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Supporting allergen-specific immunotherapy by inhibition of Janus kinases

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Conflicts of interest

JG and CS-W have an issued patent "Tofacitinib as vaccination immune modulator". CS-W reports grants and personal fees from Bencard, grants from Leti Pharma, grants and personal fees from Allergopharma, grants and personal fees from PLS Design, outside the submitted work. SB reports non-financial support from ALK-Abelló, grants, personal fees and non-

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Commentary

Allergen-specific immunotherapy (AIT) is the only disease-modifying and curative treatment for allergic diseases. While in selected indications such as Hymenoptera venom allergy the curative success of AIT is impressingly high, in other disease manifestations it only reduces the symptoms to a certain extend in a fraction of patients. Key problems leading to a reduced efficacy of AIT seem to partly depend on local inflammatory processes that are not only causing side-effects, but additionally feed-back on the specific immune response. Thus, these inflammatory processes also influence the immunologic memory and impair the induction of immune tolerance (1). During the initial phase of AIT, a further increase of Th2 inflammation, including higher levels of allergen-specific IgE has been observed frequently and can be a reason for side-effects and even anaphylactic reactions during AIT.

During a postdoctoral research fellowship at the Dermatology Branch of the US National Cancer Institute/NIH, one of our coauthors (JG) developed a murine model of spontaneous autoimmunity. In this model, transgenic sOVA mice that express soluble chicken ovalbumin under control of the keratin-14 promoter are crossed to transgenic OT-I mice, in which CD 8 T cells carry an ovalbumin-specific T cell receptor. Approximately 80% of sOVAxOT-I mice develop spontaneous lethal CD 8 T cell-mediated autoimmunity (2). In approximately 20% of sOVAxOT-I mice, a preservation of self-tolerance was mediated by two mechanisms: (i) downregulation of the CD8 co-receptor and (ii) T cell anergy, in which the cells failed to proliferate in response to IL-2. Both effects are governed mainly by IL-2 (CD8-expression also by IL-7). IL-2 and IL-7 belong to the family of common gamma-chain

cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21). These cytokines signal via the IL-2 receptor subunit gamma, which interacts with Janus kinase 3 (JAK3). In the above described autoimmune model, CD8 T cells from healthy spontaneous survivors did not respond to stimulation with common gamma chain cytokines. The role of common gamma-chain-derived JAK3 signaling in the autoimmune model was verified by successfully treating sick mice with Tofacitinib (3), a JAK inhibitor with highest selectivity for JAK1 and 3 (but which additionally weaker affects other members of the JAK family (4)), whose biological effects were studied at this time at the NIH by John O'Shea and colleagues. The interesting biologic effect of Tofacitinib was that it inhibited Th1, Th2 and Th17 effector functions, but did not inhibit the induction of regulatory T cells (Tregs).

Hence, we speculated that combining AIT with a short-term anti-inflammatory therapy using Tofacitinib might not only facilitate the reduction of side-effects, but also improve therapeutic efficacy. The inventive step was to harness the formation of Tregs for allergen-specific tolerance induction in allergic settings (and later autoimmunity), while blocking harmful Th1, Th2 or Th17 reactions that can be triggered by AIT. These Th1, Th2 or Th17 reactions are a frequent reason for AIT-related adverse events and reducing such reactions poses a great potential for making AIT a valuable treatment option for polysensitized patients or high-risk patients. Moreover, blocking antigen-specific exacerbation of Th1 and Th17 responses might pave the way for applying AIT in autoimmune diseases, such as Type I diabetes or multiple sclerosis. JAKs are key players in cytokine-mediated activation of STATs (signal transducers and activators of transcription) and, hence, of inflammatory processes. The idea was to selectively maintain the desired vaccination-induced antigen presentation and tolerogenic effects, while blocking pro-inflammatory signals. As JAK1 and 3 signaling pathways are key initiators of Th2 differentiation and allergic responses in the lung (5), Tofacitinib was an interesting candidate

for this therapeutic approach. Therefore, the invention described here relates to a pharmaceutical composition comprising Tofacitinib and at least one antigen for the treatment or prevention of immune diseases (6).

