Retraction

MEDICAL SCIENCES


The authors wish to note the following: “Recently, the Department of Rheumatology from the Leiden University Medical Centre discovered that this publication is based upon fraudulent data, as the first author manipulated several assays used in the studies presented in this publication. By adding anti-IgE antibodies into the tubes containing citrullinated fibrinogen, it seemed that the activation of basophils resulted from exposure to citrullinated fibrinogen. Likewise, controls and samples were manipulated through transfer of cells from positive controls to other tubes that now also appeared to contain a positive reaction. This behavior was done in the absence of all other investigators who did not have knowledge of these irregularities. After discovery of these events, several experiments were performed with the goal to reproduce the data indicating the presence of IgE anticitrullinated protein antibody (ACPA). The validity of these data as presented in the manuscript could not be confirmed. For these reasons, the authors wish to retract the paper from the scientific record.

The Leiden University Medical Centre has completed an investigation by an independent investigation committee into the violation of scientific integrity and irregularities in this paper and has concluded that sole responsibility for the irregularities rests with the first author. She has admitted manipulating the data presented in the paper.”

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Evidence for a functional role of IgE anticitrullinated protein antibodies in rheumatoid arthritis


Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints. Among the autoantibodies described in RA, anticitrullinated protein antibodies (ACPs) are highly specific and predictive for RA. In addition, ACPAs have been implicated in the pathogenesis of RA. However, a direct functional response of immune cells from ACPA+ RA patients toward citrullinated proteins has not been demonstrated. In this study, we show that exposure to citrullinated antigens leads to activation of basophils from ACPA+ RA patients within 20 minutes. This was not observed after exposure of basophils to noncitrullinated control antigens or after stimulation of basophils from ACPA− RA patients and healthy controls. Basophil activation was correlated with the binding of citrullinated proteins to basophils. Furthermore, serum from ACPA+ RA patients in contrast to that from ACPA− RA patients could specifically sensitize human FcεRI expressing rat basophil cells (RBL), enabling activation by citrullinated proteins. Mast cell degranulation products such as histamine levels were enhanced in synovial fluid of ACPA+ RA patients as compared with ACPA− RA and osteoarthritis patients. In addition, histamine levels in synovial fluid from ACPA+ RA patients correlated with IgE levels, suggesting degranulation of mast cells by cross-linking IgE. Immunohistochemistry on synovial biopsies demonstrated an increased number of degranulated CD117+ mast cells in ACPA+ RA patients; IgE and FcεRI expression in synovial mast cells from ACPA+ RA patients was increased. In conclusion, our results show an immunological response of immune cells from ACPA+ RA patients in a citrulline-specific manner. Moreover, these data indicate a role for IgE-ACPAs and FcεRI-positive cells in the pathogenesis of RA.

Results

IgE-ACPAs Are Present in RA. IgE antibodies against citrullinated fibrinogen but not against noncitrullinated fibrinogen could be detected in IgG-depleted sera of ACPA+ RA patients in contrast to ACPA− RA patients and controls (Fig. 1A). In parallel, a CCP2 ELISA could confirm the presence of IgE-ACPAs in ACPA+ RA patients (Fig. 1B). Sixty three percent of IgG-ACP+ RA patients were positive for IgA-ACPAs, and 62% were positive for IgM-ACPAs.

