

# Anti-Ids in Allergy: Timeliness of a Classic Concept

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**Abstract:** Anti-idiotypic antibodies (anti-ids) are part of natural immune responses with regulatory capacity. Their effect on an antigen-specific, so-called Ab1 antibody response, is dependent on 1) the original antigen, which they mirror, being Ab2 antibodies, and 2) their isotype. In the case of IgE-mediated allergy, natural anti-ids against allergen-specific IgE represent internal images of allergen molecules. A key biologic feature of allergens is that they can crosslink IgE, expressed by B-lymphocytes or passively bound via high affinity receptors to effector cells, which renders cellular activation. Therefore, the IgE cross linking capability of anti-ids determines whether they dampen or enhance immediate-type hypersensitivity. Correspondingly to classic antiallergen blocking IgG antibodies, anti-ids may also interact with inhibitory Fc $\gamma$ RIIb receptors and, thereby, down-regulate T<sub>H</sub>2-type inflammation. Anti-ids and other B-cell epitope mimetics, like mimotopes and DARPin, represent antigen surrogates, which can be used for vaccination. Intriguingly, they may induce antibody responses without activating potentially proinflammatory, antiallergen T-lymphocytes. Taken together, collective evidence suggests that anti-ids, although representing immunologic classics, are a timeless concept in allergology.

**Key Words:** anti-idiotypes, allergy, vaccination, Fc $\epsilon$ RI, Fc $\gamma$ RIIb, blocking

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## INTRODUCTION

Besides a specific antigen binding site, antibody molecules possess antigenic determinants themselves. When these epitopes are located within the variable region of an antibody, they are designated idiotopes. Hence, each Fab arm of an antibody displays a set of idiotopes, representing epitopes for complementary antibody molecules (Fig. 1), so called anti-idiotypic antibodies (anti-ids). Anti-ids are part of regular immune responses and are thought to result in a web of interacting

idiotypes. Classically, Jerne defined the order of an antibody response as follows: Antibody Ab1 is produced in response to an antigen and induces the production of anti-idiotypic Ab2, which can in turn stimulate the synthesis of an anti-(anti-idiotypic) antibody Ab3, and so forth (Fig. 1). Anti-idiotypic antibodies (Ab2) can be classified into several categories according to their fine specificity (Fig. 2): 1) conventional Ab2 $\alpha$  antibodies recognize idiotopes of Ab1 outside of its antigen binding site, but still within the variable region; 2) internal image Ab2 $\beta$  antibodies recognize idiotopes directly within the antigen binding site of Ab1 and, therefore, mimic its original antigen epitope like “internal images”; 3) in case the idiope recognized by Ab2 is not completely overlapping but close to the antigen binding site of Ab1, it may still be able to interfere with the antigen binding and is called Ab2 $\gamma$ . Consequently, binding of antigen by Ab1 is not affected by Ab2 $\alpha$ , may be blocked by Ab2 $\gamma$  and is completely blocked by Ab2 $\beta$ .

## ANTI-IDS IN EXPERIMENTAL STUDIES: THE BEGINNING

The first experiments regarding anti-ids were animal studies, which were performed to evaluate the effect of anti-ids either on an upcoming or ongoing immune response.

For instance, Cosenza and coworkers designed a mouse study where they used a monoclonal IgA antibody derived from the myeloma cell line TEPC 15, specific for the hapten phosphorylcholine.<sup>1</sup> To generate an anti-idiotypic immune response, mice were immunized with the myeloma antibody Ab1 and sera were harvested. Parallel, they immunized another group of BALB/c mice with heat-killed pneumococci to induce a phosphorylcholine-specific Ab1 response. As a proof for the induction of specific antibodies, erythrocytes attached to either pneumococcal C polysaccharide or phosphorylcholine were incubated with the splenocytes from the latter group of mice, resulting in hemolytic plaques because of immune complex-mediated complement activation.<sup>2</sup> Importantly, when spleen cells were preincubated with the anti-idiotypic serum generated upon immunization with myeloma IgA, plaque formation was specifically inhibited because of blockage of Ab1. This principle was later also shown for anti-ids directed against Ab1 specific for group A streptococcal antigen.<sup>3</sup>

However, anti-ids could not only inhibit effector responses, but also prevent de novo induction of specific immune responses in vivo. Naive BALB/c mice were administered the anti-idiotypic serum derived from immunization with TEPC myeloma IgA and subsequently intravenously immunized pneumococci. Antibody responses to phosphoryl-

