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Cutting Edge: Intravenous Ig Inhibits Invariant NKT Cell-Mediated Allergic Airway Inflammation through FcγRIIIA-Dependent Mechanisms

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Despite their increasing use in autoimmune, inflammatory, and allergic conditions, the mechanism of action of i.v. IgG (IVIg) is poorly understood. On the basis of the critical role of invariant NKT (iNKT) cells in allergic airway inflammation (AAI) and their constitutive expression of the low-affinity IgG receptor FcγRIIIA, we surmised that IVIg targets iNKT cells to exert their anti-inflammatory effect. We found that IVIg treatment significantly inhibited AAI in OVA-sensitized C57BL/6 mice and downregulated α-galactosylceramide–induced iNKT cell activation and cytokine production. Allergic responses were restored in iNKT cell-deficient mice by transferring iNKT cells from PBS- but not from IVIg-treated mice, suggesting that IVIg acts directly on activated iNKT cells that have a critical role in AAI. The inhibitory effects of IVIg on both iNKT cell activation/function and OVA-driven AAI were lost in FcγRIIIA−/− mice. Our data unravel an FcγRIIIA-dependent inhibitory effect of IVIg on activated iNKT cells that confers protection in AAI. The Journal of Immunology, 2011, 186: 000–000.

Intravenous Ig (IVIg) is a therapeutic molecule prepared from pools of plasma obtained from several thousand healthy blood donors. In addition to its use in the treatment of primary and secondary immune deficiencies, IVIg has been used as a therapeutic option in a large number of autoimmune, allergic, and inflammatory conditions including idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, myasthenia gravis, and Kawasaki disease (1–6). Considering studies conducted in mouse models, several mutually non-exclusive mechanisms may account for the therapeutic effects of IVIg (1–3, 7–11). It is believed that the beneficial effect of IVIg may be related to its content of natural polyreactive Abs reactive with a large number of immunologically relevant molecules (12–14). Notably, anti-inflammatory activities of IVIg are mediated through both FcγRIIB- and/or FcγRIIIA-dependent and FcγR-independent mechanisms. Thus, IVIg has been shown to exert a regulatory role on both the humoral and cellular compartment of the immune system.

Invariant NKT (iNKT) cells are innate lymphocytes that recognize glycolipids presented by the nonclassical MHC class Iib family member CD1d (15–17). iNKT cells release massive amounts of IFN-γ following activation through their semi-invariant TCR, but they are also unique in their ability to secrete high amounts of IL-4 in addition to other Th2 cytokines such as IL-13 (16, 18–21). iNKT cells are therefore implicated in the physiopathology of several autoimmune and inflammatory diseases (19, 22, 23), including allergic airway inflammation (AAI), in asthma (18, 24, 25).

Allergic asthma is a chronic inflammatory disease of lung, the incidence and morbidity of which has increased dramatically in the past few decades, especially in industrialized countries (26, 27). Asthma is a heterogeneous and multifactorial disease and is characterized by an airway hyperreactivity in response to...
a variety of specific and nonspecific stimuli, mucus hypersecretion, pulmonary eosinophilia, Th2 cytokine production, hyperplasia and hypertrophy of smooth muscle, and elevated concentrations of serum IgE (28). IVlg has been considered as one of the therapeutic options in the treatment of asthma, although the underlying mechanisms have not been explored (29, 30). Given the critical role of iNKT cells in AAI, we surmised that IVlg targets this cellular population to exert its anti-inflammatory effect.

Materials and Methods

Mice

Six- to 8-wk-old male C57BL/6 wild-type (WT) and Jα18−/− mice were bred and maintained in our animal facility under specific pathogen-free conditions. FcyRIIA−/− mice (N7 C57BL/6J) were purchased from The Jackson Laboratory. Jα18−/− mice were backcrossed for 13 generations onto a C57BL/6 background. Animal experiments were performed according to the French Institutional Committee.

Induction of AAI and IVlg treatment

Mice were sensitized with OVA (100 μg, i.p., per mouse; Sigma-Aldrich) absorbed to 1.6 mg aluminum hydroxide gel (Merck). After 7 d, mice were exposed to intranasal Ag (50 mg/d for OVA) or PBS on 3 consecutive d. Inhibition of AAI by IVlg in OVA-sensitized mice was assessed by injecting IVlg (10 mg, i.p., per mouse) 1 h prior to the first of the three consecutive challenges with OVA. In some experiments, OVA-sensitized WT or Jα18−/− mice were adoptively transferred with total splenocytes (2.5 × 106 iNKT cell equivalents) or sorted iNKT cells (2.5 × 106) from WT mice that were pretreated with PBS or IVlg 1 h prior to sacrifice and transfer. Bronchoalveolar lavage fluid (BALF) and lungs were recovered 24 h after the last challenge. Total and differential cell counts were determined in the BALF, and histological analysis of lung sections was evaluated in a blinded fashion by a histopathologist (M.H.) as previously described (25). Peribronchial and perivascular inflammation was graded on a subjective scale of 0, 1, 2, and 3 corresponding to no, mild, moderate, or severe inflammation, respectively, with an increment of 0.5 if the inflammation fell between two integers.

