

Chapter 10

The Targets of IgE: Allergen-Associated and Tumor-Associated Molecular Patterns

Erika Jensen-Jarolim, Diana Mechtcheriakova, and Isabella Pali-Schoell

Abstract The most studied targets of IgE immunoglobulins are termed allergens and mostly represent protein antigens from plant or animal origin. However, it has been revealed that also pathogens and self-antigens can be recognized by IgE. Thus, besides a detrimental role in allergy, IgE may exert defense and surveillance functions. Here, we discuss the display of rigid epitope patterns as a common key feature of antigens leading to both formation of high-affinity IgE antibodies by B lymphocytes and IgE-mediated triggering of effector cells. In analogy to the principle of pathogen-associated patterns as danger signals, we introduce here the terms allergen-associated molecular patterns (AAMPs) for the repetitive epitopes exposed by allergens and tumor-associated molecular patterns (TAMPs) in settings when molecular epitope patterns are displayed through overexpressed tumor antigens.

10.1 Background: IgE and the Anaphylactic Reaction

As already discussed in the previous chapters, IgE antibodies have several unique features which enable them to induce highly specific biological responses. Taken together, major features of IgE are (i) high binding strength to their target epitopes through unique affinity maturation program of the IgE-producing B lymphocytes (epsilon B lymphocytes) in an appropriate milieu of cytokine and danger signals [1–3]; (ii) high-affinity interaction of their Fc epsilon (Fcε) domain with specialized FcεRI receptors on a distinct panel of mostly cytotoxic and phagocytic effector cells conferring antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cell-mediated phagocytosis (ADCP) functions to them [4–6]; (iii) moderate affinity interactions with specific IgE receptors of the CD23 C-lectin type enabling IgE to participate in antigen uptake, transport and presentation by classical antigen-presenting cells (APCs) including dendritic cells (DCs) and B lymphocytes, as well as non-classical APCs like epithelial cells [7, 8]. Depending on the

E. Jensen-Jarolim (✉)

IPP – Department of Pathophysiology, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria

e-mail: erika.jensen-jarolim@meduniwien.ac.at

time point, duration and repetition of antigen contact IgE will either induce reactions of the immediate anaphylactic type or nourish chronic Th2-type inflammation [9–11]. In any circumstances, an ongoing IgE-mediated response will be hard to turn off, symbolic for the sensitization state of the individual and characterized by “tissue memory” of IgE [12], prolonging the survival rate of its effector cells. To counterbalance an undesired IgE response toward actually harmless allergens, today’s allergen immunotherapy aims at inducing a tolerant state in the patient [13, 14]. This immune tolerance is supported by the production of immunomodulating cytokines and the induction of non- or anti-inflammatory blocking IgG antibodies which interact with FcγRIIb receptors on epsilon B lymphocytes and on FcεRI-positive effector cells and thus are able to downregulate IgE levels [15]. In this process, immunomodulatory cytokines IL-10 and TGF-beta are driving forces [16]. Both cytokines are, however, also produced by some tumor types and may participate in undesired tolerance phenomena in cancer diseases [17]. On the other hand, it has been reported that epithelial tumors are the source of typical Th2 cytokines as IL-4 and IL-13 [18, 19] which are directly involved in IgE class switch [20]. Anecdotic reports on natural IgE present in tumor tissues indicate that they may have pathophysiological significance in tumor surveillance and attack [21, 22]. Systematic allergooncological approaches to study the prevalence of natural antitumor IgE are at this time point missing [23]. Nevertheless, the advanced knowledge derived from the studies on allergic diseases may help to understand the function of IgE in malignancies. For this, a comparison of the possible target structures of IgE on allergens and on self-antigens like tumor antigens has been undertaken in this chapter.

10.2 Crosslinking of Antigens – A Biological Tool for Immunoregulation

Antibodies, when expressed by B lymphocytes as membrane immunoglobulins (mIg; also termed B-cell receptor, BCR) and in their secreted form, represent homodimeric molecules, thus being principally equipped with two identical antigen binding sites called paratopes [24]. The paratopes are opposed to the epitopes of an antigen which represent exposed molecular surface plains, covering a relative flat area between 600 and 1,000 Å² [25, 26]. Some B-cell epitopes may be cryptic, meaning normally not accessible to the immune system, and might be exposed after physical, chemical or enzymatic processing of an antigen.