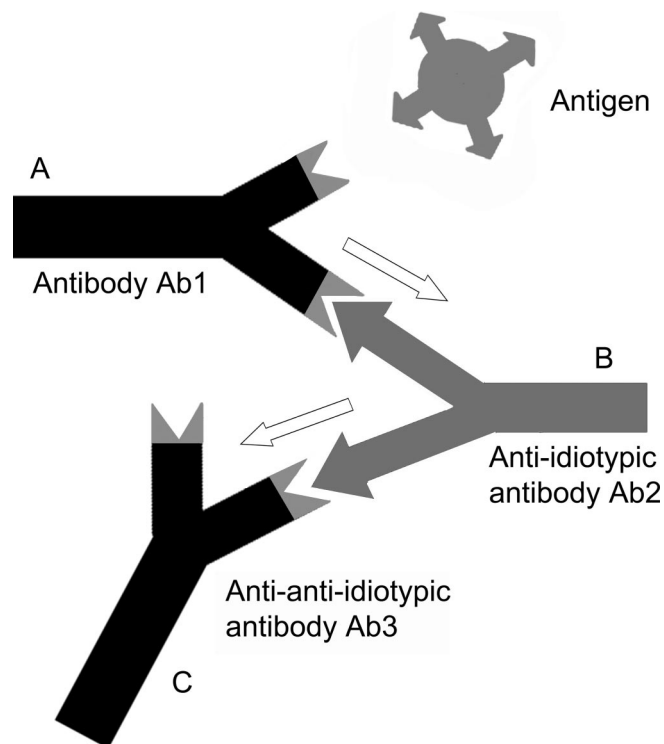
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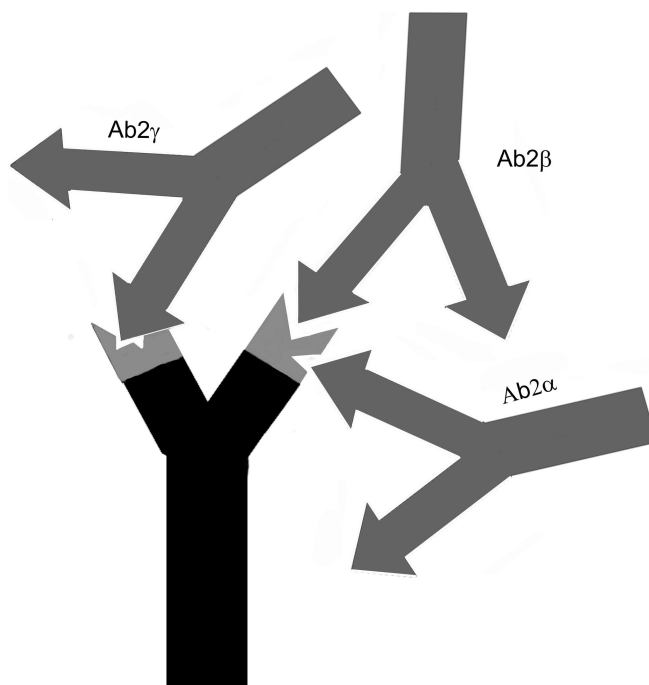


**FIGURE 1.** The anti-idiotypic network amplifies antigenic signals. (A) An antibody Ab1 is produced in response to a specific antigen. (B) With a defined idiotype, Ab1 induces the production of an anti-idiotypic antibody Ab2. This Ab2 may resemble the original antigen as an internal image. (C) Ab2 can stimulate the synthesis of an anti(anti-idiotypic) antibody Ab3 which principally is of the same specificity as Ab1.

choline but not to an irrelevant control allergen were completely blocked. Thus, anti-ids were discussed to be directed also against membrane immunoglobulins of naive antigen-specific B cells. Accordingly, it was hypothesized that membrane and secreted immunoglobulins share similar variable domains even when belonging to different isotypes.<sup>1,4</sup> The key result of these studies was, however, that anti-ids act at the cellular level to specifically suppress antibody formation.

