Epinephrine drives human M2a allergic macrophages to a regulatory phenotype reducing mast cell degranulation in vitro

To the Editor,

As the prevalence of allergies rises, the impact of social factors such as physiological stress has gained much attention. While stress is suggested to exacerbate allergic conditions, including asthma and atopic dermatitis, less is known about the effect of acute stress mediator epinephrine on allergic M2a macrophages in a Th2 environment. This study aimed to investigate whether human M2a macrophages express adrenergic receptors to respond to epinephrine and what effect epinephrine could exhibit on M2a macrophages in an in vitro Th2 environment. We further assessed whether epinephrine-treated M2a macrophages could affect IgE-mediated degranulation in human mast cells in vitro.

To study the effect of epinephrine on human M2a macrophages, we isolated monocytes from healthy donors and matured them in the presence of M-CSF according to a standard protocol into monocyte-derived macrophages (M0). M0 were subsequently treated with IL-4 and IL-13 to differentiate them into M2a phenotype, which showed higher expression of CD206 marker and IL-10 production. Detailed information on this study is available in this article’s online supplementary information. The presence of the β2-adrenergic receptor (β2-AR) was confirmed in the M2a subtype, but no expression of α2A-AR, β1-AR, and β3-AR was detected (online repository; Figure S2D-E). The 16-hour treatment of M2a macrophages with 1 µmol/L epinephrine led to a significant upregulation of the cytokines IL-10 (P = .0131), TNF (P = .0012) and IL-6 (P = .0001), while no M1 marker IL-12 was detected (Figure 1A-D). This effect was not observed in the supernatants of M2a macrophages treated with the vehicle (negative control). Also, CD86 surface marker expression was significantly upregulated (P = .0313) (Figure 1G, Figure S3), indicating an antigen presentation capacity of this phenotype. Since epinephrine can induce cytokine production already after a few hours, we also observed the mRNA production of IL-10, IL-6, TNF, IL-1β , and CCL1 after 2 hours. Other M2 markers, including CCL2, CCL22, CCL18, and TGF-β, were less affected, and expression of IFN-γ was not detected after epinephrine treatment (Figure 2A). The production of anti-inflammatory IL-10 cytokine alongside IL-6, TNF, and IL-1β and upregulation of CD86 suggest that epinephrine can drive M2a macrophages towards an immunoregulatory M2b phenotype in vitro. Since the M2b phenotype is commonly induced by exposure to immune complexes and TLR ligands, which was not the case in our study, and we did not observe CCL1 production in the supernatants of epinephrine-treated M2a macrophages,1,2 we termed this immunoregulatory phenotype "M2b-like." It is important to note that the immunoregulatory function of this phenotype was confirmed in vitro on human cord blood–derived mast cells (CBMCs), where treatment with supernatants from epinephrine-treated M2b-like macrophages significantly reduced the IgE-mediated β-hexosaminidase degranulation (P = .0013). Interestingly, this effect was significantly pronounced compared to treatment with epinephrine alone (P ≤ .05) (Figure 2B).

To the best of our knowledge, this is the first report about the presence of the β2-AR receptor on the M2a macrophage phenotype, which is an important player in allergy. We, however, acknowledge that our study has its limitations. Although µM epinephrine in mouse cells can induce regulatory macrophages3 and dose-dependent studies of epinephrine on human monocytes revealed the strongest effect on chemokine/cytokine production in 1-10 µmol/L concentration range, often used to stimulate human monocytes in vitro,4,5 our results do not necessarily translate into real human setting. However, there is reason to believe that during stress the local epinephrine concentrations at the immunological synapse are higher than in circulation due to sympathetic neuronal discharge and local catecholamine production from neighbouring immune cells (previously termed “diffusely expressed adrenergic organ”). Another limitation of results (Figure 2A) is the normalization against a single housekeeping gene. We acknowledge that under given conditions, using a second gene for normalization had been advisable. This was a study on epinephrine effect on in vitro Th2 inflammation. To translate these data and develop targeted therapies in the future, it would be important to obtain the information on the exact signalling pathway that epinephrine might have activated on M2a human macrophages and drive the M2b-like phenotype. FcR signalling known to induce the M2b phenotype by activating phosphoinositide 3-kinase (PI3K)2, may be a possible pathway induced by epinephrine in our study; furthermore, catecholamine activation of a β2-AR noncanonical pathway through phosphoinositol 3-kinase (PI3K) induced regulatory macrophages in mice.3

