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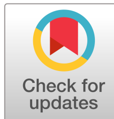

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Article type : Letter to the Editor

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

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Conflict of interest statement

Dr. Gotovina has nothing to disclose. Dr. Bianchini has nothing to disclose. Dr. Singer has nothing to disclose. Dr. Herrmann has nothing to disclose. Dr. Pellizzari has nothing to disclose. Dr. Haidl has nothing to disclose. Dr. Hufnagl has nothing to disclose. Dr. Karagiannis reports grants from NIHR Biomedical Research Centre at Guy's and St Thomas's Hospitals NHS Trust and King's College London, grants from Medical Research Council, grants from Breast Cancer Now, grants from CR UK/NIHR in England/DoH for Scotland, Wales and Northern Ireland Experimental Cancer Medicine Centre, grants from Cancer Research UK, grants from Guy’s and St Thomas’s Charity , during the conduct of the study; grants from IGEM Therapeutics Ltd, outside the submitted work; In addition, Dr. Karagiannis has a patent (IgE antibody technology) issued to IGEM Therapeutics Ltd. Dr. Marshall has nothing to disclose. Dr. Jensen-Jarolim reports grants and other from Biomedical International R+D GmbH, Vienna, grants and other from Bencard Allergie GmbH, Germany, other from Allergy Therapeutics Lt, UK, outside the submitted work.

Author contributions

J.G. wrote the article, carried out the experiments, analysed data and designed final figures; R.B. was involved in planning, supervision, and discussion of the findings of this work; J.F.S. assisted with immunofluorescence sample preparation and data analysis; I.H. assisted with sample preparation and phenotyping of M0 and M2a macrophages; G.P. assisted with RT-qPCR sample preparation for investigation on adrenergic receptor expression; I.D.H. helped supervise the CBMCs experiments; K.H. wrote the ethics project; S.N.K. supervised the experiments on M2a macrophages and adrenergic receptor expression; J.S.M. supervised the experiments on CBMCs; E.J.J. conceived the study and was in charge of overall direction and planning and helped in writing.

Keywords: allergy; beta2-adrenergic receptor; epinephrine; M2a macrophages; mast cell

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To the Editor,

As theprevalence of allergiesrises the impact of social factors such as physiological stresshave 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 Th2 environment. This study aimed to investigate whether human M2a macrophages express adrenergic receptors to respond to epinephrine and what effect couldepinephrine 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[1]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 theβ2-adrenergic receptor (β2-AR) was confirmed in M2a subtype, but no expression of α2A-AR, β1-AR and β3-AR was detected (online repository**Fig. E2d-e**).The 16h treatment of M2a macrophages with 1 μM epinephrineled to a significant upregulation of the cytokines IL-10 (p=0.0131), TNF (p=0.0012) and IL-6 (p=0.0001)while no M1 marker IL-12 was detected(**Fig. 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=0.0313) (**Fig. 1g, Fig. E3**) indicating an antigen presentation capacity of this phenotype. Since epinephrine can induce cytokine production already after few hours, we also observed the mRNA production of IL-10, IL-6, TNF, IL-1β and CCL-1 after 2 hours. Other M2 markers, including CCL2, CCL22, CCL18, and TGF-βwere less affected, and expression of IFN-γ was not detected after epinephrine treatment (**Fig. 2a**). The production of anti-inflammatory IL-10 cytokine alongside IL-6, TNF, IL-1β and upregulation of CD86 suggests that epinephrine can drive M2a macrophages toward an immunoregulatory M2b phenotype *in vitro*. Sincethe 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 CCL-1 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 macrophagessignificantly reducedtheIgE-mediated β-hexosaminidase degranulation(p=0.0013).Interestingly, this effect was significantly pronounced compared to treatment with epinephrine alone ($p \leq 0.05$) (**Fig. 2b**).