Hart and his group generated anti-ids by immunization of rabbits with *p*-azophenylarsonate-specific mouse antibodies. When naive mice were then injected with the anti-idiotypic rabbit serum and afterward challenged with KLH (keyhole limpet hemocyanin)-linked azophenylarsonate, *de novo* antibody formation was suppressed up to 97%.<sup>5</sup> However, the suppression was not complete. This might have been because of the appearance of antibody responses directed against either the carrier protein KLH or the hapten (*p*-azophenylarsonate) as such. It seemed probable that the induced antiphenylarsonate antibodies (and their anti-ids), induced by carrier-hapten immunization, possessed idiotypes other than those elicited by either KLH or the hapten. Again, the conclusion of this study was that anti-ids acted suppressive at the cellular level via binding to immunoglobulins expressed by B-lymphocytes.

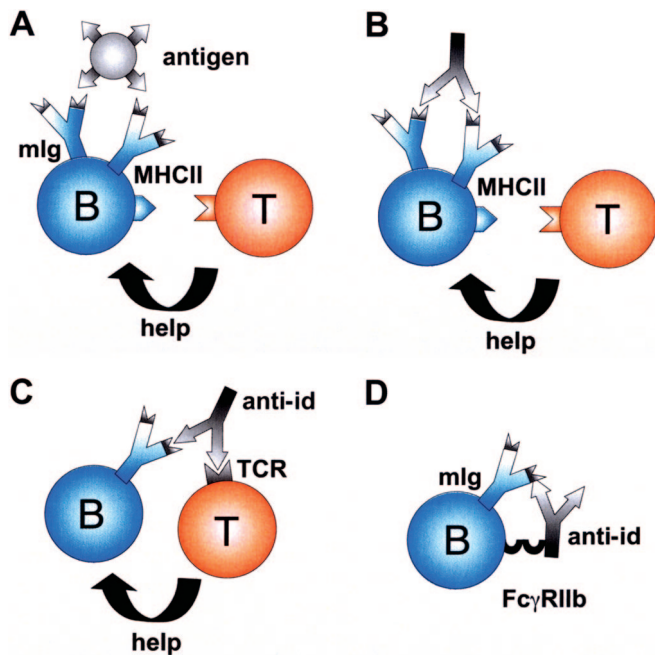
Furthermore, it became apparent that T cell receptors carry idiotypes similar to those of immunoglobulins, meaning



**FIGURE 2.** Categories of anti-idiotypic antibodies. Ab2 $\alpha$  antibodies recognize idiotopes outside the antigen binding site, but still within the variable region of Ab1. Ab2 $\gamma$  recognizes idiotopes close to the antigen binding site and thus may interfere with the antigen binding. Internal image Ab2 $\beta$  binds directly within the antigen binding site.

that anti-ids could potentially regulate both, B-lymphocyte and T-lymphocyte function.<sup>6</sup> The idiotypic relationship between hypervariable domains of T and B lymphocyte receptors has indeed been repeatedly documented.<sup>7-10</sup> Furthermore, it has been shown that anti-ids can mediate cooperation between T and B lymphocytes (Fig. 3C). Furthermore, when B-lymphocytes expressing membrane Ab1 (mAb1) are targeted by Ab2, they internalize and process the anti-id (Fig. 3B) like the original antigen (Fig. 3A). Consequently, an anti-idiotypic peptide of Ab2 is presented in an MHC II context to T cells, again resulting in T cell-help. This would be an additional explanation for how B-lymphocytes recruit T cell help. In the early 1980s, also Blaser et al identified idiotypes on specific T-helper cells similar to the antigen-specific IgG. However, they dissected an antihapten from an anticarrier response and proposed that, in contrast to the above, especially an anticarrier anti-id might block T-helper cell function, rendering reduced antibody production.<sup>11</sup>

Taken together, already at that timepoint, functional similarity between antigen and anti-id could be shown. There was mostly evidence that administration of anti-ids suppressed specific idiotype production.<sup>4,12</sup> However, other studies<sup>13</sup> suggested that under certain conditions anti-ids could enhance immune responses. For instance, administration of anti-ids of the IgG1 isotype raised in A/J mice against an antistreptococcal antigen A antibody (Ab1) could prime animals for a secondary boost to the antigen. Interestingly, anti-ids of the IgG2 isotype were rather