Analysis of iNKT cells

The proportion of iNKT cells as well as their surface expression of CD69 was determined in splenocytes by flow cytometry using fluorochrome-conjugated anti–TCR-α (clone H57-597), anti-CD5 (clone 55-7.3), and anti-CD69 (clone H1.2F3) from BD Biosciences (Le Pont de Claix, France) and an α-GalCer (CD1d: allophycocyanin-conjugated [clone H1.2F3] from BD Biosciences (Le Pont de Claix, France) and an α-GalCer [24, 25]) provided by the National Institutes of Health Tetramer Core Facility. To assess iNKT cell activation in vivo, mice received an injection of α-GalCer–pulsed dendritic cells (DC) for 48 h (20).

Levels of cytokines in the BALF, homogenate lung, serum, and cell-free culture supernatants were assessed using ELISA Duoset kits (R&D Systems).

Statistical analysis

Data are expressed as the mean values ± SEM for each group of mice. We analyzed statistical comparisons with two-tailed unpaired Student t test and Mann–Whitney test. We considered a p value <0.05 significant.

Results and Discussion

IVlg inhibits airway inflammation in OVA-sensitized and challenged mice

We first examined the protective effect of IVlg on the evolution of AAI in OVA-sensitized C57BL/6 mice. As shown in Fig. 1, IVlg treatment inhibited the recruitment of inflammatory cells in the airway passage. Thus, the total number of infiltrating cells (Fig. 1A) and, in particular, the percentage (Fig. 1B) and number (Fig. 1C) of eosinophils, one of the major mediators of allergic responses, was significantly downregulated following IVlg treatment. Despite the notion that C57BL/6 strain develops poor airway hyperreactivity compared with other strains (24, 25), IVlg treatment of OVA-sensitized C57BL/6 mice led to a significant decrease of this cardinal feature of asthma (Supplemental Fig. 1A). However, the production of pro-Th2 cytokines was unaffected by IVlg (Supplemental Fig. 1B). These results thus document the therapeutic benefit of IVlg in severe childhood asthma (4, 29, 30).

IVlg downregulates the activation and function of iNKT cells

 Activated iNKT cells have been shown to play a crucial role in development of allergic asthma following OVA sensitization and challenge (18, 24, 25). Absence of iNKT cells indeed abolishes experimental AAI. In view of the observed protective effect of IVlg in AAI, we asked whether IVlg treatment has repercussion on the activation and cytokine production by iNKT cells. Following injection of naive or α-GC–pretreated mice with IVlg, we assessed the expression level of the activation marker CD69 on iNKT cells. As shown in Fig. 1D, IVlg treatment significantly downregulated the mean fluorescence intensity (MFI) of CD69 on iNKT cells both in naive or α-GC–pretreated mice. Furthermore, IVlg treatment also reduced significantly the serum levels of IL-4 (Fig. 1E) and IFN-γ (Fig. 1F) following in vivo activation of iNKT cells by α-GC. Moreover, in vitro addition of IVlg alleviates...
IVlg treatment blocks the capacity of iNKT cells to restore AAI

Jα18<sup>−/−</sup> mice lack iNKT cells and are resistant to AAI (18, 24). To evaluate whether IVlg-mediated protection in AAI is due to its direct effect on pathogenic effector iNKT cells, we adoptively transferred iNKT cells from PBS- or IVlg-treated WT mice into iNKT cell-deficient Jα18<sup>−/−</sup> mice. As expected, OVA-presensitized Jα18<sup>−/−</sup> mice that did not receive iNKT cells did not experience AAI upon Ag challenge. OVA-presensitized Jα18<sup>−/−</sup> mice that received total splenocytes or purified iNKT cells from PBS-treated WT mice developed airway inflammation as evidenced by massive lung infiltration by inflammatory cells, including eosinophils (Fig. 2A, 2B). Interestingly, animals that were transferred with iNKT cells from IVlg-treated WT animals showed >50% reduction in total cell infiltration and eosinophilia upon Ag challenge (Fig. 2A, 2B). This result demonstrates that the inhibitory effect of IVlg on AAI can be transferred by iNKT cells and that iNKT cells are therefore less pathogenic upon IVlg treatment.

