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LETTER

Induction of immunosuppressive CD39 but not the trafficking C-C chemokine receptor 4 on Treg during LAR in mite allergic asthma

To the Editor,

The late asthmatic response (LAR) occurring during experimental allergic asthma is a recognized model for the study of asthma mechanisms.[1] It involves inflammatory processes, such as eosinophil recruitment and activation and mast cell and basophil activation, and activated lymphocytes and their Th2 cytokines.[2] It has been suggested that protective anti-inflammatory mechanisms, particularly Treg, are defective during the LAR phase.[3]

We aimed to study the (anti-)inflammatory systemic immune responses occurring at baseline and after mite exposure in allergic asthma in the early asthmatic response (EAR) vs. the LAR.

Seventeen GINA 1 well-controlled mite asthmatic subjects were included. They were exposed to placebo (saline) at visit 1 (V1) and to *Dermatophagoides pteronyssinus* (Dpt) (46 ± 20.5 ng/m^3) at visit 2 (V2) in ALYATEC environmental exposure chamber (EEC).[4] Blood samples were obtained before exposure (T0) and 6 hours after exposure (T6) at V1 and V2. Methods are detailed in the online supplement. Population characteristics are depicted in Table S1. Thirteen subjects (dual responders, DR) exhibited an EAR followed by an LAR and four (early responders, ER) an isolated EAR after Dpt exposure but not to placebo.

At baseline, discriminant variables between the DR and ER groups were the TregCCR4$^+$ (%Treg), CD4CCR4$^+$ (%CD4), TregCCR4$^+$ (%Treg)/CD4CCR4$^+$ (%CD4), Dpt skin prick test (SPT, mm), Dpt-specific IgE (kU/L), and TARC (pg/mL) (see online supplement and Figure S1).

After the Dpt challenge exposure in the EEC, we observed an increase in CCR4 expression on CD4$^+$ cells as well as increased IL-2 and IL-5 levels compared with placebo in DR, confirming the inflammatory response occurring during the LAR.[5] Among the immunosuppressive variables, we observed increased expression of CD39, which is related to Treg function,[6] in DR compared with placebo, but no significant change in Treg (%CD4), IL-10, and TGF-β levels, nor in the expression of CCR4 on Treg. Results are depicted in Figure 1. Other variables such as ICOS (%Treg), PD1 (%Treg), and the IFN and IL-4 cytokines and MDC, TARC, Eotaxin, and Rantes chemokines remained unchanged (Figure S2).

Thus, allergen exposure induced a typical inflammatory response during LAR that combined lung-homing T cells and IL2 and IL5 cytokines on one hand, and on the other the expression of CD39 on Treg cells. CD39 is an ectoenzyme that hydrolyzes adenosine triphosphate and adenosine diphosphate into adenosine monophosphate. It has been implicated in the suppressive function of Tregs in

