IL-25, a member of the IL-17 family, is an important cytokine that promotes the Th helper type 2 (Th2) response in allergic asthma. Due to its high expression in human asthma, its association with eosinophilic airway inflammation and airway hyper-responsiveness in multiple mouse models, and its association with the exacerbation of asthma by rhinovirus, IL-25 represents a key target for therapeutic intervention. However, high affinity neutralizing humanized antibodies cross-reactive to human and mouse IL-25 have not been developed, in part due to the high level of structural conservation between human and mouse IL-25 protein. In order to generate potent neutralizing antibodies against human IL-25, we have utilized our novel transgenic mouse system, AbeoMouse™, which produces an exceptional immune response that breaks tolerance for mouse proteins, and allows for the direct selection of antigen-specific B-cells, paired with single-cell antibody genome cloning, expression and screening. The AbeoMouse™ system provides plasmacytes with a 45-fold increase in antigen-specific surface immunoglobulin (Ig), an accelerated immune response, and rapid identification of lead candidates without hybrids. Using this technology, we have identified novel, potent neutralizing antibodies against IL-25, and we have demonstrated the efficacy of a lead candidate in a mouse model of chronic, IL-25-induced asthma exacerbation. In addition, we have successfully generated and optimized humanized lead candidates with high affinity and potency. The AbeoMouse™ system represents a powerful and rapid platform for generating potent therapeutic antibodies.

Abstract

Development of Potent Neutralizing Therapeutic Antibodies to IL-25 From a Novel Transgenic Mouse System

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**AbeoMouse™ RAPID ANTIBODY DISCOVERY PLATFORM**

Triple-transgenic mouse engineered to express IL-4 and the Idigil-25 B-cell receptor proteins, resulting in a hyper immune response and surface antibody expression during all stages of B-cell differentiation.

**Therapeutic Antibody Discovery Process**

1. Single-cell cloning & rapid discovery of specific, high affinity chimeric monoclonal antibodies
2. Direct Selection of AbeoMouse™ B-Cells and single cell screening
3. Purified Antigen Specific B-Cells
4. Single-cell RT-PCR and Cloning
5. Immunization with Human IL-25 and Single B Cell Cloning

**Immunization with Human IL-25 and Single B Cell Cloning**

**Efficacy in a Rhinovirus Induced Asthma Exacerbation Model**

IL-25 is elevated in human patients during rhinovirus (RV) infection, and IL-25 plays a key role in a mouse disease model of asthma exacerbation by RV18 infection. Using this model, we show that Abs against IL-25 treatment suppresses the RV18-induced type-2 immune response, resulting in a reduction of eosinophilic airway inflammation in vivo.

**Summary**

Using a novel transgenic mouse system, we have shown that we can rapidly obtain chimeric antibodies of high affinity and neutralizing potency against both human and mouse IL-25, without the use of hybrids. B-cell expressing affinity matured anti-IL-25 surface antibodies were directly selected, and recombinant chimeric antibodies were screened for IL-25 binding and neutralization. From our neutralization screen, we identified and characterized three lead mAbs – A0010, A0125, and A0215 – which all showed high affinity by SPR and high potency in cellular assays. Surprisingly, A0125 and its humanized derivatives lost nearly all binding and potency when directed against recombinant IL-25, most likely due to glycosylation at or near the IL-25 epitope. A0215, however, exhibited high affinity and potency against both human and mouse IL-25 derived from human cells. We also show that treatment with A0215 blocks RV-induced exacerbation in a mouse asthma model, providing further evidence that IL-25 is an important role in asthma exacerbation. Finally, we have developed humanized leads based on A0010 that exhibit high affinity and potency against IL-25. We are currently optimizing these leads in an effort to bring a strong candidate anti-IL-25 antibody to the clinic.

**Development of Humanized Lead Candidates**

For A0010 and A0125, mouse CDRs were grafted into several optimal human framework regions, expressed transiently and tested for affinity and neutralization potency against human and mouse IL-25.

**Functional Screening: Neutralization of IL-25 Activity**

Cell-based screening of chimeric IL-25 antibodies – Neutralization of IL-25 mediated CXCL1 secretion in HT-29 cells

**Anti-IL-25 Antibody Primary Screening**

ELISA binding to IL-25 of single B-cell cloned chimeric human IgG4 mAbs from transduced HEK supernatants

**Transfection HEK cells and screen for via surface expression**

IL-25 Neutralization – IC₅₀

Efficacy in a Rhinovirus Induced Asthma Exacerbation Model