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Mimotope vaccination – from allergy to cancer

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Abstract

Background—Mimotopes are peptides mimicking protein, carbohydrates or lipid epitopes and can be generated by phage display technology. When selected by antibodies, they represent exclusively B-cell epitopes and are devoid of antigen/allergen-specific T-cell epitopes. Coupled to carriers or presented in a multiple antigenic peptide form mimotopes achieve immunogenicity and induce epitope-specific antibody responses upon vaccination.

Objective/methods—In allergy IgG antibodies may block IgE binding to allergens, whereas other IgG antibody specificities enhance this and support the anaphylactic reaction. In cancer, inhibitory antibody specificities prevent growth signals derived from overexpressed oncogenes, whereas growth-promoting specificities enhance signalling and proliferation. Therefore, the mimotope concept is applicable to both fields for epitope-specific vaccination and analysis of conformational B-cell epitopes for the allergen/antigen.

Results/conclusions—Mimotope technology is a relatively young theme in allergology and oncology. Still, proof of concept studies testing allergen and tumour mimotope vaccines suggest that mimotopes are ready for clinical trials.

Keywords

allergooncology; allergy; cancer; epitope specificity; immunotherapy; mimotope; vaccination

1. Introduction

In both allergy and cancer biology, antibodies may be beneficial or detrimental, depending on their epitope specificity [1,2]. In allergy, specific immunotherapy (SIT) with allergens aims to induce antibodies which block, but do not enhance the allergic reaction. Similarly, in immunological targeting of antigens overexpressed by malignant cells, growth-inhibitory antibodies are preferred, whereas growth-stimulating specificities should be prevented. Therefore, in both cases it is important to direct immune responses to inhibitory antibody epitopes of the allergen/antigen. Phage display technology enables the selection of mimotopes that structurally mimic such B-cell epitopes. These mimotopes can be used to localize conformational epitopes and, more importantly, for active immunization strategies. The induced antibodies directed against the native allergen/antigen represent a polyclonal humoral immune response with memory function. Thus, mimotope technology opens new avenues for epitope-specific treatment of allergy and cancer.

2. Current status of allergy treatment

Today, an average of 23% of the population in industrialized countries suffers from seasonal allergic rhinitis [3]. Thereby IgE antibodies, elicited against harmless antigens, cause mild to severe symptoms and even life-threatening anaphylactic reactions. Current clinical strategies for the prevention of allergic symptoms involve allergen avoidance by patients, pharmacotherapy or SIT. In 1911 Noon and Freeman reported for the first time, to our knowledge, the efficacy of immunotherapy for the treatment of type 1 hypersensitivity [4]. For almost a century the principle of therapy has not changed much and is routinely applied against airborne allergens and hymenoptera venom [7]. The treatment, accepted by the WHO, comprises repeated administration of increasing doses of allergen extracts to actively induce tolerance towards allergens in patients suffering from rhinoconjunctivitis and asthma [5,6][8]. The efficacy of SIT is clinically well-documented, but the molecular background including humoral and cellular mechanisms, is to date incompletely understood [9].

During treatment an increase of allergen-specific IgG antibodies [10], particularly IgG4 and IgG1 isotypes [11], is observed, sometimes accompanied by a decrease of allergen-specific IgE serum levels. Later it was shown that the increase of allergen-specific IgG antibody levels towards the individual allergens in an extract and the alteration of specific IgE do not necessarily correlate with the outcome of therapy [12]. Furthermore, the exact determination of allergen-specific IgG is even complicated by the presence of anti-IgE autoantibodies [13]. Wachholz *et al.* reported that IgG antibodies induced by grass pollen immunotherapy were able to block the IgE-facilitated antigen uptake by B-cells and subsequently the presentation of allergens to T-cells [14]. This refers to the fact that, beside the humoral anti-allergen response, allergen-specific T-cells also play an important role in allergic inflammation. In contrast to conformational epitopes of B-cells, T-helper cells recognize short linear fragments of allergens which are presented by HLA class II molecules on antigen presenting cells (APCs). In sensitized healthy individuals, regulatory T (T_{reg}) cells are the dominant subset specific for allergens, whereas patients with allergy show a bias towards allergen-specific T helper type 2 (T_H2) cells. The induction of a tolerant state in peripheral T-cells represents an essential step in SIT [15]. On the cellular level the induction of IL-10-producing T_{reg} cells [16], and the shifting from a T_H2 to a T_H1 cytokine profile [17] are known to contribute to a successful immunotherapy.