To the best of our knowledge, this is the first report about the presence of the β2-ARreceptor 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 macrophages[3]and

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does-dependent studies of epinephrine on human monocytes revealed the strongest effect on chemokine/cytokine production in 1-10μM concentration range, often used to stimulate human monocytes *in vitro*[4,5], our results do not necessarily translate into real human settings. However, there is reason to believe that during stress the local epinephrine concentrations at the immunological synapse are higher than in circulationdue to sympathetic neuronal dischargeandlocal catecholamine production from neighbouring immune cells (even termed “diffusely expressed adrenergic organ”[6]).Anotherlimitation of results (**Fig. 2a**) is the normalisation against a single housekeeping gene. We acknowledge that under given conditions,using a second gene for normalizationhad beenadvisable. This was a study on epinephrine effect on*in vitro* Th2 inflammation. To translate these data anddevelop 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. The FcR signallingknown to induce M2b phenotypeactivatesphosphoinositide 3-kinase (PI3K)[2]may also be apossible pathway in our study;furthermore catecholamine activation of β2-AR non-canonical pathway through phosphoinositol 3-kinase (PI3K) induced regulatory macrophages in mice[3].

Even thoughM2b-likemacrophages retain the ability to produce many pro-inflammatory cytokines including IL-6, TNF, and IL-1β, the upregulation of IL-10 ($IL\text{-}10^{high}/IL\text{-}12^{low}$) is certainly a central part of this phenotype and in the range reported in previous studies[1,7]. Future studies should address theinvolvement of IL-10, but also IL-6, TNF and IL-1β in the observed reductionof β-hexosaminidase production by CBMCs, as this was beyond the scope of this work.However, IL-10 could be a possible target, since it was shown to supress the FcεRI signalling pathway and reduce histamine release in CBMCs[8]or to directly affect the FcεRI expression and reduce degranulation in human skin mast cells[9]. Although mast cells are known to express β2-AR and can respond to epinephrine stimulation (control treatment**Fig. 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 supernatants 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 M2b-like phenotype suppress the IgE-dependent release of inflammatory mediatorsfrom mast cells. In allergic patients,acute stress may drive the plasticity of macrophages towards a regulatory M2b phenotype andreduce 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[10], 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.

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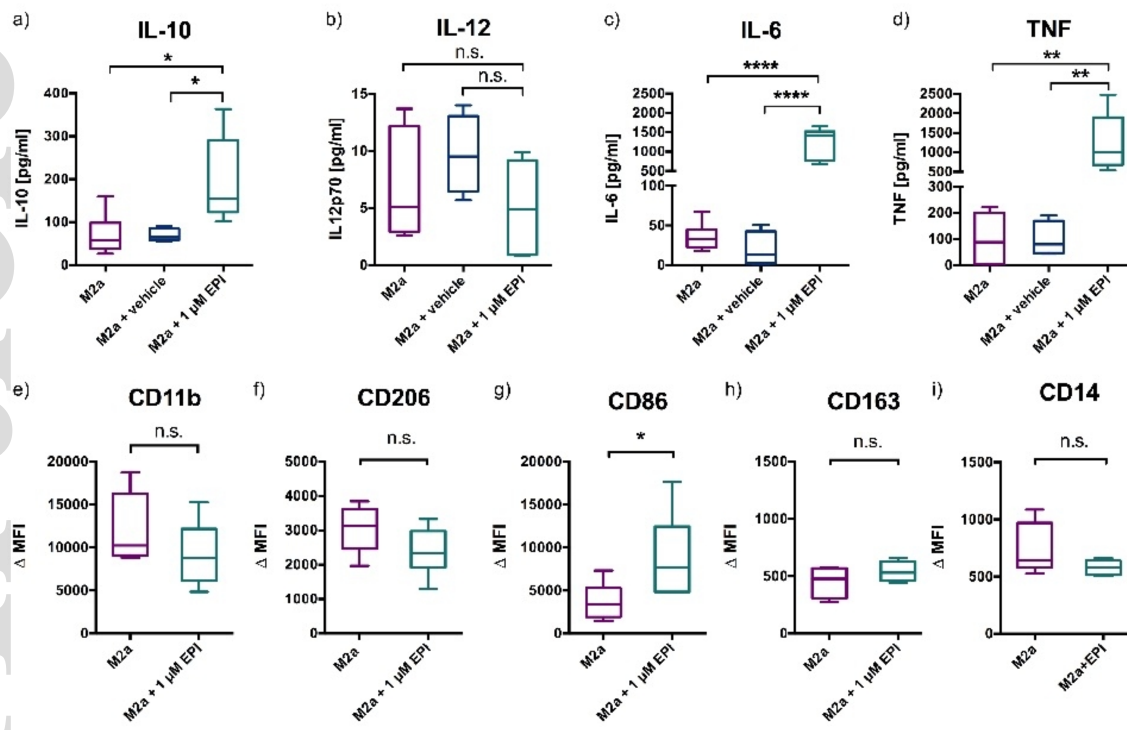