**FIGURE 3.** Mechanisms of anti-idiotypic antibodies on B (B) and T cell (T) interaction. (A): B cells recognize antigen by membrane-expressed immunoglobulins (mlg) and receive bystander help from T-helper cells. (B) Alternatively, an anti-id may bind to mlg of a B-cell. This leads to processing and MHCII display of an anti-idiotypic peptide rendering T cell activation and help. (C) Because of idiotypic similarities between B and T cell receptors, an anti-idiotypic antibody may also force cooperation between B and T cells by binding to both. (D) Inhibition of B cell proliferation is achieved by simultaneous binding of the anti-id to its corresponding idiotype on mlg and via its Fc $\gamma$  domain to Fc $\gamma$ RIIb on the B-cell.

associated with suppressive effects on naive, antigen-sensitive B cells.<sup>12</sup> Therefore, besides antigen-specificity of the anti-idiotypic system, also the induced isotype seemed to contribute to the regulatory capacity of anti-ids.

### NATURAL ANTI-IDS TO ALLERGEN-SPECIFIC IGE: QUALITATIVE AND QUANTITATIVE STUDIES

In allergy research, Malley et al reported the influence of anti-ids on primary and secondary IgE antibody responses to timothy grass (*Phleum pratense*) pollen extract.<sup>14</sup> An anti-idiotypic antibody was produced to the grass pollen-specific IgE and was administered intraperitoneally followed by immunization of mice with timothy grass pollen extract. This anti-id profoundly prevented IgE production toward the allergen extract, and suppressed even up to 75% of the IgE response to a secondary boost. This suppression persisted for at least 35 days.<sup>14</sup>

Despite optimistic results such as these, the role of anti-idiotypic immune responses in humans induced during long-time immunotherapy could at that time only be postulated.

Bose and colleagues did the first quantifications of anti-ids in rye grass-allergic patients' sera.<sup>15</sup> Rye I-specific IgG anti-ids were purified from a ryegrass allergic patient

who had been previously hyposensitized. In vitro, these IgG specifically blocked the binding of IgE and IgG to group I allergens of rye grass in sera of 20 individual allergics. This result pointed toward the recurrence of idiotypes and a constricted repertoire of human antibodies specific for an allergen. The authors discussed similar idiotypes of IgG and IgE within their antigen binding site. However, in this study it remained unclear whether the same anti-ids were able to inhibit antigen binding to both, IgE and IgG.

A functional distinction between paratope-associated idiotypes versus bystander idiotypes (ie, idiotypes located outside the antigen-binding site) of IgE and IgG was made by the group of Saint-Remy.<sup>16</sup> They observed cross-reactivity of house dust mite-specific IgE and IgG, but only with respect to bystander idiotypes.<sup>17</sup> They inhibited Ab1-Ab2 interactions with house dust mite allergens and thereby found that the majority of natural anti-ids in mite-allergic patients was of the Ab2 $\beta$  type, thus resembling internal images of the allergen. Still, the authors suggested the epitope-specificity of IgE and IgG to be different.

Interestingly, anti-ids specific for ragweed were also found in nonatopic individuals although to a much lesser extent than in allergic and immunotherapy-treated patients.<sup>18–20</sup> The authors proposed that anti-ids detected in nonatopics probably represent a basal level of Ab1/Ab2 responsiveness because of the presence of allergen-specific IgG, but not IgE antibodies, in health.

Anti-id levels in untreated patients sensitive to grass pollen allergen Lol p I and in hyposensitized patients were compared with levels in allergic donors not sensitive to Lol p I, and nonallergic donors by Bose et al using sepharose purification. Via binding to radiolabeled, affinity-purified F(ab')<sub>2</sub> fragments of Lol p I monoclonal antibodies, the amounts of anti-ids and the ratio to allergen-specific antibodies were calculated.<sup>21</sup> Again, anti-ids were observed to be present not only in allergic individuals but also in nonallergics, although the latter did not show any detectable IgG or IgE (Ab1) anti-Lol p I. Anti-id levels in allergics were shown to directly correlate with the level of pollen exposure or the hyposensitization status. In the majority of patients treated by immunotherapy an inverse relationship between serum levels of anti-Lol p I IgE and IgG antibodies (Ab1) and of anti-ids (Ab2) could be observed. An initial increase of specific idiotype (Ab1) levels was associated with a drop of the anti-id (Ab2) level. However, like during hyposensitization, the anti-id level later on increased toward a plateau. An increase of Ab2 was also reported by Castracane et al in a ragweed allergic human patient undergoing immunotherapy. They used an antiragweed specific F(ab')<sub>2</sub> fragment for coating in a solid phase assay.<sup>19,20</sup> In line with earlier observations by Oudin and Cazenave, it had to be considered that a proportion of the raised anti-ids was most probably not specific to Lol p I, but could have also encountered similar idiotypes derived from different allergens.<sup>21,22</sup> Hebert and colleagues measured elevated anti-id levels in sera of hyposensitized patients by the use of 3 different murine monoclonal anti-Lol p I antibodies.<sup>23</sup> These murine antibodies were shown to share cross-reactive idiotypes with human anti-Lol p I IgE and IgG antibodies and therefore suitable in this study.<sup>24</sup>