The cognition that several short, discontinuous stretches of an antigenic protein chain together form the 3D antibody epitopes [27] has rendered multiple efforts using structural mimics of epitopes, that is mimotopes, as a circumvention strategy for 3D epitope definition [28, 29]. (Such mimotopes can also be applied for epitope-specific vaccination and are suitable for induction of tumoricidal IgE when applied by the oral route; see also [30, 31] and Chapter 7 of this book.)

However, linear sub-epitopes in the range between 8 and 12 amino acids could be as well mapped recently with high accuracy using an antibody-based proteomics

approach [32]. On the membrane of a resting B cell the distance of BCRs has been determined to be approximately 40 nm [33]. The phenomenon that properly spaced multiple copies of an epitope are needed to turn on or turn off a B lymphocyte rendered the cognition that antigens may act as immunoregulators [34]. The collected evidence concerns predominantly the formation of IgM and, consecutively, IgG antibodies by B lymphocytes [35]. Similarly, direct isotype switch from IgM to IgE can occur [36], but depending on the antigen, IgE switch may even occur in an IgM-independent fashion [37]. Chapter 3 of this book gives a comprehensive overview on this topic, also with respect to the effects on affinities of different IgE species.

During the last four decades numerous studies have focused on the evaluation of the epitope requirements for B-lymphocyte activation. Already early immunization studies have indicated that some antigens per se rather induced tolerance, whereas others were prone to act immunogenic. This was systematically analyzed in vitro using conjugates of flagellin as a carrier with different hapten density on it [38]. Within the same series of chapters, Feldmann specified this observation by stating that antigen molecules in a monomeric fashion or with few epitopes never induced tolerance, whereas highly dense display of epitopes did [39]. He concluded that “a tolerance-inducing antigen must bind more avidly to B-cell membranes than an immunogen. . .” [39]. Obviously, the dense display of epitopes on the body’s own cell membranes could, therefore, be involved in the maintenance of tolerance against self.

It has further been documented that these immunoregulative phenomena of antigens could be enhanced by coupling or admixing the same protein antigens to immunogenic or “non-immunogenic” carriers [40]. Among carriers for antigen-specific tolerance induction polyethylene-glycol became particularly popular [41], whereas for antigen-specific immunity coupling to non-related protein carriers like keyhole limpet hemocyanin (KLH), encapsulation in particulate materials [42] or admixture to the protein-absorbing adjuvant $\text{Al}(\text{OH})_3$ has been preferred. As vaccine carriers often enhance the antigenic density, the resulting opposed types of immune responses may thus have to do with the different epitope spacing.

Among B-lymphocyte responses, one has also to dissect between the so-called T-lymphocyte-dependent (TD) and T-independent (TI) responses. According to their definition, for immune responses to TD antigens like low-dose-soluble antigen or monovalent haptenated proteins T-cell help has to be provided to induce sufficient immunity [43]. However, T-cell dependence is a relative matter: some antigens, largely TI but still needing residual T-cell help, are called TI-2 antigens. However, in complete T-cell independence, also immune responses comprising high titers of IgM antibodies may be generated by the so-called TI-1 antigens. In this model, polymeric high-molecular-weight antigens (like lipopolysaccharide, LPS) may represent polyclonal B-cell stimulators or – when exposing highly organized, rigid surfaces – be potent-specific crosslinkers of the BCRs. In a classic publication, Dintzis et al. defined in 1976 the *immunon*, that is, minimally 12–20 antigenic determinants spaced in a distance of 10 nm, as the minimal requirement of TI B-cell activation [44]. Later, Bruton’s tyrosine kinase (Btk) was found to be associated with these clusters, not only being responsible for B-cell activation in terms of proliferation

