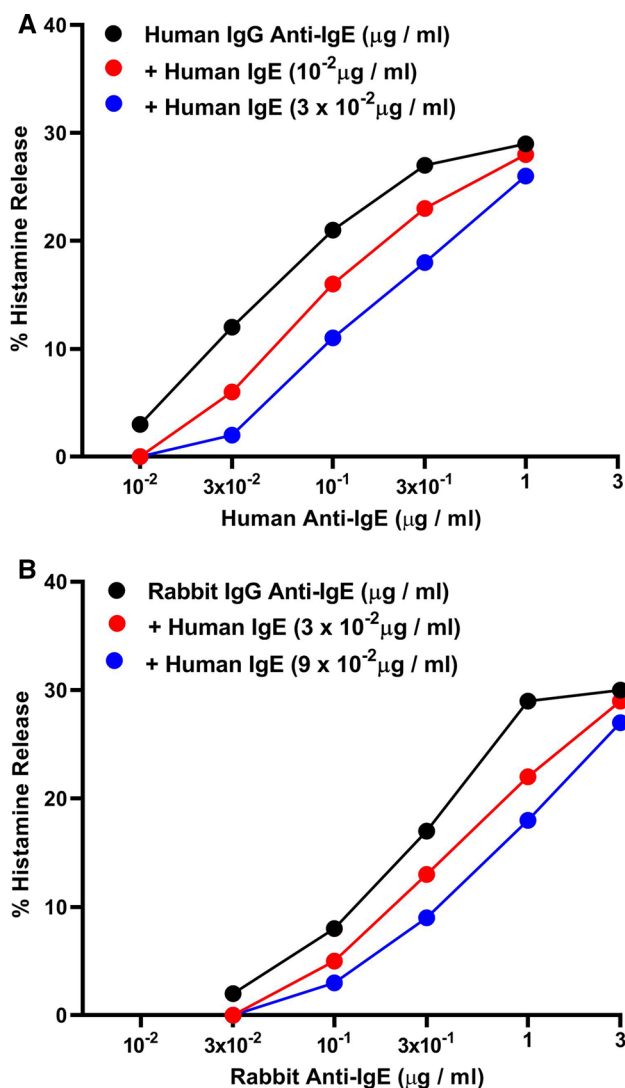Our results also demonstrate that when the human anti-IgE is functionally active, it can work as a complete

Fig. 7 A Effects of increasing concentrations of human monoclonal IgE (ADZ) (🔴: 1×10^{-2} μg/ml; 🔵: 3×10^{-2} μg/ml) on human IgG anti-IgE-induced histamine release from human cardiac mast cells (HCMCs). Cells were preincubated (5 min at 37 °C) with increasing concentrations of human monoclonal IgE and then challenged with the indicated concentrations of human IgG anti-IgE for an additional 30 min at 37 °C. Each value is the mean of duplicate determinations. **B** Effects of increasing concentrations of human monoclonal IgE (🔴: 3×10^{-2} μg/ml; 🔵: 9×10^{-2} μg/ml) on rabbit IgG anti-IgE-induced histamine release from HCMCs. Cells were preincubated (5 min at 37 °C) with increasing concentrations of human monoclonal IgE and then challenged with the indicated concentrations of rabbit IgG anti-IgE for an additional 30 min at 37 °C. Each value is the mean of duplicate determinations. Similar results were obtained in two additional experiments

secretagogue inducing the release of a wide spectrum of pro-inflammatory, vasoactive, and immunomodulatory mediators from HCMCs. For instance, histamine exerts profound cardiovascular effects in humans [70, 71] and tryptase, the most abundant secretory granule protein in human mast cells [112], stimulates collagen production by fibroblasts [113].

LTC_4 is detrimental for atherosclerosis and myocardial infarction [75] and dilated cardiomyopathies [10]. PGD_2 is also detrimental for the cardiovascular and respiratory systems [69, 76].