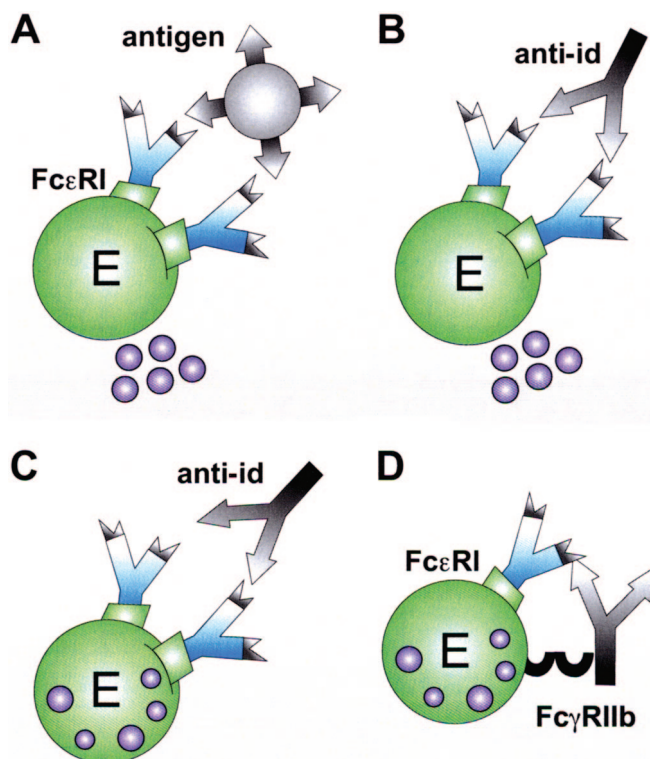
## THE FUNCTIONAL ROLE OF ANTI-IDS IN ALLERGY

Levels of anti-ids were generally found to be higher in healthy individuals than in untreated atopic patients. In the latter, levels can be elevated by immunotherapeutic treatment to the levels found in healthy volunteers.<sup>20,24</sup>

Valacer and colleagues inhibited IgE binding from allergic patients to ragweed antigen by administration of anti-ids purified from sera of nonallergic individuals. Strikingly, this effect could be achieved although no allergen-specific IgG or IgE could be detected in the sera of the healthy donors. The authors discussed that anti-ids in healthy persons could regulate the IgE response by suppressor mechanisms.<sup>25</sup> Indeed, a dramatic suppressive effect of anti-ids on the IgE response was observed in experimental studies. A single anti-id directed against an idiotope of a murine antibody specific for a major epitope of grass pollen allergen Lol p IV down regulated the allergen-specific IgE response profoundly, whereas levels of other isotypes, for example, IgG1 and IgG2 were less affected.<sup>26</sup>

The enthusiasm about potential beneficial effects of anti-ids in the course of immunotherapy was dampened by the sudden awareness that anti-ids against allergen-specific IgE antibodies potentially could also target IgE bound to effector cells. This could lead to either mediator release by IgE-cross-linking (Fig. 4B) like it is the case with the original antigen (Fig. 4A), or to the inhibition of allergen-induced release (Fig. 4D). In fact, Geha et al demonstrated that passive cutaneous administration of an antitetanus toxin anti-id in a human patient elicited positive immediate-type skin reactions.<sup>27</sup> In another study, passive cutaneous anaphylaxis experiments in rats were chosen to analyze the *in vivo* effects of anti-ids on mast cell bound IgE. Anti-ids were raised in syngeneic mice by immunization with dinitrophenol (DNP)-specific mouse monoclonal IgE. The anti-idiotypic serum induced a passive cutaneous anaphylactic reaction in skins of rats sensitized with the DNP-specific mouse IgE. In contrast, the same anti-id did not elicit a reaction in DNP-IgE sensitized rats when they simultaneously were passively administered grass pollen-specific IgE. Thus, a specific anti-id seemed to be more effective in histamine release when less IgE specificities were present in the sensitized individual. From this it was concluded that anti-idiotypic stimulation within a polyclonal response, like in human atopy, would rather inhibit mediator release (Fig. 4C). Furthermore, successful immunotherapy was considered to rely on continuous anti-id targeting of the mast cells rendering internalization and, therefore, depletion of cell bound specific IgE.<sup>28</sup>