and antibody synthesis but also controlling the cytoskeleton and thus having a function in antigen processing and presentation [45]. In further pioneer studies, Dintzis et al. specified that for the optimal spacing of at least approximately 20 haptenic epitopes within an immunon, a minimal threshold size of approximately 100 kDa of the monomeric antigen is required [46]. Therefore, antigens which are too small to display a sufficient number of epitopes to form an immunon can bind B lymphocytes but cannot stimulate them. On the other hand, it has been realized that sometimes large polymeric molecules, although exhibiting a dense epitope display, are not inducing immunity but in contrast tolerance. The explanation was that the distance between epitopes of multivalent antigens can also be too large to allow productive B-cell stimulation [46]. In that case, recognition of several epitopes far apart on the antigen, but coming together on a flexible loop, does not substitute for a rigid, optimal crosslinking. Taken together, crosslinking of the BCR occurs optimally with antigens exhibiting a repetitive (at least 12–20 copies) epitope display in rigid spacing above 5–10 nm. Interestingly, some viruses displaying a crystalline-like surface with perfectly spaced epitopes have been recognized as TI-1 antigens [47, 48]. In both TI cases, the antigenic determinants must be arranged in a properly spaced manner for optimal crosslinking, B-cell activation and IgM immunoglobulin synthesis. The degree of antigen organization is even more important for the magnitude of IgG responses and can be supported by costimulatory B-cell signals like via CD21 [49] and Toll-like receptor-4 (TLR4)–MyD88 complex [50], the latter being also susceptible for stimulation with heat shock protein 60 (HSP60) [51]. Further, the spacing of the virus epitopes is affecting the affinity of the (bivalent) IgG response and its capacity to reduce the infectivity of the virion particle [52].

The B lymphocytes are, therefore, today recognized as pattern recognizers activated not by single epitopes but by the repetitiveness and spatial organization of the antigen [53]. Importantly, the concentration or aggregation state of an antigen, for instance upon chemical modification, can determine its immunogenicity [54]; environmental stress augments expression of pathogenesis-related proteins in plants [55], and in turn, food processing may render immunogenic aggregates of proteins [56]. With increasing concentration of an antigen also the epitope density increases and may significantly improve the recognition and uptake of the antigen by B-cells as well as other professional APCs. This is important, because professional APCs take care for recruitment of specific and bystander T-helper cells and thus enhance the extent of immune responses. Importantly, it is not sufficient that TD antigens are sensed by the specific BCR but simultaneously a second danger signal is received via by TLRs, representing another class of pattern recognition receptors (PRRs) also expressed by the B lymphocytes [57]. In an experimental system using transgenic mice devoid of TLR on their B lymphocytes (but not on DCs), antigen-specific antibody production indeed was deficient and could not be substituted by adoptive transfer of CD4-positive T-cells [58].

Generally, for TD antigens, the given cytokine milieu will determine the consecutive isotype switch, and the immune response can further be promoted by natural [59] or artificial adjuvants and carriers [60, 61].

10.3 Crosslinking – Indispensable for IgE Synthesis and Pathophysiology

We hypothesize that the above reviewed principles for B-cell activation, namely, rigid and dense (but not too dense) epitope display on antigens, will similarly account for IgE production. The rationale for this is derived from the fact that in both the sensitization and the effector phase of IgE-mediated responses crosslinking is a critical biological phenomenon. Thereby, the epitopes of an antigen (or allergen if clinically undesired hypersensitivity is meant) must interact with the complementary determining regions (CDRs) of at least two IgE antibodies to induce a biological response in the form of signal transduction, protein synthesis or mediator release, respectively [62, 63].

For the mast cell, being the most typical IgE-binding effector cell, it has been shown that also the antigen amount and contact duration are decisive for the overall signal strength and thus determine whether the cell will degranulate or acquire enhanced survival [64, 65]. In contrast, interaction of two antigenic epitopes with only one homodimeric IgE molecule expressed as membrane IgE (mIgE) by a B-lymphocyte or passively bound to high-affinity FcεRI will not activate but, contrarily, lead to anergy or tolerance by the B-cell or non-reactivity of the effector cell. The same principle obviously accounts for DCs, where polyvalent but not monovalent IgE engagement resulted in internalization of antigens [66].