Basophils from ACPA+ RA Patients Become Directly Activated After Stimulation with Citrullinated Protein. To determine whether IgE-ACPAs have the potential to activate FcεRI+ cells directly, we performed a basophil activation test (BAT) on healthy controls, mediators such as ACPAs. Such information would be highly relevant because it would provide evidence for a direct functional role of ACPAs in the immune response underlying RA. Therefore, we reasoned that in case ACPAs can mediate inflammatory reactions in humans, it should be possible to visualize the inflammatory potency of ACPAs by studying the activity of IgE bound to FceRI-expressing cells. FceRI is a high-affinity Fc receptor capable of binding monomeric IgE molecules. On cross-linking two IgE molecules bound to their Fc receptor, an activatory signal is mediated. Hence, we anticipated that ACPA+ RA patients would also harbor FcεRI-bound IgE-ACPAs that can directly trigger FcεRI-expressing cells ex vivo without further manipulation, a phenomenon that would not be present in ACPA− RA. Showing such disease-, phenotype-, and antigen-specific effects would provide compelling evidence for a role of ACPA in the pathogenesis of ACPA+ disease and would substantiate the notion that different pathogenic mechanisms are underlying ACPA+ and ACPA− RA, as current genetic association studies suggest. Therefore, the aim of the present study is to verify whether IgE-ACPAs can activate FcεRI+ immune cells. Using an ex vivohuman model, we show that FcεRI+ cells from ACPA+ RA patients can be directly activated by citrullinated antigens. Moreover, we observe increased levels of histamine within the synovial fluid of ACPA+ RA patients as well as increased numbers of mast cells with a degranulated phenotype in the synovia of these patients. Together, these results indicate that ACPAs can directly recruit immune effector mechanisms and provide “translation” evidence for a role of ACPAs and FcεRI+ cells in the pathogenesis of RA.

\[R\]heumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints. Recently, a major development in RA focused on antibodies to proteins modified by citrullination (i.e., an enzyme-mediated posttranslational modification of peptidyl arginine to peptidyl citrulline). Anticitrullinated protein antibodies (ACPs) have been shown to be highly specific for RA (1), are predictive for the development of RA in patients with undifferentiated arthritis (2), and are associated with the extent of joint destruction (3, 4). In an arthritis mouse model, it has been described that transfer of monoclonal antibodies to citrullinated proteins can enhance arthritis progression, whereas tolerization for citrullinated proteins diminishes arthritis severity (5, 6). Together, these observations indicate a role for ACPAs in RA pathogenesis. In humans, however, in spite of the clinical relevance of ACPAs, the potential role of ACPAs in rheumatoid inflammation has not yet been elucidated.

Previously, it has been shown that in vitro generated immune complexes containing ACPAs are able to activate macrophages. These ACPA-containing and ELISA plate-bound immune complexes could induce TNF-α production in macrophages from healthy donors via the engagement of FcγRIIa (7). However, a direct functional and, more importantly, specific response of immune cells from ACPA+ RA patients to citrullinated antigens has not been shown, because it is very difficult to demonstrate in a human setting the direct pathological action of immune


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ACPAs RA patients, and ACPA+ RA patients. The BAT is an ex vivo human model that has repeatedly proved to be reliable in the past as a valuable instrument for investigating IgE-mediated immune responses of basophils in allergic disorders (8). In this assay, whole peripheral blood cells are stimulated with antigen and basophil activation is measured 20 min later by up-regulation of CD63. Intriguingly, stimulation with citrullinated fibrinogen resulted in direct activation of basophils from ACPA+ RA patients (Fig. 2). In contrast, addition of HBSS buffer, peptidyl arginine deiminase (PAD), and noncitrullinated fibrinogen (NC-FB) resulted in direct activation of basophils from ACPA+ RA patients (Fig. 2). In contrast, addition of HBSS buffer, peptidyl arginine deiminase (PAD), and noncitrullinated fibrinogen (NC-FB) resulted in direct activation of basophils from ACPA+ RA patients (Fig. 2). In contrast, addition of HBSS buffer, peptidyl arginine deiminase (PAD), and noncitrullinated fibrinogen (NC-FB) resulted in direct activation of basophils from ACPA+ RA patients (Fig. 2).