The capability of human anti-idiotypic IgG purified from mite allergic patients to recognize cell bound IgE idiotypes of other donors, and to induce mediator release of basophils in the absence of allergen was demonstrated.<sup>29</sup> Consequently, the possibility that degranulation in response to anti-ids could be a general event in allergy was proposed. However, the degranulating activity of anti-id preparations varied from one patient to the other. Several mechanisms were discussed: First, the precise specificity of anti-ids could play a role, because framework idiotopes could be private and, in contrast, paratope-associated idiotopes of antibodies



**FIGURE 4.** Mechanisms of anti-idiotypic antibodies on effector cells. Mediator release of effector cells (E) is triggered by cross-linkage of 2 receptor-bound IgE either via (A) recognition of the antigen or (B) the anti-id. (C) Effector cell inhibition is achieved by binding of the anti-id to its specific idiotope within a polyclonal response. (D) Cross-linkage of FcεRI and the inhibitory FcγRIIb receptor via an anti-idiotypic IgG antibody also inhibits degranulation.

were found to be more common among different individuals. Hence, the relative proportion of these 2 types of anti-ids could explain the varying degranulating activity of single anti-id preparations on basophils of different donors. Secondly, the authors discussed the repertoire of anti-ids to be dependent on the individually varying anti-allergen IgE response, limiting their degranulation capacity when testing basophils of other donors.

Taken together, the impact of anti-ids in the regulation of IgE antibody responses to allergens has at that time not been revealed to the same extent as for infectious diseases or in malignancies.<sup>30–32</sup> However, genetic engineering of antibodies opened novel methodological possibilities for precise investigation of the effects of anti-ids in the effector phase of type I allergy. As many studies previously were based on mouse monoclonal anti-ids, it was difficult to extrapolate the findings to the human setting. Therefore, the group around Shakib intended to engineer murine/human chimeric anti-ids and the corresponding anti-allergen IgE,<sup>33</sup> taking Der p 1 as a model allergen. Testing these chimerized IgE-anti-idiotypic antibodies on FcγRIIb transfected cells and purified basophils, they found an inhibition of the degranulation through the anti-ids. Interestingly, a novel mechanism of action was proposed, involving FcγRIIb-binding by the Fcγ domain of the anti-idiotypic IgG, and,

consequently, cellular inhibition through the associated ITIM-motif of this receptor (Fig. 4D).<sup>34</sup> In this context it is of interest that also allergen-specific IgG may recognize allergen already complexed with mIgE on B-lymphocytes or with IgE bound to the high affinity receptor. Upon simultaneous binding to Fc $\gamma$ RIIb, allergen-specific IgG has been shown to down regulate not only effector cells, but also B-lymphocytes.<sup>35,36</sup> Therefore, we postulate here that similarly anti-ids may exploit this mechanism and act on the B cell level, too (Fig. 3D).

Interestingly, anti-ids may not only dampen the allergic response after allergen immunotherapy, but may also have a protective function in offsprings of allergic mothers. In a mouse model, the maternally derived anti-id against allergen-specific IgE was shown to induce IgE suppression in the offspring which was long-lasting and dose-dependent.<sup>37</sup> As molecular modeling ruled out an internal image function of the anti-ids, possibly also here the binding to Fc $\gamma$ RIIb could contribute to the protective function.