Therefore, the spacing of epitopes will critically affect antigenicity and allergenicity of an agent with respect to its capacity to induce immunoglobulin synthesis. The single, monoclonal B lymphocyte is uttermost restrictive, because each single immunoglobulin expressed on its membrane will recognize the same epitope. Consequently, as discussed above, recent work suggests that B lymphocytes are pattern recognizers [53]. Primarily, upon antigen contact and TI activation, the B lymphocyte will produce a soluble form of IgM antibodies through alternative splicing and secrete them. However, if simultaneously with repeated antigen contact the CD40 ligation and/or IL-4 and IL-13 stimulation occurs, the specific B lymphocyte has several options to switch toward IgE: (i) direct deletional switching [67, 68] and (ii) sequential switching via gamma 4 [68, 36] or even in an IgM-independent fashion, which was at least demonstrated in mice infested with helminths [37]. The (IgE-producing) εB-lymphocytes undergo somatic hypermutation and affinity maturation in spite of their location outside germinal centers [3]. Thereby, recognition of the original antigen epitopes is improved substantially, rendering outstanding IgE affinities, with equilibrium dissociation constants ($K_D = k_d/k_a$) between 10^{-10} and 10^{-11} M [2]. Obviously, also for the reactivation and expansion of the memory subset of the B lymphocytes at later points of time it is crucial that epitopes are presented in a repetitive manner. Indeed, the antibody response in sensitized mice was successfully re-challenged with a dimeric allergen, but not monomeric allergen [69]. For memory εB lymphocytes, contacts with TLR activators may possibly be important for productive activation too, as previously demonstrated for IgG [50]. Moreover, also the achieved affinity to the epitopes will affect surface binding of

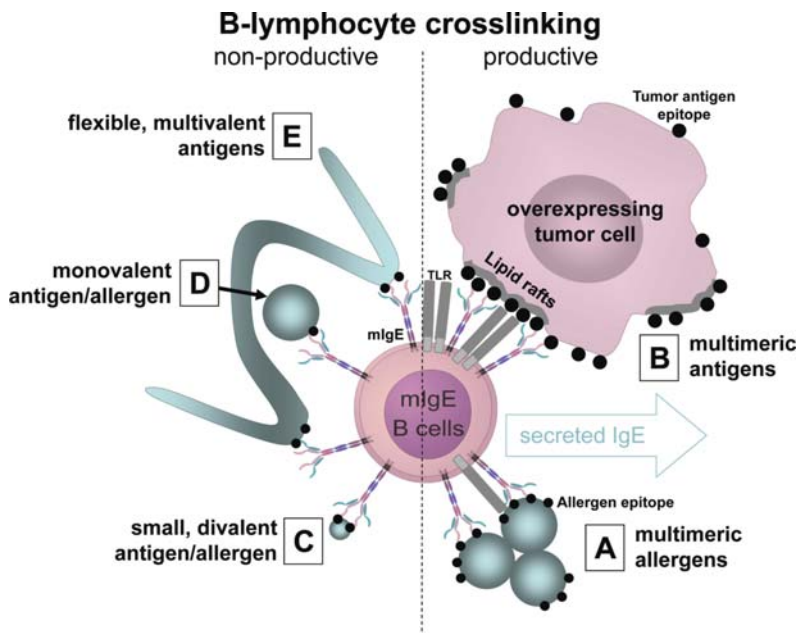


Fig. 10.1 The antigen determines immune reactions through the spacing of its B-cell epitopes. *Right side:* Productive activation (including antibody synthesis) will take place when a multivalent antigen is able to crosslink more than one mIgE by the rigid display of repetitive epitopes. These requirements are fulfilled by multimeric allergens (A), and overexpressed tumor antigens, e.g. accumulating in lipid rafts (B). Other pattern recognition receptors, as TLR family members, may contribute to the extent of activation. *Left side:* Non-productive activation (cytokine production, prolonged survival, but no IgE synthesis) will take place when mIgE remains monomeric. This may happen upon contact with a too small divalent antigen (C), with a single molecular unit of a monovalent antigen (D), and upon contact with loosely connected epitopes (E)

allergens and level of activation [70, 71]. Contrarily, antigens may also induce tolerance in IgE-producing B lymphocytes, possibly in case of multivalent polymers, improper epitope spacing, high flexibility of antigen, or at too small molecular size of an antigen, being monomeric [72] (Fig. 10.1).

Spacing of allergenic epitopes is also critical for the effector phase of the established immediate-type allergy, when IgE is already bound with high affinity to its receptors on mast cells, eosinophils or basophils. According to the textbook, allergens have to be at least bivalent, but should rather be multivalent to increase their potency to activate FcεRI-bearing effector cells, that is, exposure of more than one IgE epitope (Fig. 10.2). Moreover, the FcεRI signal depends on concentration and affinity of both the antigen and the receptors [73].