To provide evidence that these findings are attributable to a specific reaction to citrullinated proteins and not to some peculiarity of citrullinated fibrinogen, evaluation of another citrullinated protein, myelin binding protein (MBP), was performed in healthy controls, ACPA+ RA patients, and ACPA+ RA patients (Fig. 3). In parallel with results for citrullinated fibrinogen, stimulation with citrullinated MBP also resulted in basophil activation in ACPA+ RA patients. Because it might be possible that basophils are activated by immune complexes or rheumatoid factor (RF), basophil activation was assessed by incubation with monomeric IgG or heat-aggregated IgG immune complexes, which did not result in basophil activation, as measured by the up-regulation of CD63 (Fig. 3). Moreover, basophils of ACPA+ RA patients who were RF+ (n = 4) did not show activation by citrullinated proteins, whereas basophils of ACPA+ RA patients who were RF− (n = 2) showed activation after incubation with citrullinated proteins. Next, we wished to assess whether the observed activation is mediated by antibodies bound to high-affinity Fc receptors. Therefore, we eluted IgGs by means of acid glycine buffer (9) and analyzed whether activation of basophils by citrullinated fibrinogen was inhibited. Flow cytometric analysis of eluted samples showed a complete loss of IgE expression on basophils after elution. This was paralleled by the fact that basophils from ACPA+ RA patients could no longer be activated by citrullinated fibrinogen after elution of IgGs (Fig. 4). Basophils still responded to N-formyl-L-methionyl-L-leucyl-phenylalanine (fMLP) activation via fMLP receptors by CD63 up-regulation both before and after elution, indicating that elution of IgGs did not impair Ig-independent functions of basophils.

**Fig. 2.** Basophil activation. Whole heparinized blood was stimulated with HBSS buffer, 1 μg/mL anti-immunoglobulin E (anti-IgE), 0.2 U/mL PAD, 10 μg/mL noncitrullinated fibrinogen (NC-FB), and 10 μg/mL citrullinated fibrinogen (C-FB) in control (CTR; n = 26), ACPA+ RA (n = 15), and ACPA+ RA (n = 29) patients. Bars represent median values. $P < 0.0001$ for HBSS vs. alglE in CTR, ACPA+ RA, and ACPA+ RA. $P < 0.0001$ for C-FB vs. NC-FB in ACPA+ RA. $P < 0.0001$ for C-FB in ACPA+ RA vs. ACPA+ RA. $P < 0.0001$ for C-FB in ACPA+ RA vs. CTR.

**Fig. 1.** IgE-ACPAAs. (A) Anti-IgE antibodies against noncitrullinated fibrinogen (NC-FB) (●) and citrullinated fibrinogen (C-FB) (○) in IgG-depleted serum samples from control (CTR; n = 5), ACPA+ RA (n = 4), and ACPA+ RA (n = 8) patients. (B) Anti-IgE-ACPAAs in IgG-depleted serum samples from CTR (n = 5), ACPA+ RA (n = 4), and ACPA+ RA (n = 6) patients. $P = 0.003$ in ACPA+ RA vs. CTR. $P = 0.007$ in ACPA+ RA vs. ACPA+ RA.

**IgE-ACPAAs Sensitize FcεRI-Expressing RBLs to Citrullinated Proteins.** To investigate further whether IgE-ACPAAs cross-link FcεRI, human FcεRI-transfected rat basophil cell lines (RBLs) were sensitized for 18 h with sera from healthy controls, ACPA+ RA patients, and ACPA+ RA patients. Sensitization with ACPA+ RA sera led to β-hexoaminidase release by RBLs on stimulation with citrullinated fibrinogen but not with noncitrullinated fibrinogen (Fig. 5). Importantly, the β-hexoaminidase release by RBLs was not observed when sera from healthy controls and ACPA+ RA patients were used for sensitization. Likewise, nontransfected RBLs were not activated, indicating that this process relies on the presence of FcεRI. In parallel with the findings for citrullinated fibrinogen, citrullinated MBP could also activate sensitized RBLs (Table 1). Moreover, preincubation of an ACPA+ RA serum with neutralizing anti-IgE antibodies (Omalizumab; Novartis) prevented sensitization of RBLs, because no β-hexoaminidase release was demonstrated after stimulation with anti-IgE, citrullinated fibrinogen, or citrullinated MBP (Table 1). In addition, IgG-depleted serum was still able to sensitize RBLs, further confirming that IgG-ACPAAs do not activate basophils (Table 1).

**Basophils from ACPA+ RA Patients Bind Citrullinated Proteins.** To confirm further that citrullinated fibrinogen does indeed bind basophils of ACPA+ RA patients, flow cytometric staining of basophils with citrullinated fibrinogen coupled to a fluorochrome...
patients or RA patients were RA Patients. and 27 were ACPA RA patients display higher levels of both cell-bound = 6) patients. RA patients (Fig. 7). Although total IgE levels in RA patients compared with controls (i.e., OA and RA patients) (Table 2). Intriguingly, when we assessed degranulation by analyzing the difference between CD117 and CAE staining, degranulation was more frequently observed both in absolute as well as relative numbers (i.e., percentage of mast cells) in ACPA RA synovium compared with ACPA RA synovium. Overall, these results reveal a higher number of degranulated mast cells in ACPA RA, suggesting activation of mast cells in ACPA RA.