SIT harbours a certain risk for side effects, such as the possibility of large local inflammatory [18] and anaphylactic reactions [19]. Allergen extracts used for SIT are prepared from natural allergen sources and show high variation in their protein composition and amounts. They consist of allergenic and nonallergenic components [20,21], therefore they cannot be adjusted precisely to the patient's profile. Subsequent induction of new sensitization to irrelevant components has been observed after this treatment. Thereby, IgE antibodies are produced *de novo* against previously unrecognized B-cell epitopes on the allergen [22-24]. To make SIT more safe several attempts have concentrated on the concept of component-resolved diagnosis and therapy, which is based on recombinant allergens and hypoallergenic allergen derivatives. The advantage of recombinant allergens is the production of molecules with defined quality devoid of irrelevant components, allowing treatment according to the patient's sensitization profile [25]. Nevertheless, recombinant allergens still may present novel epitopes that possibly elicit new IgE antibody specificities. To bypass this unwanted reaction other studies focus on the concept of epitope-specific immunotherapy.

2.1 The importance of epitope specificity in allergy

The blocking capability of IgG is due to an antigen trapping or due to a blockade of the IgE-facilitated allergen presentation by B-cells to T-cells [14,26]. Furthermore, co-crosslinking

of FcεRI and FcγRIIb receptors by allergen-IgG complexes can negatively regulate allergy effector cells [27]. To be able to compete with IgE being a high affinity antibody, one would expect that high affinity of the IgG to the allergen represents a desirable feature of a potential blocking antibody. In surface plasmon resonance studies, however, none of the tested purified human or murine monoclonal IgG antibodies were able to reach the affinity of IgE antibodies to allergens [28]. Moreover, an affinity matured anti-allergen IgG antibody recognizing the wrong epitope will, even though present in molar excess, not successfully interfere with the binding of IgE to the allergen [29]. It was demonstrated that among different monoclonal antibodies specific for birch pollen major allergen Bet v 1 some antibodies were able to block IgE binding to Bet v 1, whereas others were able to even promote IgE recognition of Bet v 1 [30] *in vitro* [1] and *in vivo* [31]. The mechanism might be linked to the promotion of homodimerization and thus the IgE crosslinking capability of allergens [32] (Figure 1).

Consequently, SIT with whole allergen extracts or recombinant proteins, respectively, will produce both types of anti-allergen IgG, anaphylactogenic and blocking antibodies.

Therefore, it is crucial to systematically analyse the structural details of IgE epitopes on allergens. In principle, epitopes can be either of the linear (continuous) type, or of a conformational nature. Conformational, discontinuous epitopes are formed when amino acid residues that are not neighbouring within the sequence become spatially juxtaposed in the folded protein. For several allergens it could be demonstrated that effective IgE binding depends on their three-dimensional structural integrity [33]. In contrast, linear epitopes are formed by adjacent amino acid residues and may be inaccessible in the native conformation. A number of studies have tried to delineate B-cell epitopes from the primary amino acid sequence of proteins. This strategy is limited to the identification of solely linear epitopes, whereas B-cell epitopes cannot be predicted from these analyses because of their conformational nature [34]. Epitope-mimics, so-called mimotopes, represent a close approximation of the original 3D-epitope, even though their amino acid composition rarely shows similarities. This is due to the fact that mimotopes mimic an epitope by their biochemical and electrostatic properties, and not necessarily by sequence homology [35]. With the phage display technology it is possible to generate such structural mimics of B-cell epitopes.

3. Current status of cancer treatment

Whereas active immunotherapy is the preferred option in allergy treatment today, in cancer therapy passive immunotherapy is state of the art. Advances in recombinant DNA technology enabled the development of chimeric and humanized monoclonal antibodies that are far less antigenic than the original mouse monoclonal antibodies and exhibit longer half-lives within the serum [36]. With this invention a new area of anti-cancer therapy based on monoclonal antibodies additional to the classical treatments of cancer including surgery, radiation and chemotherapy began. Targets for monoclonal antibodies are growth factor receptors and surface protein and non-protein antigens overexpressed on malignant cells. Furthermore, antibodies have been armed with cytokines, chemotherapeutic agents, toxins, and radionuclides to augment their tumouricidal efficacy [37]. In 1997, rituximab (MabThera®, Roche in EU; Rituxan®, Biogen Idec/Genentech in USA) directed against CD20 on B-cells for the treatment of non-Hodgkin's lymphoma, became the first approved monoclonal antibody; since then nine monoclonal antibodies have received approval from the United States (FDA) to date for anti-cancer therapy, all of them of the IgG class [38,39].

