LETTER TO THE EDITOR

The Fcε receptor I pathway is crucial but not exclusive for basophil activation in patients with autoimmune forms of chronic spontaneous urticaria

To the editor

Ibrutinib, an irreversible and specific Bruton's tyrosine kinase (BTK) inhibitor, leads to complete inhibition of IgE/FcεRI-BTK degranulation of human basophils without affecting the signalling via G protein-coupled receptors (C5a, fMLP).[1,2] Since autoantibodies in chronic spontaneous urticaria (CSU) either against the IgE/FcεRI complex or IgE play a relevant role in CSU,[3,4] ibrutinib could, therefore, be a promising asset in basophil assays.[5] This *in vitro* study aimed to differentiate between FcεRI-dependent and FcεRI-independent pathways in autoimmune CSU (aiCSU).

Serum from patients with aiCSU (total $n = 24$, age range 24–71 years, 62.5% female) was investigated in two basophil assays with donor basophils (CU-BAT) according to the literature[6,7]; either with releaser basophils of six well-characterized healthy donors under ibrutinib inhibition (phase 1, $n = 20/24$) or with non-releaser basophils of two healthy donors, unresponsive to FcεRI stimulation without IL-3 priming (phase 2, $n = 13/24$). In nine out of 24 cases, both experiments were performed. Anti-IgE (100 ng/mL; Beckman Coulter, Marseille, France), C5a (Sigma-Aldrich, St. Louis, MO, USA) and fMLP (Sigma-Aldrich) served as positive and a serum mix of healthy donors ($n = 5$) as a negative control. CD63 served as an activation marker and was expressed as the percentage of activated basophils. Only serum samples with a CD63 up-regulation of at least 7.7% at baseline in CU-BAT were evaluated, to only include aiCSU.

Ibrutinib experiments (phase 1, $n = 20/24$): Compared to baseline, the percentage of activated donor basophils after stimulation with patient serum was significantly reduced under ibrutinib (CD63 29.1% ± 18.2 vs. 5.1% ± 6.4, $P < 0.0001$): a complete inhibition could be observed in 16 of 20 patients. Interestingly, four out of 20 patients' sera could partially activate donor basophils despite ibrutinib inhibition (Fig. 1). Non-releaser experiments (phase 2, $n = 13/24$): When using non-releaser basophils without IL-3 priming, nine out of 13 aiCSU patients' sera were not able to stimulate unprimed basophils (Fig. 2, subgroup A). In contrast, the sera of four patients led to significant CD63 up-regulation of non-releaser basophils (2.8 ± 1.4% vs. 28.1 ± 12.8%, $P = 0.0028$). Even after priming with IL-3, CD63 expression was significantly higher in the subgroup B (6.9 ± 4.0% vs. 44.6 ± 24.6%, $P = 0.0028$). Importantly, serum IL-3 levels in aiCSU sera were low and below the level required for priming. A comparison of both experiments showed that sera of two patients who were only partially suppressed by ibrutinib in CU-BAT could not activate non-releaser basophils. Interestingly, the serum samples of two cases with complete ibrutinib inhibition were able to activate non-releaser basophils without IL-3 priming, a circumstance already observed by MacGlashan.[5]

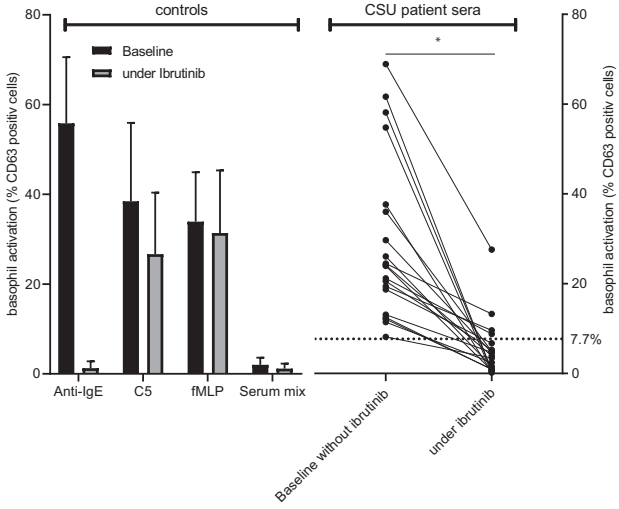

Figure 1 Phase 1: Releaser basophils of well-characterized donors primed with optimal IL-3 concentration with and without pretreatment of ibrutinib (100 nmol/L for 15 min) were incubated with the serum of patients with chronic spontaneous urticaria (CSU), $n = 20/24$. Values represent the measurement of CD63, expressed as the percentage of activated basophils (*CD63 %: 29.1% ± 18.2 vs. 5.1% ± 6.4, $P < 0.0001$). 7.7% was defined as the cut-off of basophil activation for study inclusion. Anti-IgE, C5a, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and serum mix of six healthy donors were used as controls.