Discussion

In this study, we demonstrate that IgE-bearing basophils from ACPA RA patients are directly activated on exposure to citrullinated antigen. These results most likely reflect the presence of FcεRI-bound IgE-ACPAs on the surface of basophils, because the high-affinity FcεRI will bind available free IgE. After cross-linking adjacent IgE molecules by antigen, aggregation of FcεRI triggers an immediate intracellular signaling process resulting in rapid cellular activation (12). In line with these observations, we also show that IgE-ACPAs are present in sera of ACPA RA patients, because these sera could readily sensitize an FcεRI-transfected RBL for recognition of citrullinated antigen. These observations are highly relevant because they show that immune cells from ACPA RA patients but not from ACPA patients or healthy controls can recognize and become activated on binding citrullinated antigens in a highly specific manner.

The presented data do not show that FcεRI-expressing cells, such as mast cells, are involved in the pathogenesis of ACPA RA, because the final evidence for this hypothesis can only be obtained by a specific intervention study. However, we believe that our results, obtained from independent lines of evidence, provide a considerable amount of independent data that support the role of IgE-ACPAs and mast cells in the pathogenesis of ACPA RA. First, only basophils from ACPA RA patients

(FITC) was performed (Fig. S2). Basophils from ACPA RA patients were stained by citrullinated fibrinogen-FITC, whereas basophils of ACPA RA patients did not bind citrullinated fibrinogen-FITC. Noncitrullinated fibrinogen-FITC did not bind basophils, again demonstrating the potential of basophils from ACPA RA patients to bind citrullinated antigens.

Enhanced Synovial Mast Cell IgE Expression and Histamine Release in ACPA RA Patients. The previously discussed results suggest the potential of IgE-ACPAs to activate FcεRI+ immune cells such as mast cells, which are present in synovium of RA patients. Because higher levels of human IgE result in higher expression of both IgE and FcεRI (10, 11), we next analyzed whether synovial mast cells from ACPA RA patients display higher levels of both cell-bound IgE and FcεRI as compared to ACPA RA patients and osteoarthritis (OA) patients. Significantly higher IgE expression on synovial mast cells of ACPA RA patients was observed compared with OA patients and ACPA RA patients (Fig. 6). In parallel, FcεRI expression was also up-regulated in ACPA RA patients ($P = 0.04$ vs. ACPA RA, $P = 0.003$ vs. OA) (Fig. S3).

To verify if activation of mast cells in the synovium had occurred in ACPA RA patients, histamine levels in synovial fluid from OA, ACPA RA, and ACPA RA patients were determined. Histamine levels were significantly increased in ACPA RA patients compared with controls (i.e., OA and ACPA RA patients) (Fig. 7). Although total IgE levels in synovial fluid were not different between groups, a significant correlation between histamine levels and total IgE in synovial fluid was present in ACPA RA patients only (Fig. S4).

Synovial Mast Cells Are Degranulated in ACPA RA Patients. To substantiate further the finding that citrullinated proteins might directly activate mast cells and contribute to inflammation, biopsies of inflamed knees of RA patients were microscopically examined. RA synovial biopsies were obtained of 50 patients, of whom 23 were ACPA+ and 27 were ACPA−; age and disease duration were not significantly different between groups (Table 2). Representative pictures of immunohistochemical staining for CD117 demonstrating c-kit/stem cell receptor on the surface of mast cells, chloroacetate esterase (CAE) staining, and double CD117/CAE staining are shown (Fig. 8). Mast cells were found (although not exclusively) near the synovial vessels. A higher number of mast cells was observed both by semiquantitative scoring and absolute numbers of CD117+ mast cells in ACPA RA patients (Table 2). Intriguingly, when we assessed degranulation by analyzing the difference between CD117 and CAE staining, degranulation was more frequently observed both in absolute as well as relative numbers (i.e., percentage of mast cells) in ACPA RA synovium compared with ACPA RA synovium. Overall, these results reveal a higher number of degranulated mast cells in ACPA RA, suggesting activation of mast cells in ACPA RA.