One of the clinically most used monoclonal antibodies, trastuzumab (Herceptin®, Roche), is directed against the extracellular domain of human epidermal growth factor receptor 2

(HER-2) [40,41], a member of the EGFR family. HER-2 constitutively exists in an activated conformation [42], lacks ligand-binding activity and its signalling depends on heterodimerization with other members of the EGFR family, preferentially with HER-3 [43,44]. Trastuzumab was originally selected from a series of monoclonal antibodies, among which were detrimental, growth-promoting species. Trastuzumab acts via mediating antibody-dependent cellular cytotoxicity (ADCC), blocking the shedding of the extracellular domain of HER-2, reducing HER-2 signalling by physically inhibiting homo- or heterodimerization, initiation of G1 arrest and inhibition of angiogenesis [45,46].

Generally, high costs, repeated administration to achieve sufficient antibody titres and anti-tumour activity, as well as inadequate tissue distribution in the patients are the limiting factors in passive immunotherapy. An alternative, more patient-friendly approach is active immunotherapy with mimotopes, producing a polyclonal immune response consisting of the desired antibody specificity and additionally stimulating immunologic memory.

3.1 The importance of epitope specificity in cancer

From the above it is clear that epitope specificity plays a crucial role in the development of immunotherapy with monoclonal antibodies. To focus on trastuzumab, one has to mention that originally a number of anti-HER-2 antibodies had been isolated [47]. Among them, 4D5 (later trastuzumab) was selected due to its growth-inhibitory functions [48], in contrast to others, which even stimulated cancer cell growth [49]. The difference in their biological activity is strictly dependent on the respective targeted epitope on HER-2 and has to do with their ability to hinder or promote heterodimerization of HER-2, the latter inducing signal transduction and cell proliferation [2] (Figure 1). For this reason the characterization of tumour antigen epitopes is a basic step in developing epitope-specific immunotherapy. Yip *et al.* identified large and conformational B-cell epitopes on the tumour antigen HER-2 [2]. In 2003 Cho *et al.* successfully defined the binding site of trastuzumab as an epitope composed of three loops on the HER-2 molecule [50]. The work by Riemer *et al.* [51] showed that it is feasible to imitate such complex epitopes using peptide mimics generated by phage display technology: five characterized HER-2 mimotopes indeed matched to loop three, as defined by Cho *et al.*

4. Phage display technology

Phage display technology, first introduced by G. Smith in 1985 [52], is an advanced tool to define peptide structures that mimic natural epitopes including both conformational and linear epitopes. These peptides are called mimotopes [53]. The strength of the phage technology lies in the display of up to 10^9 different peptides in a library form enabling the selection of mimotopes in a repetitive screening procedure. A large number of protein–antibody, virus–antibody and ligand–receptor interfaces have already been mapped by the use of phage display technology [54].

For the creation of a peptide phage display library random oligonucleotides are inserted into the genome of the filamentous bacteriophage fd using either the minor coat protein pIII (display of 3 – 5 copies/phage) [52] or the major coat protein pVIII (display of up to 2700 copies/phage) [55] as display system on the surface of the phages. The peptides can be presented in either linear or circular form. To construct circular peptides the sequence has to be flanked by two cysteine residues. By building a disulfide bond a constrained cycle is formed and presented on the phage surface [56]. Beside the different structural presentations, the length of the peptides can vary from 6 to 38 amino acids [57]. Thus, within this phage system only the presentation of relatively short peptide sequences is possible, whereas larger gene insertions of proteins or antibody fragments appear to prevent pIII and pVIII functions necessary for phage reproduction [57]. To overcome this problem

phagemid systems have been developed [58]. The phagemid itself carries only the phage gene *gIII* or *gVIII*, containing the foreign sequence, and needs a helper phage with all the necessary genes for phage production including also a copy of the wild-type gene used for display. Therefore, both recombinant and wild type proteins will be produced and incorporated into the phage particle. For *pIII* one of five copies, and for *pVIII* from 1% to 30% of the 2700 copies will display the foreign protein [59]. For the production of combinatorial antibody libraries primarily phagemid systems are used resulting in the display of 'giant mimotopes', most often in association with *pIII*. Antibody display can be performed: i) by antibody fragment (Fab) systems, including two light chain domains (variable and constant) and the variable and first constant domain of the heavy chain; ii) by single-chain variable fragment (scFv) systems where only the variable domains of each chain are presented; or iii) by single-chain Fab (scFab) [59].