There are limitations of our in vitro study: since our study was conducted in two phases, not all patient sera were subjected to both experiments, which make it difficult to find a correlation between the two effects. Especially in the assays with non-releaser basophils, the number of participating patients was low.

In summary, this in vitro study shows that the IgE/FcεRI-BTK pathway seems to be dominant for the degranulation of

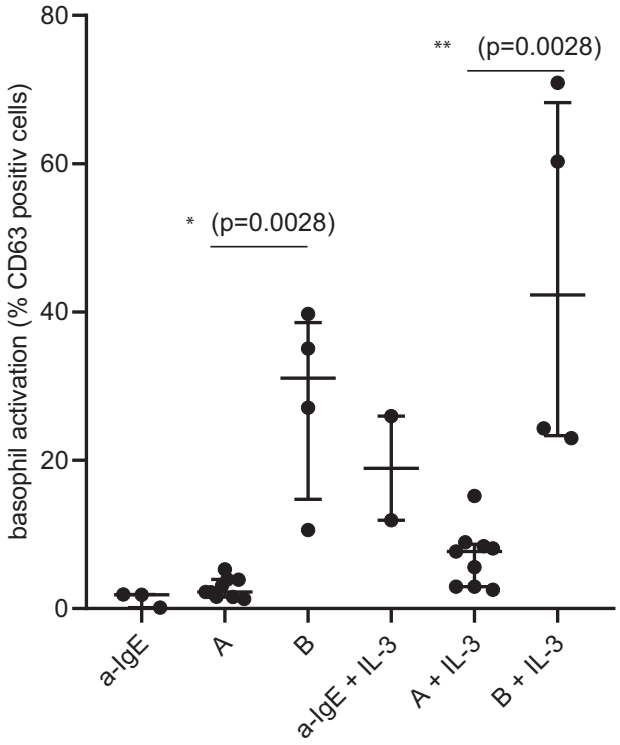

Figure 2 Phase 2: Evaluation of non-releaser donor basophils (unresponsive to FcεRI stimulation without priming) stimulated with the serum of CSU patients (n = 13/24). Group A represents CSU sera not active on non-releaser basophils, in contrast to group B whose sera activate non-releaser basophils without the addition of IL-3 primer. All experiments with non-releaser basophils were done with and without IL-3 priming. Anti-IgE served as a control. Values represent the measurement of CD63 up-regulation, expressed as the percentage of activated basophils (%).

basophils treated with aiCSU sera, but its inhibition does not completely abrogate basophil activation in all patients. Incomplete inhibition in four aiCSU serum samples by ibrutinib and the ability of four sera to elicit activation in non-releaser basophils suggest that in some aiCSU, sera pathways or factors beyond or in addition to FcεRI triggering of basophils may exist. It seems that the serum of some aiCSU patients contains a factor ('second signal') that may act as a basophil primer, enhancing signalling through FcεRI and inducing CD63 up-regulation. The exact nature of factors outside the IgE/FcεRI-BTK signalling remains still unclear and will be the focus of future studies.

Conflicts of interest

WJP and OH are employees of ADR-AC GmbH, a specialized laboratory offering basophil activation tests for routine diagnostics in Switzerland.

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Ethics approval and consent to participate

This study was approved by the local ethics committee (Kantonale Ethikkommission Bern). All patients included in this study gave informed consent.

L. Jörg,[1,*,†] N. Mueller-Wirth,[2,†] T. Pecaric-Petkovic,[2] C. Diaz,[2] W. Pichler,[2] O. Hausmann[2,3]

[1]Department of Rheumatology, Immunology and Allergology, Inselspital, Bern University Hospital,University of Bern, Bern, Switzerland, [2]ADR-AC GmbH,Adverse Drug Reactions, Analysis and Consulting, Bern, Switzerland, [3]Löwenpraxis Luzern, Lucerne, Switzerland

*Correspondence: L. Jörg. E-mail: lukas.joerg@insel.ch

[†]Contributed equally

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