Fig. 3. Basophil activation with citrullinated (C)-MBP, heat-aggregated IgG (HAIgG), and IgG. Whole heparinized blood was stimulated with HBSS buffer, 1 μg/mL anti-IgE, 10 μg/mL noncitrullinated (NC)-MBP, 10 μg/mL C-MBP, and 10 μg/mL monomeric IgG, and 10 or 100 μg/mL HAiG in healthy control (CTR; $n = 3$), ACPA RA ($n = 2$), and ACPA RA ($n = 6$) patients. $P = 0.02$ in ACPA RA vs. CTR and ACPA RA.

Fig. 4. Elution of IgG. Basophil activation before and after elution of Ig with acid glycine buffer in ACPA RA patients ($n = 5$). Basophils were stimulated with HBSS buffer, 1 μg/mL anti-IgE, 10 μg/mL noncitrullinated fibrinogen (FB), 10 μg/mL citrullinated fibrinogen (C-FB), and 1 μg/mL fMLP before and after elution of Ig with acid glycine buffer in ACPA RA patients ($n = 5$). $P = 0.04$ for C-FB PRE elution vs. C-FB POST elution.

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could be triggered by citrullinated antigens. Second, we demonstrated a higher number of mast cells in the inflamed synovia of ACPA⁺ patients as well as higher FcεRI and IgE expression per cell, which stabilizes FcεRI expression on tissue-resident mast cells. More importantly, we also observed more degranulated mast cells, indicating that these mast cells have released their granule content to the external environment. Because these granules contain many potent biological mediators such as serine proteases (e.g., chymases, tryptases), cytokines (e.g., IL-1, TNF-α), lipid-derived mediators (e.g., prostaglandins, thromboxane A2, leukotrienes), and histamine, it is highly conceivable that these mast cells have contributed to the signs and symptoms present in RA. Among this plethora of mediators, TNF-α is a more prominent mediator, because anti-TNF therapy has already proved to be effective in RA.

Intriguingly, IgE levels were correlated with histamine levels in synovial fluids from ACPA⁺ RA patients only, further strengthening the notion that mast cells, via triggering of IgE-ACPA containing FcεRI, have been activated and contribute to the inflammatory milieu found in the joints of RA patients. IgE and mast cells are often associated with acute allergic reactions and early-phase reactions in allergic inflammation (13). However, they also play a role in chronic allergic inflammation, where they are thought to be involved in many processes such as tissue remodeling, recruitment of other immune cells, and neo-vascularization (13). These processes are probably a reflection of their “physiological” role in the control of parasitic infections, which can also be associated with higher IgE levels, an activated mast cell phenotype, and the persistent presence of antigen. A role for antigen-specific IgE in autoimmunity has previously been demonstrated (14, 15). Bullous pemphigoid (BP) is an autoimmune skin disease characterized by subepidermal blistering. The disease is mediated by autoantibodies against hemidesmosomal proteins such as BP180, and a direct pathogenic role for IgG and IgE has been demonstrated. Intriguingly, many immunological features of BP resemble those seen in RA, because most BP patients display higher levels of total IgE, basophils isolated from the peripheral blood of BP patients were shown to be activated on exposure to BP180, and mast cells present in BP lesions appear to have a degranulated phenotype. For this reason, it is very tempting to speculate that although many differences exist, the biological process present in BP and RA is essentially similar but that the antigen specificity of the antibodies (putatively) driving the disease is responsible for the highly different clinical phenotypes.