4.1 Biopanning-selection and characterization of the mimotope

In 'biopanning' [60] allergen- or antigen-specific antibodies of interest, monoclonal or polyclonal, are adsorbed on microtiter plates and incubated with a phage display library containing the complete repertoire of the respective library. Phages displaying peptide or protein domains which bind to the antibodies are caught whereas unbound phages are removed by washing steps. Bound phages are then eluted from the complexes by acidic solutions (such as HCl or glycine buffer) or by competition with the original antigen, if available. Amplified phage particles of the preceding round are used as starting material for the next panning round. Thereby, specifically interacting ligands can be amplified with great efficacy. Eluents from the biopanning rounds are tested by ELISA or other immunological methods. An increase in the titre of phage particles specifically binding to the selection antibody during the panning rounds is a first indicator of successful selection. Subsequently, the colony screening method is used to identify specifically interacting phage clones for further analysis. Generally, a strong signal in these tests predicts good mimicry of the original antigen, but this has to be additionally proven by competition assays with the original antigen or allergen [61]. After sequencing the most promising clones, computational matching studies can be performed using a software program rendering visualization of the location and the structural features of the epitope of interest [51,62]. Subsequent immunization studies with the mimotopes must prove molecular mimicry and should lead to antibodies recognizing the original allergen/antigen. Figure 2 illustrates the principle of mimotope selection, starting from the choice of the antibody used for biopanning to the desired allergen/antigen-specific immune response in mice.

5. Utilizing mimotopes to localize epitopes by computer modeling

Several groups have invented algorithms and programs that assist epitope mapping. They work with mimotope and antigen sequence information [63], or with mimotope sequence and antigen structure [64-66], or integrating the different approaches. A recent publication presents the Pep-3D-Search approach for predicting the epitope area through mimotopes or a motif sequence derived from a set of mimotopes [67]. Among all, MIMOX was the first free web tool for structural mapping of an antibody epitope by mimotope information [68].

During our own early work with mimotopes these programs were not available and we were forced to develop different methods to approach the question of 3D-epitope mapping: i) In an attempt to characterize a structural IgE epitope on birch pollen allergen Bet v 1, a computer-aided, 3-dimensional coarse-grained epitope search was developed [69]; ii) More recently, when we characterized the IgE epitopes of house dust mite allergens Der p 1 and Der p 2 [62] epitope matching was performed using BALL (Biochemical Algorithms Library) [70]. Two criteria were used to assess the quality of the potential epitope: solvent exposure and amino acid similarity assessed by a modified Gonnet matrix [71]; and iii) Even

more challenging was the alignment of mimotopes to HER-2 due to the 10 times larger molecular size than with allergen molecules resulting in the need for higher calculation power. Based on the antigen molecule's spatial structure from the RCSB Protein Data Bank [72] the algorithm took into account the relative flexibility of short peptides when scanning a given protein surface for matching sites [51]. Further, for investigations of mimotope-antibody interactions we took advantage of AutoDock 3.0.5 [73]. With this program it is possible to dock the oligopeptides to the Fab fragment of interest [35]. Taken together, the computational approaches available today allow allocation of B-cell epitopes with a high predictive accuracy.

6. Making the mimotope immunogenic

After mimotope characterization the obtained information must be applied to design an immunogen for successful induction of an epitope-specific immune response. Peptides are too small to elicit an immune response and are, therefore, immunologically ignored [74] or may induce tolerance [75]. Phage particles themselves are highly immunogenic because they carry sufficient T-helper cell epitopes on their surface [76]. On the other hand, phage-displayed mimotopes are not suitable for clinical studies in humans because the phage particles carry foreign DNA and antibiotic resistances. Therefore, alternative carrier systems have to be chosen which are necessary for achieving sufficient immunogenicity for vaccination.

Keyhole limpet hemocyanin (KLH) is a large protein that acts as a carrier, causes a strong immune response and provides additional T-helper cell epitopes, which is important as T-helper cells B-cells stimulate to increase their production level and quality of antibodies [77]. In parallel, also cytotoxic T-lymphocytes (CTLs) may be activated through T-helper-cell-derived IL-2. Tetanus toxoid (TT) represents a carrier protein similar to KLH with T-helper activity and has also been used as a peptide carrier previously [78,79]. In order to prevent crosslinking of preexisting IgE in allergy, the choice of the appropriate carrier system of an allergen mimotope has to be taken into consideration when constructing a vaccine. In an allergology study albumin binding protein (ABP) was used to construct a monovalent fusion protein with a mimotope of the birch pollen allergen Bet v 1 [80].

Another option to present a mimotope in a way suitable for immunization is the multiple antigenic peptide system (MAP), where linear peptides are synthesized in a straight-forward manner on a tyrosine backbone allowing a dense display of the peptides [81]. Recently, we synthesized a linear mimotope for the carcinoembryonic antigen (CEA) in MAP form and termed it "multiple antigenic mimotope" (MAM). This CEA-MAM was used for successful anti-cancer vaccination in a murine tumour transplant model [82]. Generally, for the vaccination with mimotopes one has to consider that the orientation and the structure of the mimotope in the vaccine should be the same as it was when displayed on the phage during biopanning. Table 1 summarizes the advantages and disadvantages of mimotope vaccination.