Tofacitinib was applied together with AIT in a murine model of allergic airway inflammation (7). Interestingly, the co-administration of Tofacitinib 48 hours prior and post AIT injections was significantly more potent than classical AIT in improving key parameters of allergic airway inflammation. These include total cell and particularly eosinophilic infiltration into the lung as well as the levels of IL-4 and IL-13. Moreover, specific IgE (sIgE) and total IgE levels were clearly reduced. Particularly, sIgE levels were only reduced with high significance by combining AIT with Tofacitinib administration, but not by AIT alone.

Importantly, the Tofacitinib-mediated improved efficacy of AIT is not caused by a general inhibition of immunologic effector functions, as the induction of potentially protective IgG antibodies is not affected by Tofacitinib (7). It might be speculated that Tofacitinib administration inhibits the formation of IgE-producing plasmablasts and generates a window of opportunity for IgG class-switching. Moreover, Tofacitinib administration without accompanying AIT is not able to influence inflammatory cell infiltration into the lung as well as immunoglobulin or cytokine levels, a fact verifying that the observed effects are not a result of transient immunosuppression.

As it additionally could be demonstrated that Tofacitinib favors the induction of human $FOXP3^{+}CD4^{+}$ T cells *in vitro* (7), Tofacitinib treatment during the up-dosing phase of AIT inhibits the STAT-dependent induction of unwanted Th2, Th1 and Th17 responses while not impairing the generation of tolerance-inducing regulatory T cells (8) (Figure 1). In contrast, Cyclosporine A inhibits FOXP3-mediated Treg induction by direct inhibition of NFAT (nuclear factor of activated T cells) binding to the proximal FOXP3 promoter (9).

However, this effect is also not observed applying other immunosuppressants such as steroids or rapamycin.

Of note, the approach described here does not aim at a long-lasting modification of the JAK/STAT pathway during the whole period of AIT. The administration of Tofacitinib for short periods during the up-dosing phase, which is uncritical with respect to Tofacitinib-mediated side-effects, rather aims at short-term blockade of this signaling cascade to restrict local inflammation and to support the AIT-mediated tolerogenic memory effect.

Conclusion

Although AIT is an effective treatment of allergic diseases, it is of considerable interest to enhance its therapeutic efficacy, to improve patients' compliance by shorter treatment regimens and less side-effects as well as to expand its use to additional disease manifestations and patient groups. The invention described here, shows that inhibition of the JAK/STAT pathway for short periods of a few days during AIT is able to shift the balance of newly induced T cell reactivity by restricting local inflammation. A potentially favored induction of regulatory T cells might contribute to build-up a basis for a long-lasting modulation of the immunological memory. The improved control of local inflammation might help to extend the application of AIT to more severe conditions such as polyallergy, severe asthma and high-risk patients suffering from mastocytosis or anaphylaxis. Such an approach even might allow a seasonal therapy by AIT or to better manage patients suffering from perennial symptoms. Moreover, comparable concepts might be beneficial for the treatment or prevention of other immune diseases such as autoimmunity. The main criteria for patent filing, novelty (prior art), inventive step and applicability are given by the tolerance-supporting effect of Tofacitinib, the combination of Tofacitinib with AIT (which is not immedeately obvious even to experts in the field) and the possibility to extend the novel

combinatory use of two approved drug to other diseases states that are to date not sufficiently treated.

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Figure legend

Figure 1. Simplified overview of the hypothesized mechanism of Tofacitinib-supported tolerance induction during allergen-specific immunotherapy. While Tofacitinib blocks pro-inflammatory T cell responses (Th1, Th17, Th2, Th9) by the inhibition of STAT signaling, it favors the NFAT-dependent formation of anti-inflammatory regulatory T cell (Treg) responses and, thus, builds an "immunologic filter" for T cell memory. GATA3, GATA-binding protein 3; FOXP3, forkhead box P3; NFAT, nuclear factor of activated T cells; RorγT, RAR-related orphan receptor γt; STAT, signal transducer and activator of transcription; T-bet, T-box expressed in T-cells.