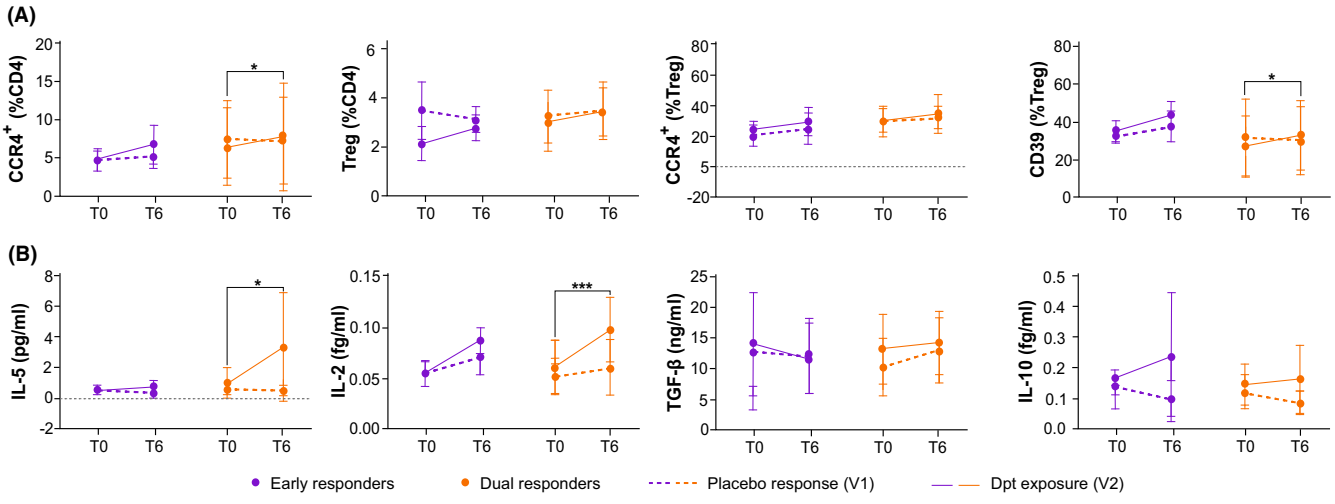

FIGURE 1 (Anti-)inflammatory systemic response. Evolution of variables between T0 and T6 according to the visit and the asthmatic response. T0: baseline; T6: 6 h after the early asthmatic response. (A) Lymphocytes, CD4$^+$, and Treg. (B) Cytokines

human. In a murine model of asthma, it has been shown that CD39$^+$ Treg cells control allergic inflammation,[6] but no data were available regarding allergen exposure in human. However, Treg (%CD4) and their expression of CCR4 and its ligands MDC and TARC, which induce Treg lung homing, remained stable. These observations may explain the lack of Treg recruitment to the lung during LAR that has been reported by Kinoshita et al. who showed that the ratio of the number of Treg cells to CD4+ cells at 24 h was significantly smaller in DR compared with that in ER. He also showed that the decrease of Treg at 24 h correlated with the magnitude of the LAR.[3] Results were reinforced by the analysis of the immunosuppressive: proinflammatory ratio and particularly the Treg:IL5, TregCCR4$^+$:IL2, and TGF_β:IL2 ratios, which showed an imbalance in favor of inflammation in DR (Figure S3). We cannot differentiate an allergen or a bystander effect on the observed inflammatory response but previous in vitro studies has shown that, mite-specific proliferative response of peripheral T lymphocytes correlated significantly with the magnitude of the LAR and with serum IL-5 after bronchial allergen challenge.[7]

The limitation of this study is the imbalance between the groups and the sample size requiring confirmation on a larger sample. However, our proportion of DR and ER subjects reflects the real-life situation, as it is accepted that around 60% of adult mite-asthmatic subjects exhibit a LAR.[1]

Taken together, our results suggest that DR subjects exhibited a higher T2 profile at baseline and after allergen exposure compared with ER subjects. In the stable state, but not during a LAR, the immune response is balanced by CCR4-expressing Treg cells, although Treg express immunosuppressive CD39 during a LAR.

AUTHORS CONTRIBUTIONS

VD, FdB, ND: conception and design of the study; ND, AP, VD: acquisition and analysis of the data; VD, MM, FB: analysis of the data; VD, FdB, AP, NK: interpretation of the data; VD, AP, FdB: writing the paper. All the authors have contributed to the conception and revision of the manuscript and have approved the submitted version.

FUNDING INFORMATION

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CONFLICT OF INTEREST STATEMENT

ND is cofounder and an Alyatec employee. FdB is a medical expert, cofounder, and shareholder of Alyatec. FdB reports grants from Aimmune, Stallergenes Greer, Chiesi, and Regeneron, and personal fees from ALK-Abello, Mundipharma, and Novartis, and is a member of the Board at Stallergenes Greer, Novartis, ALK-Abello, Mundipharma, Medapharma, Boehringer, AstraZeneca, and Calor. VD, AP, MMB, FB, and NK: none.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Virginie Doyen[1]
Anh Poirot[2]
Myriam Maumy-Bertrand[3,4]
Frederic Bertrand[3,4]
Naji Khayath[2,5,6]
Nathalie Domis[7]
Frederic de Blay[2,5,6,7]

[1]*Department of Chest Medicine, CHU UCL Namur, Université Catholique de Louvain, Yvoir, Belgium*
[2]*Chest diseases Department, Strasbourg University Hospital, Strasbourg, France*
[3]*LIST3N, Université de Technologie de Troyes, Troyes, France*
[4]*IRMA, CNRS UMR 7501, Labex IRMIA, Université de Strasbourg, Strasbourg, France*
[5]*EA 3070 Federation of Translational Medicine, FHU Homicare, University of Strasbourg, Strasbourg, France*
[6]*CRISALIS / F-CRIN INSERM Network, Toulouse, France*
[7]*ALYATEC, Environmental Exposure Chamber, Strasbourg, France*

Correspondence
Virginie Doyen, Department of Chest Medicine, CHU UCL Namur, Université Catholique de Louvain, Yvoir, Belgium.
Email: virginie.doyen@chuuclnamur.uclouvain.be

ORCID

Frederic de Blay https://orcid.org/0000-0001-5678-2214

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.