Once pharmacologically activated by their ligand, iNKT cells are protective rather than pathogenic during AAI (31, 32). For this reason, we also examined whether IVlg-preconditioned iNKT cells were able to transfer protection to normal naive mice upon challenge with OVA. To address this issue, we transferred IVlg-preconditioned iNKT cells from naive mice to OVA-presensitized recipient animals. Neither PBS- nor IVlg-preconditioned iNKT cells from naive mice were able to confer protection to recipient mice upon

production of IL-4 by sorted iNKT cells that were activated by α-GC–loaded DC (Supplemental Fig. 2). These data indicate that IVlg modulates the activation and function of iNKT cells.
challenge with OVA (Supplemental Fig. 3). These results demonstrate that IVIg has a suppressive effect on effector iNKT cells but is unable to modulate these cells and render them protective.

The inhibitory effect of IVIg on activated iNKT cells and AAI is FcRIIIA dependent

The blockade of cell-surface FcyR is a possible mechanism for the anti-inflammatory properties of IVIg. In view of the fact that iNKT cells constitutively express surface FcγRIIIA but not FcyRI, -IIb, or -IV (33), we examined the anti-inflammatory effect of IVIg on iNKT cells in FcγRIIIA−/− mice. iNKT cell counts were normal in FcγRIIIA−/− mice compared with WT mice (Supplemental Fig. 4A, 4B). In addition, iNKT cells from both strains produced similar levels of IL-4 after in vitro stimulation with α-GC–pulsed DC, suggesting normal functioning of iNKT cells in FcγRIIIA−/− mice (Supplemental Fig. 4C).

To determine whether FcγRIIIA are involved in the inhibitory effect of IVIg on iNKT cells, FcγRIIIA−/− and WT mice were injected with IVIg 1 h before α-GC treatment. Interestingly, the inhibitory effect of IVIg on iNKT cell activation as measured by the expression of CD69 (Fig. 3A) and by the production of IL-4 (Fig. 3B) and IFN-γ (Fig. 3C) was lost in FcγRIIIA−/− mice as compared with PBS-treated mice. These findings indicate that the inhibitory effect of IVIg on iNKT cells requires FcγRIIIA.

These findings led us to determine whether IVIg inhibits iNKT cell-mediated AAI through FcγRIIIA-dependent mechanisms. Consistent with this view, we found that FcγRIIIA deficiency led to a quasicomplete loss of the anti-inflammatory effect of IVIg treatment in OVA-sensitized and challenged animals (Fig. 4). Indeed, the total number of infiltrating cells (Fig. 4A) and eosinophils (Fig. 4B), as well as peribronchial and perivascular inflammation (Fig. 4C–K), was significantly decreased following IVIg treatment in WT mice but not in FcγRIIIA−/− mice. It is of note that WT and FcγRIIIA−/− mice had a similar capacity to develop AAI in response to OVA immunization and challenge. Together, our data demonstrate an FcγRIIIA-dependent inhibitory effect exerted by IVIg on activated iNKT cells that confers protection in AAI. Together, these data allow us to infer that the beneficial effect of IVIg on iNKT cell-mediated allergic airway inflammation implicates FcγRIIIA, although we do not provide evidence of a direct effect of IVIg on FcγRIIIA expressed by iNKT cells in vivo in our model.

A well-accepted paradigm is that the inhibitory functions of IgG are mediated by FcγRIIB, which contains a cytoplasmic immune tyrosine-based inhibitory motif that recruits phosphatases, leading to suppression of intracellular signal transduction (34). By contrast, FcγRIIIA is established as an activating receptor that is required for multiple Ab–immune complex-dependent inflammatory responses in vitro and in vivo. However, Park-Min et al. (35) have shown that inhibition of IFN-γ responses by immune complexes is independent of FcγRIIB. These authors revealed that immune complexes that are contained in IVIg inhibit IFN-γ signaling in macrophages by mechanisms dependent on FcγRIIIA, although being an activating receptor, and suggest that inhibition of IFN-γ signaling contributes to the therapeutic efficacy of IVIg (35, 36). Consistent with these data, our results demonstrate an inhibitory role for FcγRIIIA in IVIg-mediated suppression of iNKT cell activation. This suppressive effect on iNKT cells contributes to the anti-inflammatory effects of IVIg in airway inflammation. Interestingly, the NK cell-dependent effect of IVIg has also been suggested to be of relevance in the prevention of recurrent pregnancy loss (5). Strategies aimed at functional inhibition of iNKT cells by targeting through IVIg alone or in combination with other appropriate therapeutics should help in paving the way for fighting allergic conditions and in maintaining a critical balance between tumor immunity and self-tolerance.

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Disclosures

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References


