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Genetic restriction of antigen-presentation dictates allergic sensitization and disease in humanized mice

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ABSTRACT

Background: Immunoglobulin(Ig)E-associated allergies result from misguided immune responses against innocuous antigens. $CD4^+$ T lymphocytes are critical for initiating and perpetuating that process, yet the crucial factors determining whether an individual becomes sensitized towards a given allergen remain largely unknown.
Objective: To determine the key factors for sensitization and allergy towards a given allergen.
Methods: We here created a novel human T cell receptor(TCR) and human leucocyte antigen (HLA)-DR1 (TCR-DR1) transgenic mouse model of asthma, based on the human-relevant major mugwort (*Artemisia vulgaris*) pollen allergen Art v 1 to examine the critical factors for sensitization and allergy upon natural allergen exposure *via* the airways in the absence of systemic priming and adjuvants.
Results: Acute allergen exposure led to IgE-independent airway hyperreactivity (AHR) and T helper(Th)2-prone lung inflammation in TCR-DR1, but not DR1, TCR or wildtype (WT) control mice, that was alleviated by prophylactic interleukin(IL)-2-αIL-2 mAb complex-induced expansion of Tregs. Chronic allergen exposure sensitized one third of single DR1 transgenic mice, however, without impacting on lung function. Similar treatment led to AHR and Th2-driven lung pathology in >90% of TCR-DR1 mice. Prophylactic and therapeutic expansion of Tregs with IL-2-αIL-2 mAb complexes blocked the generation and boosting of allergen-specific IgE associated with chronic allergen exposure.
Conclusions: We identify genetic restriction of allergen presentation as primary factor dictating allergic sensitization and disease against the major pollen allergen from the weed mugwort, which frequently causes sensitization and disease in humans. Furthermore, we demonstrate the importance of the balance between allergen-specific T effector and Treg cells for modulating allergic immune responses.

1. Introduction

Immunoglobulin(Ig)E-associated allergic diseases are characterized by an aberrant immune response to usually innocuous environmental antigens (Larche et al., 2006). While major effector mechanisms of the disease are triggered by allergen-specific IgE antibodies, effector T lymphocytes play a pivotal role in the initiation and propagation of the allergic phenotype (Romagnani, 2004; Valenta et al., 2018). Apart from the recently discovered type 2 innate lymphoid cells (ILC2) (Maggi et al.,

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2017), $CD4^+$ T helper cells represent the main source of interleukins (IL)-4 and IL-13, which promote immunoglobulin class switching towards IgE (Larche et al., 2006). In addition, results from clinical studies clearly demonstrated that T cells also play a major role in late-phase and chronic allergic reactions contributing to organ pathology in the airways, skin and gastrointestinal tract (Haselden et al., 1999; Karlsson et al., 2004; Werfel, 2009).

One major question is why certain individuals develop an allergic sensitization towards certain allergens. There are at least three mutually not exclusive hypotheses to answer this question: First, it is possible that certain individuals are genetically prone to preferentially recognize certain allergens. In fact, early studies in patient populations suffering from allergy to pollen (ambrosia, birch, mugwort), animal dander (cat) and mold (*Alternaria*) provided evidence that allergen-specific IgE production could be MHC-restricted (Fischer et al., 1992; Jahn-Schmid et al., 2005; Marsh et al., 1982; Young et al., 1994). This was confirmed when T helper cell clones from allergic patients were isolated and the existence of an allergen-specific genetic restriction of the allergen-specific immune response was demonstrated. For example, Th cell clones specific for the major mugwort (*Artemisia vulgaris*) pollen allergen Art v 1 were found to recognize one major T cell epitope, *i.e.,* Art v $1_{25–36}$, in the context of a dominant MHCII allele, *i.e.,* HLA-DR1 (Jahn-Schmid et al., 2005; Jahn-Schmid et al., 2002).

The second possibility why certain subjects develop allergy towards a given allergen would be an imbalance between effector and regulatory T cell responses towards the allergen. A study analyzing the frequency of IL-4 producing $CD4^+$ T effector cells (Teff) and IL-10-producing T regulatory cells (Treg) in allergic and non-allergic subjects suggested that allergic subjects present with higher numbers of IL-4-producing $CD4^+$ effector cells whereas IL-10-producing allergen-specific Tregs are increased in non-allergic subjects (Akdis et al., 2004). Since it was then demonstrated that $CD4^+CD25^{high}Foxp3^+$ allergen-specific Treg cells are present and functionally active in both non-atopic and atopic individuals the question regarding the specific contributions of allergen-specific $CD4^+$ effector cells and Tregs in the regulation of the allergen-specific IgE response arises. In fact, it is well established that extrathymically induced Treg subsets but also Tregs engineered by overexpression of the transcription factor *FOXP3* are extremely potent in controlling T cellular immune responses against environmental antigens including allergens (Schmetterer et al., 2011a, b; Shevach and Thornton, 2014; Verhagen et al., 2015). Moreover, expansion of $CD4^+$ Treg using immune-complexes of IL-2 and anti-IL-2 antibodies, can be used to treat hypersensitivity diseases but also transplant rejection in experimental settings (Shevach, 2012; Webster et al., 2009).