7. Epitope-specific vaccination with mimotopes

7.1 Mimotopes in allergy

Phage display technology was first applied in allergology to isolate a mimotope of the plant panallergen profilin by performing a biopanning experiment with purified IgG/IgE antibodies from the serum of profilin allergic patients. Constrained and linear random nonapeptide libraries were screened with the anti-profilin antibodies. The selected mimotope represented a cross-reacting epitope mimic for IgE antibodies directed against mugwort, birch pollen and celery tuber profilin and it was concluded that therapies with such peptides

might block the antigen binding sites of IgE without crosslinking and subsequent mediator release [83]. Consecutively, a similar panning experiment was performed with two murine monoclonal IgG antibodies, BIP1 and BIP4, directed against the major birch pollen allergen Bet v 1 [84], and with purified IgE from birch-pollen-allergic patients. As expected, alignments with the Bet v 1 amino acid sequence showed no linear homologies, but the epitopes could be localized by a computer-aided mathematical approach based on three-dimensional structure and chemical character of the amino acids [69]. Immunization with IgE mimotopes coupled to ABP in a monovalent fashion did not elicit type I skin reactions and, furthermore, induced allergen-specific IgGs that were able to inhibit IgE binding to Bet v 1 [80]. Again indicating the importance of epitope specificity, immunization with the BIP1 mimotope yielded anaphylactogenic antibodies [31].

Besides Bet v 1, which is recognized by 95% of birch pollen allergic patients, Phi p 5, the major grass pollen allergen, represents another important target for immunotherapy in allergy. Using Phl p 5a-specific IgE antibodies purified from allergic patients and screening a peptide as well as a combinatorial Fab library, conformational IgE epitopes could be identified with the help of the selected mimotopes. Further, Fabs and peptide mimotopes induced anti-Phi p 5a-specific immune responses in mice [85,86], even when injected in a DNA minigene format [87].

Furthermore, B-cell epitopes were successfully defined for parvalbumin, the major fish allergen [88] and for the two major house dust mite allergens, Der p 1 and Der p 2 [62]. The latter confirmed a previous study defining a Der p 1 epitope by a biopanning approach with a non-constrained 15mer- and a constrained 9mer-peptide library [89]. The selected Der p 1 and Der p 2 mimotopes determined epitopes within the conserved regions of the allergens. As crossreactive allergens not only share similar amino acid sequences but also structural homology, the generation of such mimotopes could be useful for the development of SIT targeting epitopes within a clinically cross-reactive allergen group [62]. A recent study identified two relevant conformational IgE-binding regions of Pru p 3, the major peach allergen, using a 12mer-random peptide phage display library [90].

The role of T-cell help for the formation of allergen-specific antibodies after immunization with phage-displayed mimotopes was investigated in proliferation studies with murine splenocytes. As expected, it was shown that the 11mer-mimotope did not crossreact with the allergen at the T-cellular level. It is tempting to speculate that this might be an ultimate advantage, as allergen-specific T-lymphocytes, which nourish chronic allergic inflammation [91] cannot be activated by the applied mimotopes. However, phage-displayed mimotopes were able to drive spleen cells to proliferation indicating that the carrier contributes to the immune response in a mimotope vaccine by recruiting bystander T-cells that help B-cells to generate an appropriate humoral immune response [92].

7.2 Mimotopes in cancer

Much more than in allergy, mimotope work has accelerated in cancer research since the first application of this technology in 2000: one group screened a phage display library with the mouse monoclonal antibody BCD-F9, which recognizes an unknown antigen found on the surface of many tumour cells. After four rounds of biopanning they selected one linear decapeptide, which was able to inhibit the binding of BCD-F9 to HT-1080 fibrosarcoma cells. Moreover, the polyclonal antibodies generated by immunization of rabbits bound specifically to HT-1080. Importantly, in a tumour metastasis model with CD-1 nude mice, passively applied anti-mimotope sera led to a prolonged life span [93].

7.2.1 Tumour-associated carbohydrate antigens (TACAs)—Carbohydrates are primarily T-cell independent antigens that elicit low antibody responses and poor memory.

The immunogenicity of these antigens has to be enhanced either by coupling to an appropriate carrier or by turning the carbohydrate into a peptide structure mimicking the original antigen. Mimotopes for different carbohydrate antigens such as GD3/GD2, Lewis Y and sialyl-Lewis X have been developed [94]. GD2, a disialoganglioside, is a relevant tumour antigen expressed on various tumours including neuroblastomas. Using the chimeric anti-GD2 antibody 14.18, which is applied for diagnostic purposes and had already entered clinical trials [95], a biopanning screening was performed. The selected circular mimotopes were analysed in a computer modelling program and fit well into the GD2 binding groove of an anti-GD2 Fab antibody [35]. In the subsequent study these mimotopes were coupled to KLH and administrated as active immunotherapy in mice, inducing a GD2-specific humoral immune response [96]. These and other GD2 mimotopes have been successfully used for immunization in DNA form [97,98]. The DNA-vaccine-induced antibodies recognized GD2-positive tumour cells, mediated complement-dependent cytotoxicity (CDC) and exhibited a reduction of GD2-positive melanoma growth in SCID mice after passive administration [98].