Fig. 1. Epinephrine effect on M2a cytokine production assessed by ELISA and surface marker expression assessed by flow cytometry. M2a macrophages (purple) were incubated overnight (16h) with 1 µM epinephrine (EPI) (teal) or vehicle (blue). IL-10 (a), IL-12 p70 (b), IL-6 (c) and TNF (d) cytokines were 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). ΔMFI is calculated after isotype control MFI subtraction

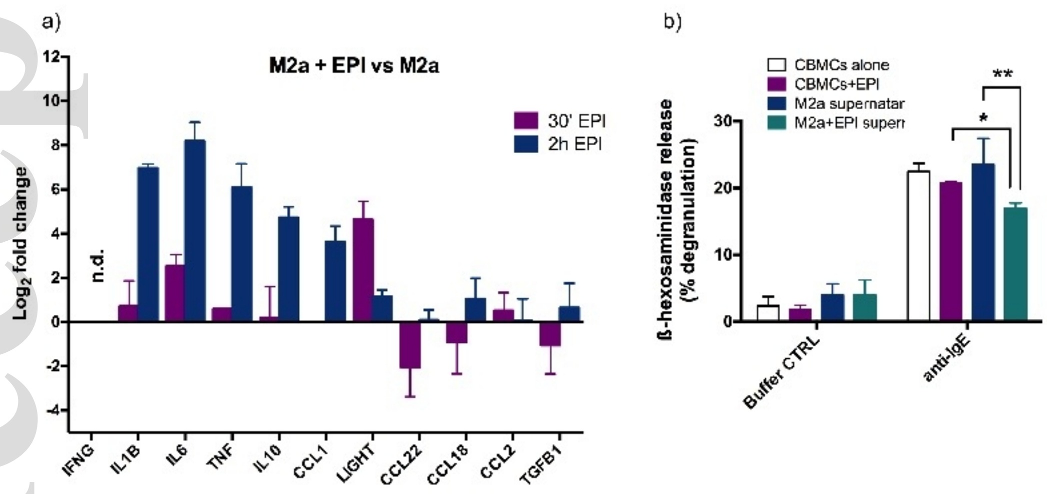

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Fig. 2. Transcriptional profiling of epinephrine-treated M2a genes (30 min and 2h) versus untreated M2a macrophage genes (a) and FcεRI-mediated β-hexosaminidase release in CBMCS (b). Incubation with 1 μM epinephrine (EPI) for 30 min (purple bar) and 2h (blue bar) (mean±SD of three independent donors) (a). FcεRI-mediated β-hexosaminidase release assessed in CBMCs after overnight incubation with supernatants from M2a macrophages (blue bar), epinephrine-treated M2a macrophages (teal bar) or with 1 μM epinephrine (purple bar) (two different CBMCs batches and three different PBMCs donors (n=6) (b).

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