Even though M2b-like macrophages retain the ability to produce many pro-inflammatory cytokines including IL-6, TNF, and IL-1β, the upregulation of IL-10 (IL-10<sub>IL-12<sub>10<sup>2</sup></sub></sub> is certainly a central part of this phenotype and in the range reported in the previous studies.1,2 Future studies should address the involvement of IL-10, but also IL-6, TNF, and IL-1β in the observed reduction of β-hexosaminidase production by CBMCs, as this was beyond...
FIGURE 1  Epinephrine effect on M2a cytokine production assessed by ELISA and surface marker expression assessed by flow cytometry. M2a macrophages (purple) were incubated overnight (16 h) with 1 µmol/L epinephrine (EPI) (teal) or vehicle (blue). IL-10 (A), IL-12 p70 (B), IL-6 (C) and TNF (D) cytokines were assessed in supernatants (mean ± SD of six independent donors), and CD11b (E), CD206 (F), CD86 (G), CD163 (H) and CD14 (I) surface expression (mean ± SD of six independent donors) was assessed on M2a macrophages (purple) or EPI-treated M2a (teal). ∆MFI is calculated after isotype control MFI subtraction.

FIGURE 2  Transcriptional profiling of epinephrine-treated vs untreated M2a macrophage genes (A) and FcεRI-mediated β-hexosaminidase release in CBMCs. (A) Incubation with 1 µmol/L epinephrine (EPI) for 30 min (purple bars) and 2 h (blue bars) (mean ± SD of three independent donors). (B) FcεRI-mediated β-hexosaminidase release assessed in CBMCs after overnight incubation with supernatants from M2a macrophages (blue bars), epinephrine-treated M2a macrophages (teal bars) or 1 µmol/L epinephrine (purple bars) (two different CBMC batches and three different PBMC donors (n = 6))
the scope of this work. However, IL-10 could be a possible target, since it was shown to suppress the FcεRI signalling pathway and reduce histamine release in CBMCs or to directly affect the FcεRI expression and reduce degranulation in human skin mast cells. Although mast cells are known to express β2-AR and can respond to epinephrine stimulation (control treatment; Figure 2B), the observed effect on degranulation of CBMCs with supernatants from M2b-like macrophages was significantly higher than the impact of epinephrine alone. Due to its short half-life and its instability under supernatant storage conditions (−20°C), epinephrine is not expected to be present in the supernatants of M2b-like macrophages.

In conclusion, the treatment of human allergic M2a macrophages with epinephrine led to a phenotypic switch to a macrophage subtype, which we term “M2b-like.” In vitro data suggest that the M2b-like phenotype suppresses the IgE-dependent release of inflammatory mediators from mast cells. In allergic patients, acute stress may drive the plasticity of macrophages towards a regulatory M2b phenotype and reduce allergic symptoms, but further studies are needed to translate the results of this in vitro study into real life. However, as recently demonstrated in a clinical study in which the effects of acute stress on skin prick testing greatly varied among individuals, the net outcome of short-term acute stress in patients seems to be more complex and also depends on coping mechanisms. Together, our findings support further studies on the role of acute stress mediators in allergies.

KEYWORDS
allergy, beta2-adrenergic receptor, epinephrine, M2a macrophages, mast cell

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To the Editor,

Platinums, consisting of carboplatin, cisplatin and oxaliplatin, are generally used as the front-line or second-line chemotherapy in ovarian, colorectal, endometrial, lung and pancreatic cancers. These drugs can cause immediate hypersensitivity reactions (HRs) in repeated cycles. In the majority of cases, it has been reported that a median of eight courses of carboplatin is required to induce an initial HR; however, in patients with BRCA1 and BRCA2 gene mutations, the risk of developing HRs is expected to be increased and can occur even with fewer exposures. The severity of symptoms may vary, but >50% of patients can develop at least moderately severe symptoms. Unfortunately, fatality has also been reported in case of carboplatin-induced HRs.

HRs caused due to platinum-based therapy can induce mast cell- or IgE-mediated mechanisms based on certain clues such as the clinical phenotype of patients, including the immediate occurrence of symptoms, the high rate of positivity in skin tests, the high levels of specific IgE in some patients and the prolonged time period required for sensitization, which corresponds to a six course of exposure particularly observed in carboplatin hypersensitivity.

HRs caused due to chemotherapeutics can limit their use, and alternative regimens may not serve as first-line therapy or may have less efficacy. The Brigham and Women’s Hospital Rapid Drug Desensitization Program (BWH), a 6-hour, 12-step rapid drug desensitization protocol, was introduced to enable the administration of platinums in patients with immediate HRs 15 years ago. 4

This protocol was based on in vitro and in vivo models that proposed that mast cells and basophils can be induced to predominantly inhibitory pathways by small incremental antigen doses, preventing the internalization of antigen/IgE/FcεRI complexes, calcium entry, deactivating signal transduction and mediator release. Additionally, the conversion of positive skin test results to a negative response after the RDD protocol in some patients who had tolerated complete doses of carboplatin confirms an antigen-specific cutaneous mast cell desensitization.

Although there is evidence indicating an important role of mast cells and basophils in the pathomechanism of RDD, the molecular...