### ANTI-IDS PRIME AND ENHANCE ALLERGEN-SPECIFIC IMMUNITY

It had been suggested that anti-ids function differently in healthy, allergics, or hyposensitized patients. Bose proposed 1986 that anti-ids in allergics might function as “network antigens”, mimicking the allergen and therefore being able to accelerate an immune response.<sup>21</sup> In accordance, an anti-idiotypic Ab2 $\beta$  antibody raised by a monoclonal anti-Lol p 1 antibody was able to successfully prime an allergen-specific antibody response, as a subsequent boost injection with the antigen Lol p I resulted in a further increase of specific IgE and IgG antibody levels.<sup>38</sup> The administration of anti-ids augmented both, idiotype specific and total antibody responses against the allergen,<sup>38</sup> a result that had been predicted before by studies outside the field of allergy.<sup>39–41</sup> However, the exact mechanism for the enhancing effect of an anti-id resembling only a single epitope, remained unclear. One may speculate that upon the administration of Ab2 the induced Ab3 facilitates subsequent antigen presentation, leading to epitope spreading. Especially the IgM isotype was considered to potentiate the immune response against low doses of antigen via the idiotypic network. A further explanation could be that immune complexes formed after antigen immunization directly stimulate antigen-specific B cells.<sup>42</sup>

### VACCINATION WITH ANTI-IDS TO CONTROL TOTAL IGE LEVELS

The immunotherapeutical potency of anti-ids for allergic patients was not only investigated with respect to the allergen-specific IgE response, but also regarding total IgE levels in allergics. It had been shown previously that natural anti-IgE isotype antibodies exist, which, depending on the epitope specificity of the Fc $\epsilon$  domain, may down-regulate or enhance the effector function of IgE.<sup>43</sup> Stadler et al aimed to generate nonanaphylactogenic IgG antibodies through active immunization with an anti-idiotypic antibody as an antigen surrogate. When they used a beneficial, nonanaphylactogenic anti-IgE antibody (termed BSW17) as a template, anti-ids could be generated from a combinatorial phage library displaying the Fab

repertoire of a grass pollen allergic patient.<sup>44</sup> According to the principle of molecular mimicry, these anti-ids resembled an epitope of the constant domain of IgE and actively induced BSW17-like anti-IgE specificities in mice.<sup>45</sup> Therefore, as an alternative to passive anti-IgE therapy with, for example, omalizumab, vaccination with anti-idiotypic molecules for active induction of a protective anti-IgE response was envisaged by the authors.

### VACCINATION WITH ANTI-IDS TO CONTROL ALLERGEN-SPECIFIC IGE LEVELS

In our own study, we generated anti-idiotypic Fab antibody fragments for IgE, which was specifically directed against timothy grass pollen allergen Phl p 5.<sup>46</sup> The phage library containing the repertoire of a grass pollen sensitized individual, which is described above,<sup>47</sup> was this time used for screening with allergen-specific IgE. Several high-binding Fab clones with mimicry potential to the allergen's IgE epitope could be isolated. Upon immunization with clones in mice, antiallergen IgG could be induced via molecular mimicry. Furthermore, these anti-idiotypic Fabs resembled naturally occurring IgE epitopes of the allergen as observed by sequence analysis and molecular modeling.<sup>46</sup> More recently, we applied one of the selected anti-idiotypic clones in a memory mouse model of acute allergic asthma to test whether it would be suitable for immunotherapy. According to a protocol by Mojtabavi et al<sup>48</sup> acute allergic asthma was induced by injecting recombinant Phl p 5 intraperitoneally, followed by aerosol challenges with this allergen. Subsequently, groups of mice with acute asthma were vaccinated with 1) the anti-idiotypic Fab fragment, or 2) control antigen KLH before reinduction of acute asthma by an additional allergen aerosol challenge.

Whereas the IgE and IgG1 antibody levels of all groups remained unchanged during treatments, the extent of acute eosinophilic inflammation upon rechallenge with aerosolized allergen was much lower in the Fab-treated group compared with the untreated asthmatic and the nonasthmatic groups (data not shown). As the specific IgE levels were not affected, it is tempting to speculate that the reason for this profound anti-inflammatory property might be (similarly as with peptide mimotopes; see below) the absence of allergen-specific T cell epitopes in the anti-ids.<sup>49</sup> Surface plasmon resonance studies indicated that high affinity polyclonal IgE may exhibit a binding behavior similar to monoclonal antibodies, indicating that each Phl p 5 allergen might only harbor a low number of IgE epitopes.<sup>50</sup> Consequently, the anti-idiotypic strategy is expected to down-regulate IgE specificities being relevant and occurring frequently in the grass pollen allergic population.