Recently, another provocative possibility for developing allergy against a given allergen was introduced. It was claimed that the intrinsic properties of allergens (Bacher et al., 2016) are pivotal for the development of tolerance *versus* allergy against aeroallergens. Specifically, it was suggested that allergens, which rapidly dissociate from inhaled particles (*e.g.*, pollen) and become soluble in aqueous solutions, escape Treg-mediated suppression and thus drive allergen-specific Teff responses and allergic sensitization (Bacher et al., 2016).

In order to investigate the three hypotheses for allergic sensitization and to decipher the contribution and interplay of i) MHC-dependent recognition of allergen-specific T cell epitopes, ii) the corresponding activation and expansion of allergen-specific Teff as well as Treg cells in the allergic sensitization process and in immune pathology and iii) the role of natural antigenic exposure, we established a unique humanized mouse model. This model is based on a human-relevant major pollen allergen and simultaneous expression of the corresponding human T cell receptor (TCR) and human leucocyte antigen (HLA) molecules. We selected mugwort allergy driven by the major mugwort pollen allergen of *Artemisia vulgaris*, Art v 1, as the human-relevant model system. Mugwort represents an important aeroallergen source growing in the European temperate climate zone, throughout North America and parts of Asia (Charpin et al., 1974; D'Amato et al., 1998; Wopfner et al., 2005). It is one of the main causes of hay fever and asthma in late summer and fall (Himly et al., 2003; Torio et al., 2003). Notably, sensitization to mugwort nearly exclusively depends on the major mugwort pollen allergen Art v 1 (in 95% of affected allergic individuals) and the presentation of its immunodominant T cell epitope, Art v $1_{25–36}$, which is highly restricted by HLA-DRA*01-HLA-DRB1*01 (Jahn-Schmid et al., 2002, 2005). Importantly, an unusually high odds-ratio of 8.45 (range 4–17) for the presence of the HLA-DRB1*01 allele and the probability of getting sensitized against mugwort (Art v 1) was observed by comparing mugwort allergic patients with healthy control populations (Jahn-Schmid et al., 2005). This represents the single strongest association at the T cell epitope level between the presence of an MHC class II allele and allergic sensitization.

For the generation of TCR tg mice we took advantage of a human mugwort-specific TCR which was cloned and functionally characterized by us previously (Leb et al., 2008). Using this TCR and HLA-DRA*01-HLA-DRB1*01 (DR1) transgenic mice (Rosloniec et al., 1997) we generated allergen-specific TCR-DR1 transgenic mice to investigate the contribution of allergen-specific MHCII as well as of allergen-specific $CD4^+$ Teff and Treg to the initiation of allergen-specific sensitization. In addition, we studied the importance of Tregs for the prevention and treatment of allergy in this model.

2. Material and methods

2.1. Recombinant allergens and peptides and preparation of pollen extracts

Purified recombinant Art v 1.0101 and Bet v 1.0101 allergens were purchased from Biomay AG (Vienna, Austria). Immunodominant peptides from major mugwort (Art v $1_{25–36}$) and birch (Bet v $1_{142–153}$) pollen allergens were obtained from ProImmune (Oxford, UK).

Preparation of pollen extracts: Artemisia vulgaris pollen (Allergon AB, Engelholm, Sweden or Greer Laboratories, Lenoir, NC) were used for the preparation of aqueous mugwort pollen extracts according to standard procedures. Briefly, 10 g of mugwort-pollen were incubated in 100 ml of PBS (1×) by stirring at 4 °C overnight. After centrifugation at 52,000*g* at 4 °C for 60 min, the supernatants were filtered and subsequently dialyzed (Spretra/Por Dialysis Membrane, MWCO: 6–8000, Spectrum Laboratories, Rancho Dominues, CA) against 1× PBS for 48 h. The total protein concentration of the dialysate was determined by standard procedures (BCA-bicinchoninic acid protein Kit, Pierce, Rockford, IL). The lipopolysaccharide (LPS) content of the mugwort pollen extract was ≤0,024 U/mg. The extracts were lyophilized and aliquots were stored at −80 °C.

2.2. PCR amplification of TCR sequences

Amplification of TCR specific DNA sequences from the original T cell clone SSR20 was performed using the oligonucleotide primers 5′-CGC GGG CCC GGG AGG TCT TCT GTG ATT TCA ATA AGG A-3′ (sense) and 5′-CCC GCG GCG GCC GCC CCC ATG AGG ACT GCA TTT TG-3′ (antisense) for the α-chain and 5′-CGC GGG CTC GAG GTG CCT TTG CCC TGC CTG T-3′ (sense) 5′-CCC GCG CCG CGG ACA CCC AGC TCC TCC AGC-3′ (antisense) for the β-chain. Both PCR fragments (size: 653 bp and 809 bp, respectively) were digested with appropriate restriction enzymes (α-chain: *Xma I/Not I*; β-chain: *Xho I/Sac II,* New England Biolabs, Ipswich, MA) and cloned into the pUC19 derived pBluescript SK^+ vector (Stratagene, Heidelberg, Germany).

2.3. Generation of TCR transgenic mice

To generate TCR tg mice, rearranged V(D)J regions of the TCR from the human Art v 1-specific and HLA-DRB1*01:01-restricted TH0 cell clone SSR20, as described previously (Jahn-Schmid et al., 2005; Leb et al., 2008), were cloned into the TCR cassette vectors pTαcass and pTβcass (kindly provided by Dr. Diane Mathis, Harvard Medical School,

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