7.2.2 HMW-MAA and Mel-CAM in melanoma—In melanoma, the human high-molecular-weight melanoma-associated antigen (HMW-MAA) [99-101] and the melanoma cell-adhesion molecule (Mel-CAM) [102] have been used for mimotope vaccination studies.

In parallel studies the anti-HMW-MAA monoclonal antibody 225.28S which is known to suppress the metastatic potential of melanoma cells [103,104] was used for mimotope selection in biopanning experiments. We generated a mimotope-fusion protein with ABP and immunized BALB/c mice [100], whereas the group of Wagner *et al.* [99] performed immunization studies in rabbits administering mimotopes coupled to TT. Independently, both groups showed a specific anti-HMW-MAA anti-body response blotted and on the surface of intact melanoma cells. Even more important, the elicited antibodies inhibited the *in vitro* growth of melanoma cells and exhibited ADCC potential. Both studies demonstrated the principal potential of a mimotope vaccine in melanoma. In 2008, Wagner *et al.* published a study where they purified anti-HMW-MAA antibodies in rabbits after mimotope immunization and tested them *in vivo* in a SCID mouse melanoma xenograft model. A prophylactic and a therapeutic approach revealed that tumour growth was inhibited significantly [105].

Monoclonal antibody MAd18-5D7 against the melanoma antigen Mel-CAM was used for generation of mimotopes using a 28mer-peptide library. The authors showed that the 28mer-mimotope peptides alone were sufficiently antigenic to elicit a specific IgG response against Mel-CAM, but at lower titres than obtained with peptides linked to TT as a carrier. They further observed the production of T_H1-type cytokines from mimotope-stimulated proliferating spleen cells [102]. This may be due to mimotopes acting themselves as T-cell epitopes after antigen processing and presentation.

7.2.3 Growth factor receptors—Numerous studies in cancer have concentrated on the epidermal growth factor receptor family including four members, HER-1 (EGFR, ErbB1), HER-2 (ErbB2), HER-3 (ErbB3) and HER-4 (ErbB4), which are important for regulation of cell proliferation and differentiation [106]. Early studies using phage display methodology with three different tumour inhibitory anti-HER-2 antibodies indicated that due to the absence of sequence homology the epitope was most probably of conformational nature [107]. Our group has focused on the production of mimotopes for EGFR and HER-2 and, performed biopannings with the monoclonal antibodies cetuximab (Erbix®), MerckKGaA) and trastuzumab [108,109]. Both antibodies are currently applied for passive immunotherapy: cetuximab is approved for therapy in EGFR-overexpressing advanced colorectal cancer and trastuzumab for the treatment of patients with metastatic breast cancer

overexpressing HER-2 [38]. In both cases, the mimotope-induced antibodies mediated ADCC and CDC reactions, receptor internalization from the cell surface and a dose-dependent inhibition of tumour cell proliferation in highly specific manner and at a similar intensity to the original antibodies [108,109].

Besides the biopanning approach various groups have defined peptide mimics of tumour B-cell epitopes via computer-aided analysis, Dakappagari *et al.* designed *in silico* B-cell epitope peptides of HER-2, facilitated their folding mimicking the native protein structure and synthesized them collinearly with a promiscuous T-helper cell epitope derived from the measles virus fusion protein. Active immunization with one of these peptide constructs prevented the development of tumours in mice transgenic for the rat *neu* gene [110]. Alternatively, they combined two HER-2 B-cell epitope constructs, applied this multi-epitope vaccine in combination with IL-12 as a T_H1 adjuvant and observed a significant reduction in the number of HER-2 positive lung metastases [111].

Another group also generated HER-2 peptides representing B-cell epitopes of HER-2, which specifically induced anti-HER-2 antibodies able to mediate ADCC reactions [112]. This multi-peptide vaccine coupled to TT significantly delayed tumour formation and progression *in vivo* in FVB/N mice carrying the activated rat *c-neu* oncogene, under the control of a mouse mammary tumour virus promoter (MMTV-*c-neu* mice), which develop spontaneous breast tumours. Furthermore, the co-administration of IL-12 led to elevated IFN- γ levels and an increase in IgG2a antibody titre supporting the anti-tumour activity [113].