Moreover, a role for mast cells in RA was suggested over 20 years ago after observing a strong positive correlation between mast cell numbers and several inflammatory markers in synovial tissue of RA patients (16). Likewise, it was noted that mast cells numbers are increased in RA synovium (17). More recently, a role for mast cells has also been demonstrated in mouse models for arthritis. For example, in the K/BxN model, which predominantly depends on pathogenic antibodies and neutrophils, a pivotal role for mast cells was observed, because mast cell-deficient mice are protected against disease development (18). Mast cells were not only involved in arthritis via production of IL-1 (19) but also produced the mast cell-restricted Mouse Cell Protease-6, the ortholog of human tryptase-b, that served as an important intensifier of inflammation within the joint via enhancing the recruitment of neutrophils. Furthermore, previous animal studies have show an arthritogenic potential of IgE-mediated activation of mast cells in rat joints (20), together with the detection of specific collagen IgE in a cartilage collagen-induced mouse arthritis model (21). In human RA, IgE-RF and

Table 1. Activation of FcεRI-transfected RBLs by RA serum

<table>
<thead>
<tr>
<th>Buffer</th>
<th>algE</th>
<th>NC-MBP</th>
<th>C-MBP</th>
<th>NC-FB</th>
<th>C-FB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACPA⁺ RA serum</td>
<td>0</td>
<td>31</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>IgG-depleted RA serum</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Anti-IgE preincubated RA serum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

After sensitization with ACPA⁺ RA serum, IgG-depleted RA serum, or anti-IgE incubated RA serum, RBLs were stimulated with Thyrod's buffer, 1 μg/mL anti-IgE, 10 μg/mL noncitrullinated fibrinogen (NC-FB), or 10 μg/mL citrullinated fibrinogen (C-FB), 10 μg/mL NC-MBP, and 10 μg/mL C-MBP. Results are expressed as the percentage of total β-hexoaminidase release (Triton-X-treated RBLs) after correction for spontaneous release in Thyrod's buffer.

Fig. 5. Passive sensitization of FcεRI-transfected RBLs. After sensitization with sera from control (CTR; n = 10), ACPA⁺ RA (n = 39), and ACPA⁺ RA (n = 50) patients for 18 hours, RBLs were stimulated with Thyrod's buffer, 1 μg/mL anti-IgE, 10 μg/mL noncitrullinated fibrinogen (NC-FB), or 10 μg/mL citrullinated fibrinogen (C-FB). Results are expressed as the percentage of total β-hexoaminidase release (treated with Triton-X) after correction for spontaneous release in Thyrod's buffer. P < 0.0001 for C-FB vs. NC-FB in ACPA⁺ RA. P < 0.0001 for C-FB in ACPA⁺ RA vs. ACPA⁺ RA. P = 0.001 for C-FB in ACPA⁺ RA vs. CTR.

Fig. 6. Surface IgE expression on CD117-allophycocyanin (APC⁺) synovial mast cells. Synovial mast cells of ACPA⁺ RA (n = 10), ACPA⁺ RA (n = 5), and OA (n = 18) patients were stained with Alexafluor-conjugated anti-IgE. Results are expressed as increase of mean fluorescence intensity (MFI) between isotype control MFI and anti-IgE MFI. P = 0.008 in ACPA⁺ RA vs. ACPA⁺ RA. P < 0.0001 in ACPA⁺ RA vs. OA.
anti-collagen II-IgE antibodies were observed in several studies (22–24). In contrast to these earlier reports, we now show a direct cellular immune response toward citrullinated antigens.

Because degranulation is the clearest histological hallmark of mast cell activation, the higher number of degranulated mast cells in synovial tissue from ACPA– RA patients as compared with ACPA– RA patients is in keeping with the recent realization that RA represents two main syndromes: ACPA– and ACPA– disease. These two forms of RA are most likely etiologically distinct, because distinct genetic risk factors underlie these distinct disease phenotypes. This notion was initially demonstrated by our finding that different HLA genes predispose to either ACPA– or ACPA– disease (25, 26), and it was subsequently boosted by the results of recent Whole Genome Association (WGA) studies (27). Furthermore, some environmental risk factors underlie only one form of RA but not the other, and the initial evidence is now appearing that ACPA– and ACPA– patients respond differently to therapy (4). Moreover, our observations are in keeping with previous data that RA can be considered a heterogeneous disease with different synovial infiltration patterns (e.g., augmented lymphocytes) (28, 29). Our data further boost this concept by showing that immune cells from ACPA– and ACPA– patients respond differently to citrullinated antigens. Our observation that degranulated mast cell numbers and the level of mast cell products in synovial fluid differ between ACPA– and ACPA– patients provides further support for distinct pathogenic pathways for ACPA– and ACPA– disease.