### DISTINCT TYPES OF ANTI-ID LIKE MOLECULES

Although antibodies are widely used for therapeutic applications, they have some potential drawbacks in large scale production including low expression yields and aggregation tendency. In this context, phage display technology does not only enable the selection of anti-ids, but also of peptide molecules, which structurally mimic B cell epitopes and therefore are called mimotopes.<sup>51</sup> Several allergen mimotopes were already defined by other working groups and ourselves, like for the

panallergen profilin,<sup>52</sup> the major fish allergen parvalbumin,<sup>53</sup> birch pollen allergens,<sup>54</sup> grass pollen allergens,<sup>46,55</sup> or house dust mite allergens.<sup>56</sup> Mimotopes lack allergen-specific T-cell epitopes and have been demonstrated to induce blocking IgG antibodies without stimulation of allergen-specific T-helper cells.<sup>49,54</sup> Furthermore, when therapeutically applied in a murine model of allergic asthma, they could, like reported here for an anti-idiotypic Fab fragment, prevent acute lung hypersensitivity and inflammation upon allergen rechallenge.<sup>57</sup>

Still, to achieve good quality of the immune response towards the relatively short peptide mimotopes, bystander T-cell help was needed. The immunogenic carrier for the mimotopes was in that case KLH providing bystander T-helper cell epitopes. Alternatively, tetanus toxoid (TT) could also be chosen as an immunostimulatory agent because of its promiscuous T-cell epitopes.<sup>58</sup>

Another possibility to enhance antigenic density is the attachment of linear peptides to tyrosine backbones. The so called multiple antigenic peptides (MAPs) can be synthesized in a straightforward manner and allow a dense display, most often as tetra- or octameric constructs.<sup>59</sup> Taken up by antigen presenting cells (APC), they are processed to T-cell epitopes and can activate T-cells too. However, controversial studies have raised the question whether or not the addition of a promiscuous T-helper epitope to a MAP is needed for achieving a sufficient immune response upon MAP-vaccination.<sup>60</sup>

Another option to overcome the limitations of antibody libraries are designed ankyrin repeat proteins (DARPin). These DARPins are a source of  $\beta$ -hairpin structures, which mimic naturally occurring repeat proteins, and play an important role in innate immunity<sup>61</sup>; reviewed in.<sup>62</sup> In a study by Vogel et al, DARPins were used to isolate ligands against the variable region of the nonanaphylactogenic anti-IgE antibody BSW17. Confirming the high specificity of the identified anti-idiotypic DARPins, they successfully prevented binding of BSW17 to IgE-sensitized rat basophils expressing human Fc $\epsilon$ RI $\alpha$ .<sup>63</sup> Recent molecular studies indicated that hairpin scaffold libraries render mimetics, which closely mimic the crystal structure of a native protein.<sup>64</sup> We are convinced that these novel developments will open up new avenues for anti-idiotypic strategies in allergy research.

## SYNOPSIS

From several studies in the past it has emerged that natural anti-ids take part in the regulation of the immune response including immediate-type hypersensitivity. Obviously, each antibody response to an allergen is accompanied by the production of anti-idiotypic antibodies and anti-antibodies, together composing an idiotypic regulatory network. As predicted by Jerne, several studies could indeed show on the molecular level that anti-ids function as internal images of allergens. Thus, it may be hypothesized that anti-ids for allergen-specific IgE potentially nourish IgE memory in periods of allergen absence, like for instance outside the pollen season. In experimental studies, anti-ids have been able to specifically down-regulate, or vice versa, boost allergen-specific immune responses. This can on the one hand be because of the fact that they act on B- and T-lymphocyte antigen receptors in either a nonproductive

(monovalent) manner or in a productive way by cross linking B cell receptors, or crosslinking B with T-lymphocytes, thereby forcing cellular crosstalk. Furthermore, anti-ids have been found in nonallergic individuals, pointing toward a protective regulatory mechanism.

Despite controversial in vitro results of the influence of anti-ids on the allergic response, levels of anti-ids usually get elevated during the course of specific immunotherapy (SIT). Therefore, anti-ids that mimic the relevant structural epitopes of an allergen are possibly attractive candidates for immunotherapy. For vaccination, mimotopes or DARPins represent novel anti-idiotypic alternatives. We propose that with anti-idiotypic tools, being synthetic or generated from the patient's antibody repertoire, immunologic disorders like allergies could be manipulated.

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