7.2.4 CD20—Another interesting tumour target is CD20 which is expressed on all mature B-cells, but not on their progenitor cells. Therefore, monoclonal antibodies to this antigen will destroy both benign and malignant mature B-cells, but allow the progenitor cells to re-establish normal hematopoietic cell lines [114]. Li *et al.* have generated mimotopes of CD20 using rituximab in a biopanning approach. They screened a 12mer peptide library and coupled the best mimotope with respect to mimicry potential, to KLH and TT for immunization experiments in BALB/c mice. With immunofluorescence staining and flow cytometry assays they showed that the immunized mice produced antibodies recognizing the native CD20 protein on tumour cell surfaces. Furthermore, in a CDC assay they proved the biological activity of the induced rituximab-like antibodies. None of the peptides showed sequence homology to CD20 in database alignments confirming that conformational epitopes play an important role also in this type of anti-cancer therapy [115].

7.2.5 CEACAM-5—Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM-5) is a poorly immunogenic glycoprotein and an important tumour marker, especially for colorectal cancer. It is expressed at up to 100 times higher levels in malignant tumour tissue samples compared with healthy tissues. A biopanning performed with the monoclonal anti-CEA antibody Col-1 led to the generation of a CEA mimotope which was synthesized as an octameric MAM and further studied in a mouse model. When BALB/c mice were prophylactically immunized with the MAM before subcutaneously receiving transplants of a tumour cell line overexpressing CEA, Meth-A/CEA, a specific and significant inhibition of tumour growth could be observed [82].

7.3 Mimotopes in allergooncology

The assumption on an inverse association between allergy and IgE levels and cancer derives from epidemiological data. Among other reports, a cohort study of 1,102,247 US men and women suggested a significant inverse association between a history of hay fever, and asthma and cancer mortality [116]. Engineered anti-tumour IgE antibodies have a high cytotoxic capacity due to interaction with potent effector cells. However, natural IgE has

also been described in squamous cell carcinoma of the head and neck [117] and in pancreatic cancer where its cytotoxic potential was demonstrated also [118]. The mechanisms of anti-tumour IgEs have already been studied in detail in several previous experiments [119-121], among them ADCC and antibody-dependent cell-mediated phagocytosis (ADCP) [122] seem to be most important. The IgE subclass has several advantages compared with IgG: it is bound with high affinity to receptors on its effector cells, the half-life of IgE in the periphery is longer than that of IgG and the effector cells expressing IgE receptors have been described within and around the tumour tissue [123]. Therefore, IgE antibodies directed against a tumour-associated antigen (TAA) could specifically trigger an immediate local effector cell response against the tumour cells [122]. Consequently, also the threat of systemic anaphylaxis has to be taken into consideration. However, in the presence of natural anti-tumour IgE antibodies (see above) no anaphylactic events were observed. This may be due to the fact that circulating TAAs which were shed from the tumour cell membrane are monovalent and thus not able to crosslink cell bound IgE. In contrast, overexpressed TAAs on the tumour membrane can crosslink IgE and release tumouricidal mediators from effector cells exactly at the site where they are needed.

To establish an active vaccination for the directed induction of tumour-specific IgE antibodies the above mentioned HER-2 mimotope [108] was applied orally in connection with anti-ulcer medication. The rationale for this derives from the observation that IgE antibodies are preferentially induced when peptic digestion is hindered under anti-acidic treatment [124]. As expected, BALB/c mice immunized with the HER-2 mimotope via the oral route under anti-acidic treatment developed IgE antibodies cross-reactive with the original antigen HER-2. Moreover, the HER-2-overexpressing breast cancer cell line SKBR-3 specifically crosslinked the induced IgE in a rat basophilic leukemia (RBL) assay. Furthermore, the released mediators, potentially through TNF- α action [125], acted tumouricidal on SKBR-3 cells. The effect was highly specific, as with a HER-2-negative control cell line no cytotoxicity was observed [126]. Thus, depending on the route and adjuvant, mimotopes may be used for a directed induction of anti-tumour IgE antibodies.

8. Conclusion

Epitope specificity can of decisive importance in the development of vaccines, which are primarily based on a humoral immune response. In the fields of allergy and cancer, it has been demonstrated by preclinical studies that epitope mimics can be used to define B-cell epitopes on allergens/antigens of interest and, more importantly, upon immunization actively induce epitope-specific antibodies. The elicited antibodies can block the allergen-IgE interaction. Moreover, as the mimotopes do not contain allergen-specific T-cell epitopes, reactivation of inflammatory allergen-specific T-lymphocytes is not supported through a mimotope vaccine. In cancer, the mimotopes induce biological activities similarly to the original monoclonal antibody, including *in vitro* and *in vivo* growth inhibition, ADCC, CDC and ADCP. In the near future, mimotopes could be an alternative prophylactic, adjuvant and therapeutic strategy against allergy and cancer.

