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COMMENTARY:

Exploiting the innate immune system to control allergic asthma

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NKT cells are key actors at the interface between innate and acquired immunity. Indeed, upon activation by glycolipid antigens, they rapidly secrete both Th1 and Th2 cytokines, which affects the development of later immune responses. Previous studies have shown that NKT cells are essential for the development of allergic asthma, a prototypical Th2-mediated pathology. By contrast, three papers, two of which are published in this issue of the *European Journal of Immunology*, demonstrate, in mouse models, that treatment with α -galactosylceramide, a specific NKT ligand, inhibits most of the parameters associated with the disease, including airway hyperreactivity, eosinophilia and IgE production. Increased IFN- γ synthesis, rather than regulatory IL-10, accounts for this paradoxical effect.

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Allergic asthma is a multifactorial respiratory disease involving both genetic and environmental factors which, in combination, lead to the development of a strongly polarized Th2 immune response towards innocuous antigens, characterized by IgE production and tissue inflammation including eosinophilia [1, 2]. In airways, inflammation is accompanied by tissue remodelling (fibrosis) and mucus hypersecretion leading to airway hyperreactivity (AHR). In the classical view, the immunological factors involved in the development of asthmatic reactions include, but are not limited to, IL-5

(which is associated with eosinophilia) plus IL-4 and IL-13 (which are individually or jointly associated with IgE production). At the cellular level, in addition to bronchial epithelial cells which contribute to the pathology, DCs (prototypical antigen presenting cells) are considered to be important actors in the initiation and, according to some authors, the maintenance of the inflammatory phenotype [3]. CD4⁺ lymphocytes provide the necessary help to B cells for IgE production, while some CD4⁺ subpopulations are endowed with a regulatory role [4]. The role of eosinophils, as the main effector cells, is more controversial and greatly depends upon the species and/or the experimental model [5–7]. Mast cells (and basophils) potentiate the disease by releasing eosinophil chemoattractants, including histamine, which also affects polarization of the immune response [8]; however, mast cells/basophils, through expression of various membrane receptors, particularly toll-like receptors (TLR), might also participate in the innate immune response and play a regulatory role [9].

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Abbreviations: α -GalCer: α -galactosylceramide · AHR: airway hyperreactivity

Likewise, CD8⁺ lymphocytes have been demonstrated to be either protective [10] or detrimental to the pathology [11]. Along the same lines, three papers [12–14], including two published in this issue of the *European Journal of Immunology* [12,13], demonstrate that NKT cells appear to play a dual role in asthmatic pathology.

NKT cells classically encompass distinct subpopulations [15]. The major subpopulation being invariant NKT cells (referred to as NKT cells from now on in this manuscript), a CD1d-restricted lymphocyte subpopulation arising in the thymus from double positive precursors and expressing a semi-invariant TCR (V α 14J α 18, V β 8,7,2 in mice and V α 24-J α Q, V β 11 in humans) able to recognize glycolipid antigens, in particular α -galactosylceramide (α -GalCer), extracted from a marine sponge [16], bacterial glycosylceramide [17] and mammalian isoglobotrihexosylceramide [18]. Besides their cytotoxic properties, NKT cells are characterized, as innate immune cells, by the very rapid production, upon TCR stimulation, of high amounts of both Th1 (IFN- γ) and Th2 (IL-4 and IL-13) cytokines [19], which enables these cells to regulate asthmatic reactions. Indeed, using NKT-deficient mice (J α 18^{-/-}, J α 281^{-/-} and Cd1d^{-/-}), two groups independently demonstrated that NKT cells were essential for the development of AHR and other asthma-associated parameters [20–22].

