

Letter to the Editor

Differential effect of human rhinovirus 1B (RV1B) on IL-4-primed IgE synthesis by PMBCs from allergic patients and healthy subjects

Human rhinovirus (HRV) is a small-sized positive-strand RNA virus of the family *Picornaviridae* and it consists of over 100 types classified into one of three species (A, B, C) (1). HRV exposure is usually asymptomatic or it may evoke symptoms of the common cold. However; wheezing illnesses in infancy and early childhood caused by HRV infections are the most significant predictor of a subsequent development of asthma (2,3). HRV is also believed to be the main trigger of asthma exacerbations in children and adults (4–7).

Airway epithelium, especially ciliated epithelial cells with high ICAM-1-expression, is the main target for HRV. However, it has been documented that HRV might also infect T and B cells. *Ex vivo* data indicate that HRV infections may induce Th2 type immune inflammation with enhanced IgE synthesis (8–14). Tam et al. found that individuals exposed to HRV had detectable levels of specific anti-HRV IgE in plasma and children with asthma exacerbations caused by HRV infections had a rise in IgE levels (15–17). Furthermore, Esquivel et al. documented that blocking IgE by omalizumab decreased susceptibility to HRV infection and illness (18). The aim of this study was to assess if HRV may affect IgE synthesis by PMBCs from allergic patients and healthy subjects *in vitro*.

The study included 12 subjects: 6 adult patients (3 males and 3 females, mean age 41.1 ± 5.5 years) with allergic bronchial asthma and/or allergic rhinitis confirmed by positive skin prick tests to relevant allergens, and a group of 6 healthy, non-allergic subjects (2 males and 4 females, mean age, 38.3 ± 4.0 years), with no history of allergy and with negative skin prick test to a panel of inhaled allergens (Table 1). Inhaled glucocorticoids and long-acting beta-2-agonists were withheld for 48 h and antihistamines for 7 days before the study. Each patient gave written informed consent for participation in the study. The study was approved by the Local Ethics Committee of the Medical University of Lodz, Poland. PMBCs were separated from the peripheral blood as described earlier (19). PMBCs (2 mL/mL) were primed with human recombinant IL-4 (10 ng/mL, Sigma-Aldrich) and exposed to RV1B at 3 MOI (0.1, 0.5 and 1.0) on the days 2 or 7 of culture in order to establish the optimal time-point of induction with virus. PMBCs were also exposed to inactivated RV1B at the same MOI and

time-points. Virus was inactivated using a centrifugal filter device (Amicon Ultra-0.5 Centrifugal Filter Unit; Merck, Cork, Ireland). Additionally, PMBCs were stimulated a mix of TLR3, TLR7/8 and TLR9 agonists in the same manner (PolyI:C – 16.4 µg/mL, R848 – 3.3 µg/mL and CpG – 16.4 µg/mL, respectively). After 11 days of culture, cell-free supernatants were collected and stored at -70°C . Total IgE concentrations in supernatants from PMBCs cultures were determined using the fluorimmunoassay method UniCAP (Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol. Quantitative variables are presented as means and standard deviation and compared using non-parametrical test. The statistical analysis was performed using Statistica (StatSoft, Tulsa, OK, USA). *p* values < 0.05 were accepted as statistically significant.

PMBCs from allergic patients cultured with medium alone released significantly more IgE as compared to cells from healthy subjects (0.39 ± 0.09 vs 0.03 ± 0.03 kU/L, respectively, $p < 0.01$; Fig. 1). Priming of PMBCs from allergic patients with IL-4 caused a slight increase of IgE generation (from 0.39 ± 0.09 to 0.64 ± 0.1 , $p = 0.07$; Fig. 1). The mean IgE synthesis by IL-4-primed PMBCs from allergic patients upon induction with RV1B at MOI 0.1 and 0.5 was significantly higher as compared to medium alone, but not to IL-4-primed cells (data not shown). However, a significant individual variation in the magnitude of IgE production in response to RV1B depending on virus MOI was observed. Therefore, the highest, regardless of the virus MOI, IgE concentrations generated upon induction of PMBCs with RV1B were selected and combined together for analysis. Such approach revealed that in allergic patients the mean IgE synthesis by PMBCs following the infection with the virus was three times higher than in unstimulated PMBCs (1.4 ± 0.22 vs 0.39 ± 0.09 kU/L, $p < 0.001$), and two times higher as compared to IL-4-primed cells (1.4 ± 0.22 vs 0.64 ± 0.1 kU/L, $p < 0.05$). In contrast, in healthy subjects, we did not observe any increase in IgE generation ($p > 0.05$; Fig. 1). Inactivated RV1B did not influence IgE synthesis by IL-4-primed PMBCs

Table 1. Clinical characteristics of patients included to the study

	Allergic patients	Healthy donors
Number	6	6
Women	3	4
Men	3	2
Age: mean $\pm$ SD (years)	41.1 ± 5.5	38.3 ± 4.0
(range)	(30–68)	(30–57)