9. Expert opinion

Although the term 'immunotherapy' is used in allergy and cancer, the meaning is different. Cancer immunotherapy mainly focuses on passive administration of monoclonal antibodies directed against a defined part of a tumour antigen, whereas generally in allergy, immunotherapy means the injection of whole-allergen extracts inducing allergen-specific non-responsiveness. A principle of SIT in allergy for downregulation the allergic hyper-responsiveness is the induction of blocking IgG antibodies, which should optimally be directed against IgE epitopes for successfully interfering with allergen-IgE interactions.

Instead of whole allergen molecules, or even whole allergen extracts, it might be sufficient to perform immunotherapy with a subunit vaccine, such as mimotopes of B-cell epitope on the allergen.

The early trials using murine monoclonal antibodies for cancer therapy were accompanied by human anti-mouse antibody (HAMA) immune responses and relatively low efficacy due to the lack of effector functions of murine antibodies in concert with human effector cells [127]. Possibly connected with this problem at that time, much attention was rather given on the cytotoxic T-cell-dependent immunity. However, these therapies have limitations as tumour cells have developed escape strategies to avoid T-cell responses, such as downregulation of HLA [128,129]. Moreover, the applicability of T-cell-based peptide vaccines is restricted to the patient's HLA haplotype.

In the meantime, antibody immunotherapies have, due to technical advances, made considerable progress: currently chimeric and humanized monoclonal antibodies with minimal antigenicity are applied as passive immunotherapeutics, mostly in combination with chemotherapy. A major advantage of passive immunotherapy is that it can be turned off at any time point. Disadvantages are side effects (most often delayed-type hypersensitivity infusion reactions mostly due to the high antibody load leading to immune complex formation [130]), but also the high costs. To offer an alternative choice and to improve quality of life for the patients during the course of tumour disease, vaccination should be considered.

With mimotope vaccines, complete-self-antibodies with the desired epitope specificity can be induced representing a continuously available, polyclonal antibody response. Although the anti-mimotope antibodies are dominated by a certain subclass depending on the route, formulation and cytokine environment of the vaccine, the overall immune response is constituted by several isotypes mediating several immune effector mechanisms. So far, promising animal studies are available, and pre-clinical and clinical studies are on the way. We suggest that minimal residual disease or prophylactic approaches in high-risk patients will be the favourable setting. These studies may break the ground for active tumour immunotherapy according to the successful concept of vaccines against infectious diseases.

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Figure 1. The effects of IgG in allergy and cancer

Depending on their epitope specificity IgG antibodies can either inhibit or promote allergic reactions or tumour growth. **A.** In allergy, specific IgGs enhance the IgE binding to allergens and the anaphylactic reaction, which could be due to supporting the crosslinking capability of allergens. **B.** On the one hand, inhibitory IgG antibodies which recognize the IgE epitope on the allergen directly block the binding of IgE to the allergen, thus, preventing mediator release. On the other hand, IgGs can act via the FcγRIIb receptor: On B-cells this results in an inhibition of IgE-facilitated antigen presentation. The inhibitory effect on FcεRI positive effector cells is not illustrated. **C.** In cancer, heterodimerization of HER-2, HER-3 and other family members leads to signalling and proliferation. Therefore, antibodies promoting heterodimerization are detrimental. **D.** In contrast, IgG antibodies hindering heterodimerization prevent signal transduction and cell growth.

Figure 2. The principle of mimotopes

The first step in generating mimotopes is the choice of an antibody recognizing the allergen/antigen of interest. In allergy, biopanning can be carried out with allergen-specific IgE whereas in cancer, an inhibitory monoclonal antibody against the tumour antigen of interest is chosen. During biopanning mimotopes are selected and further investigated for specificity to the original antibody. Afterwards, mimotopes are tested for their ability to compete with the native allergen/antigen for binding to the original antibody. The selected mimotope is coupled to a carrier and used for immunization studies. After successful immunization, mice produce antibodies against the same epitope of the original allergen/antigen which is recognized by the antibody used for biopanning.

Table 1

Advantages and disadvantages of mimotope vaccination in allergy and cancer.

Advantages of mimotopes
Easily obtained by the phage display technology
High stability in storage
Cheaper than the production of monoclonal antibodies
Free of contamination with toxic substances (e.g., from expression systems)
Coupling to immunogenic carrier molecules enhancing their antigenicity
Induction of an epitope-specific humoral immune response
Do not contain allergen/antigen T-cell epitopes
Induction of a long-lasting immune response/ no turn off
Disadvantages of mimotopes
Induction of a long-lasting immune response/ no turn off
Lower affinity than original antibody