In conclusion, our data show a direct biological response to citrullinated antigens of immune cells in ACPA– RA patients only. Elucidating this activation pathway mediated by IgE-ACPAs in mast cells could lead to previously undescribed treatment strategies in patients with RA.

![Image](Image)

**Table 2.** Characteristics of synovial biopsies in ACPA– and ACPA– RA patients

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 50)</th>
<th>ACPA– RA (n = 23)</th>
<th>ACPA– RA (n = 27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57 (15)</td>
<td>57 (14)</td>
<td>56 (16)</td>
<td>0.79</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>17/33</td>
<td>8/19</td>
<td>9/14</td>
<td>0.48</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>8 (7)</td>
<td>7 (6)</td>
<td>9 (9)</td>
<td>0.36</td>
</tr>
<tr>
<td>Median no. CD117+ MCs</td>
<td>27 (0–178)</td>
<td>33 (0–178)</td>
<td>11 (0–100)</td>
<td>0.003</td>
</tr>
<tr>
<td>MC score CD117 = 0–1 (semiquantitative)</td>
<td>16</td>
<td>4</td>
<td>12</td>
<td>0.004</td>
</tr>
<tr>
<td>MC score CD117 = 2–3 (semiquantitative)</td>
<td>34</td>
<td>23</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Median no. granulated MCs by CAE</td>
<td>3 (0–86)</td>
<td>4 (0–86)</td>
<td>2 (0–33)</td>
<td>0.63</td>
</tr>
<tr>
<td>MC score CAE = 0–1 (semiquantitative)</td>
<td>44</td>
<td>24</td>
<td>20</td>
<td>0.83</td>
</tr>
<tr>
<td>MC score CAE = 2–3 (semiquantitative)</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Median no. CAE+ CD117+ MCs</td>
<td>4 (0–76)</td>
<td>3 (0–75)</td>
<td>6 (0–47)</td>
<td>0.66</td>
</tr>
<tr>
<td>Median no. degranulated MCs</td>
<td>21 (0–173)</td>
<td>28 (0–173)</td>
<td>6 (0–67)</td>
<td>0.005</td>
</tr>
<tr>
<td>% degranulated MCs</td>
<td>82 (0–100)</td>
<td>89 (0–100)</td>
<td>58 (0–100)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Results are expressed as mean (SD) or median (minimum-maximum). MC, mast cell, M/F, male/female. Semiquantitative scores are represented as the number of patients with a score of 0 or 1 and the number of patients with a score of 2 or 3.
Detection of Isotype ACPAs. The presence of total IgG-ACPAs was tested in all RA patients by an anti-CCP2 ELISA (Eurodiagnostica) according to manufacturer instructions. IgA-ACPAs and IgM-ACPAs were measured by a previously described ELISA (34) (see SI Materials and Methods).

BAT. As described previously (35), heparinized whole blood was stimulated at 37°C for 20 min with 10 μg/mL native noncitrullinated fibrinogen (Sigma Aldrich), 10 μg/mL citrullinated fibrinogen, 0.5 U/mL PAD (Sigma Aldrich), 10 μg/mL noncitrullinated MBP, 10 μg/mL citrullinated MBP, 10 μg/mL monoclonal IgG (Jackson ImmunoResearch Laboratories, Inc.), 10- or 100-μg/mL heat-aggregated IgG, HBSS as a negative control, and 1 μg/mL anti-IgE (Pharmingen) as a positive control. Human fibrinogen or MBP was citrullinated as described previously (34).

Elution of Igs. Basophil-bound Ig was eluted with acid elution buffer as previously described (8). As control, noneluted samples were treated with 1% HBSS/BSA instead of elution buffer. Basophils were stained after elution with monoclonal PE-conjugated FcRII (bEbioscience), IgE-Alexafluor 488 (Molecular Probes), and allophycocyanin (APC)-conjugated CD203c antibodies.

RBL Mediator Release. The sensitization and stimulation of RBLs were performed as described earlier (9) (see SI Materials and Methods).

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