The presence of spontaneously occurring autoantibodies to IgE/FcεRI has been described by several investigators since the mid-80s, but the relevance of these observations continues to generate some controversies in the field [56, 109]. We still do not know the prevalence and functional relevance of IgG autoantibodies to IgE and FcεRI in different inflammatory disorders [114]. To the best of our knowledge, autoantibodies to IgE have not yet been investigated in patients with cardiovascular diseases. Interestingly, serum IgE concentrations are increased in patients with myocardial infarction [101, 102], coronary artery disease [100], and heart failure [54]. Moreover, epidemiological studies have found an increased risk of coronary artery disease and/or heart failure in patients with IgE-mediated allergic disorders [50–52]. Increased IgE levels are associated with atherosclerosis [53], and the IgE-FcεRI network has been implicated in pathological cardiac remodeling and dysfunction [54]. Finally, it has been reported that serum IgE concentrations were higher in a preclinical model of heart failure [54]. Omalizumab, a monoclonal antibody (mAb) anti-IgE, is highly effective in patients with CSU [115] and severe asthma with high levels of IgE [116–118]. Future studies should investigate the effects of omalizumab in preclinical models of heart failure associated with high serum IgE [54]. Further clinical and experimental studies are needed to investigate the presence and functional activity of autoantibodies to IgE and/or FcεRI in patients with different cardiovascular diseases.

Increasing concentrations of human monoclonal IgE concentration-dependently shifted to the right the activating properties of both human and rabbit anti-IgE. These results are compatible with the hypothesis that soluble monoclonal IgE was a competitive antagonist of both human and rabbit antibodies to IgE. The specificity of this observation was supported by the finding that human polyclonal IgG did not interfere with the capacity of either human and rabbit anti-IgE to induce histamine secretion from HCMCs.

Our study has several limitations that should be pointed out. The experiments were performed using primary mast cells isolated from myocardial tissue obtained from patients undergoing heart transplantation. These mast cells might have biochemical characteristics different from those of cells obtained from healthy donors. In the past we addressed this issue by comparing the release of mediators from mast cells isolated from failing hearts and from subjects who died without cardiovascular diseases [31]. In this study, we found quantitative, but not qualitative differences in the release of mediators from "normal" cardiac mast cells when compared with those from explanted hearts. In addition,

our experiments were performed with partially purified (29–61%) HCMCs. There is the possibility that subsets of contaminating cells expressing (e.g., macrophages, monocytes, dendritic cells, basophils) or non-expressing FcεRI (e.g., fibroblasts, cardiomyocytes) may have directly or indirectly affected some of our results. However, the excellent correlations between the release of histamine and tryptase, exclusively present in mast cells, and other mediators (i.e., LTC_4, PGD_2) induced by human and rabbit anti-IgE, suggest that HCMCs are the targets of these antibodies.

Our results might have translational relevance in several cardiovascular disorders. Cardiac mast cells seem to play a role in experimental myocarditis [98, 119, 120], myocardial infarction [30], and post-ischemic myocardial remodeling [30, 121]. In humans, cardiac mast cells might play a role in human dilated cardiomyopathies [31], aortic valve stenosis [122], different phases of atherosclerosis [123], and autoimmune myocarditis [124]. Moreover, cardiac mast cell activation has been suggested to be implicated in the severe clinical presentations of anaphylaxis (e.g., hypotension, arrhythmias, ventricular dysfunction) [125, 126]. Our results indicating that IgG autoantibodies to IgE from some patients with allergic disorders potently activate HCMCs might explain, at least in part, the cardiovascular involvement in patients with anaphylaxis or severe asthma [125, 127, 128].

In conclusion, although functional autoantibodies to IgE rarely occur in patients with AD, when these antibodies are present, they are able to trigger the release of proinflammatory and vasoactive mediators from HCMCs.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication Patients signed informed consent regarding publishing their data.

Ethics approval The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee) of the University of Naples Federico II (protocols N.198/18 and 7/19) for studies involving humans.

Informed consent Informed consent was obtained from all individual participants included in the study.

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