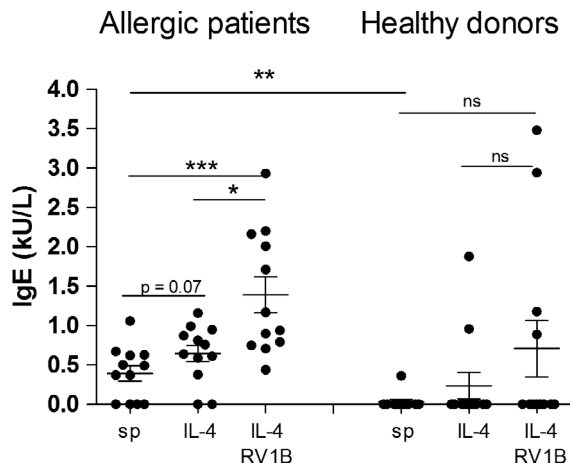


Fig. 1. RV1B-induced IgE synthesis by PBMCs from allergic patients and healthy donors – individual values. Mean $\pm$ SD, allergic patients $n = 6$, healthy donors $n = 6$; * $p < 0.05$, *** $p < 0.001$, ns, not significant.

neither in allergic patients, nor in healthy subjects, suggesting that RV1B-induced IgE synthesis requires active virus (data not shown).

In order to analyse whether TLR receptors may mediate RV1B-induced IgE synthesis observed in allergic patients, PBMCs were cultured with TLR3, TLR7/8 and TLR9 agonist mix (Poly:C, R484 and CpG DNA, respectively) added on the day 2 or day 7. We found that in allergic patients, TLR agonist mix added on the day 2 to PBMCs caused 2-fold decrease of IL-4-primed IgE synthesis ($p < 0.05$; Fig. 2). Induction of PBMCs with TLR agonist mix from the day 7 did not affect IgE synthesis (data not shown). In healthy subjects, TLR agonist mix decreased IgE

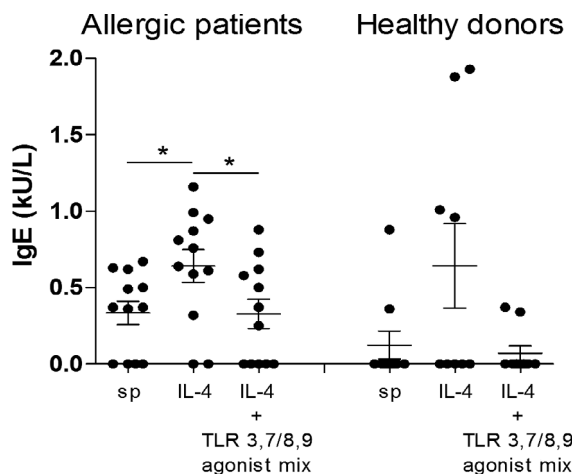


Fig. 2. The effect of TLR3 (PolyI:C), TLR7/8 (R484) and TLR9 (CpG DNA) agonist mix on IgE synthesis by PBMCs from allergic patients (A) and healthy donors (B). Mean $\pm$ SD, individual results, allergic patients $n = 6$, healthy donors $n = 6$; * $p < 0.05$.

synthesis only in these patients in which IL-4-primed IgE production.

This is the first study reporting that RV1B may significantly enhance IL-4-primed IgE synthesis by PBMCs from allergic patients, but not from healthy subjects. The magnitude of IgE synthesis in response to the induction with RV1B individually varies depending on virus MOI. Our observations are in line with results of Aab et al. showing that RV1B may increase IgM production and induce B-cell proliferation (11). However, the mechanism of both IgM and IgE synthesis enhancement by RV1B remains unknown. Interestingly, we noticed that RV1B added on the day 7 did not affect IgE synthesis in any MOI neither in allergic patients, nor in healthy subjects. This may suggest that RV1B may affect early mechanisms of IgE synthesis by PBMCs, possibly during class switching period. The increase of IgE synthesis by the rhinovirus shown in our study may be one of mechanisms involved in the virus-induced exacerbations of asthma (20,21).

Human rhinovirus was shown to modulate the epithelial cells immune response by stimulation of Toll-like receptors: TLR3, TLR7/8 and TLR9 in the endosomal compartment (22). In order to check if TLRs may be involved in IgE synthesis induced by RV1B, PBMCs were stimulated with TLR3, TLR7/8 and TLR9 agonists in the same manner as with RV1B, thus reproducing the effect of HRV pathogen-associated molecular patterns on innate receptors of immune cells. In contrast to the effect of RV1B, TLR agonist mix decreased IL-4-primed IgE synthesis in allergic patients and in those healthy subjects, in which IL-4 induced IgE synthesis. This data suggest that TLR3, TLR7/8 and TLR9 may not be involved in the induction of IgE synthesis caused by RV1B. These data are in line with Greiff et al. who showed that TLR7 stimulation led to the reduction of symptoms of allergic rhinitis (23).

In summary, results of our study suggest that HRV infections may affect IgE synthesis in allergic milieu, thus contributing to the development of IgE-mediated allergic responses.

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