In contrast, three papers now demonstrate that NKT cell activation by α -GalCer, whose administration suppresses tumor growth and autoimmune pathologies in various experimental models, inhibits allergic airway inflammation in an IFN- γ -dependent manner [12–14]; however, the papers differ regarding some of the parameters affected by the treatment, as well as the mechanisms involved. Hachem *et al.* [12] demonstrate that both intranasal and intravenous treatments with α -GalCer immediately prior to intranasal antigen-challenge inhibit AHR, eosinophilia (and IL-5) and IgE (and IL-4) production, an effect abrogated by treatment with anti-IFN- γ but not with anti-IL-10 receptor. The effect of α -GalCer injection is mimicked by the transfer of sorted NKT cells or splenocytes from α -GalCer-treated wild type (WT) but not α -GalCer-treated J α 18^{-/-} or IFN- γ ^{-/-} animals. The total NKT cell frequency and cytokine balance within lung or spleen NKT cells was directly affected by the treatment, with a decreased IL-4-producing and increased IFN- γ -producing NKT cell frequency. In a model using aerosol antigen challenges, Matsuda *et al.* [14] demonstrate that, in addition to the parameters mentioned above (with the exception of IgE concentrations), IL-13 production and goblet cell hyperplasia are also decreased upon intraperitoneal α -GalCer injection, while IgG_{2a} (a Th1 isotype in mouse) levels are increased. Most of these effects are abrogated in IFN- γ ^{-/-} animals, again suggesting a Th2 to Th1 shift

in the immune response. Intriguingly, Morishima *et al.* [13] demonstrate the opposite effects of α -GalCer treatment with continuous α -GalCer administration increasing eosinophilia and IgE production, while a single subcutaneous delivery at the time of intranasal antigen challenge abrogates eosinophilia, fibrosis and AHR in WT but not in J α 281^{-/-} mice, which nevertheless develop AHR in contrast to previous findings. A stronger sensitization protocol and a more Th2-prone background might explain these differences. In addition to the altered Th1/Th2 balance observed following α -GalCer treatment, VCAM-1 expression, which promotes eosinophil recruitment, is inhibited by α -GalCer treatment. Interestingly, while the two first papers [12,14] indicate a direct effect of α -GalCer on cytokine production by NKT cells, the last one [13] shows that NK, rather than NKT, cells account for the increased IFN- γ production. It is likely that NKT cells are involved in the initial trigger of the process thanks to a very rapid, but transient, cytokine production, while NK cells have been associated with induction of asthmatic pathology [23].

In the light of these new findings, it appears that NKT cells might exert antagonistic effects on the outcome of asthma, at least in mouse experimental models. Various parameters might account for these contrasting results: the nature, strength and to a lesser extent the timing and means of delivery of the triggering signal. Indeed, in experiments based on the comparison between NKT-deficient and WT mice and those demonstrating the essential role of NKT in the development of antigen-induced hypersensitivity, NKT triggering is indirect. It is suspected that sensitization by a protein antigen induces release/unmasking of a glycolipid self antigen (possibly isoglobotrihexosylceramide), which activates NKT cells to produce predominantly IL-4 and IL-13. NKT cell stimulation by DC- and monocyte-produced IL-18 might also contribute to the Th2 polarization [24]. Stimulation with α -GalCer provides direct activation, which leads to significant IFN- γ production, first by NKT cells, then by NK, DC and other cell types, shifting an otherwise Th2 polarized response and thus decreasing the pathology. This effect is analogous to the beneficial effect of α -GalCer treatment on anti-tumor responses [19] and on immunity against infectious agents such as *Plasmodium yoelii* and *berghei* [25]; however, it differs from the IL-4- (and to some extent IL-10-) dependent protective effect observed in most autoimmune diseases such as diabetes and experimental autoimmune encephalomyelitis [16]. Finally, besides the total amount and the bioavailability of α -GalCer received by the animals, the opposite effects of continuous α -GalCer delivery during the entire course of antigen sensitization and challenge *versus* a single administration immediately prior to challenge might be due to the very different immune status of the animals, in particular the cytokine environment, at the time of

delivery. In the first instance, α -GalCer is delivered to naïve animals, while in the second case α -GalCer is delivered to ovalbumin/alum primed and boosted animals. Furthermore, repeated α -GalCer administrations are known to promote IL-4 synthesis [24].

In humans, the link between NKT cells and atopic/asthmatic status is far from clear [26]. In addition to its potential adverse effects, it is therefore premature to conclude from the three papers [12–14] discussed in this Commentary that α -GalCer might soon be used as a drug against asthma.

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