Interestingly, activation via FcεRI and TLR-4 both induce a shared core response of gene expression toward Th2 in mast cells [74]. Thereby, also monovalent IgE-allergen interactions can induce signaling and prolong the lifetime of mast cells [64, 74, 75] and induce FcεRI surface expression [76, 77]. The higher the epitope density, the more likely effective crosslinking will occur. Whether or not an allergic

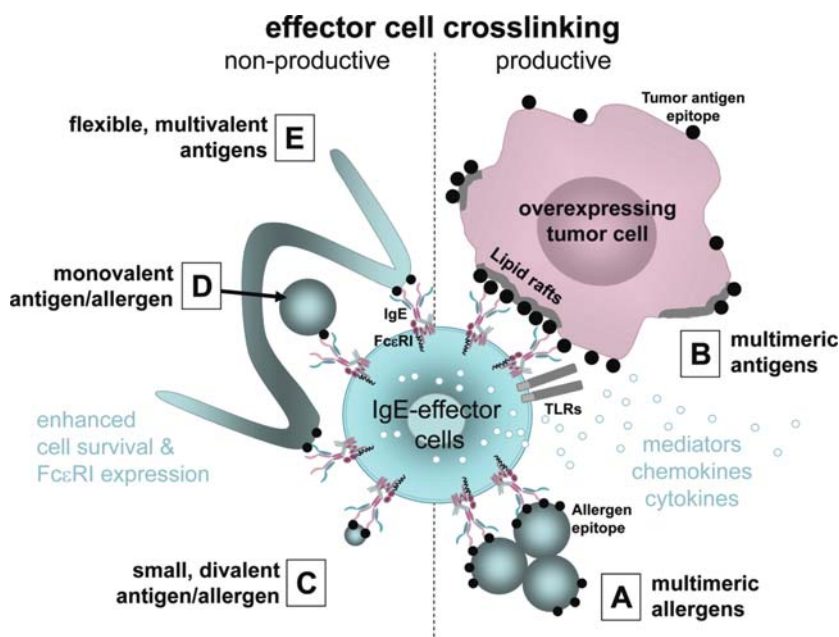


Fig. 10.2 The antigen determines the extent of immediate type inflammation upon crosslinking through the spacing of its B-cell epitopes. A typical effector cell, for instance a mast cell, is illustrated with multiple IgE molecules bound in bent shape to its high affinity receptors FcεRI, constituted of four chains. *Right side:* Productive activation rendering mediator release will take place when a multivalent antigen is able to crosslink more than one FcεRI-bound IgE by the rigid display of repetitive epitopes. These requirements are fulfilled by multimeric allergens (A) and overexpressed tumor antigens, e.g. accumulating in lipid rafts. The later may lead to antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP) (B). Other pattern recognition receptors, as TLR family members (dark grey bars), may contribute to the extent of activation. *Left side:* Non-productive activation (cytokine production, prolonged survival) will take place when FcεRI-bound IgE remains monomeric. This may happen upon contact with a too small divalent antigen (C), with a single molecular unit of a monovalent antigen (D), and upon contact with loosely connected epitopes (E)

reaction will be elicited may, however, finally depend on the structural integrity of the allergen and on the distinct spacing of its IgE epitopes (reviewed in [78]).

In early IgE crosslinking studies with rigidly spaced bivalent avidin constructs, molecules with a length of 49.4 Å were poorer triggering agents than those of 92.7 and 135.8 Å; the best cellular degranulation effect was achieved with dimers and polymers from the length of about 80–240 Å [79] (Fig. 10.3). In contrast, more recent studies indicate a shorter distance of allergen epitopes: performing in vitro studies with double-stranded DNA as a versatile rigid spacer, Paar et al. were able to show that for effective crosslinking of specific IgE on rat basophil leukemia cells, an inter-epitope distance of 44–51 Å was optimal to stimulate degranulation [80].

Further, the interaction of an antibody with its specific epitope depends on the flexibility of the Fab arms, which again depends on the respective immunoglobulin isotype. For the Fab arms of IgE in solution there is limited segmental flexibility, and

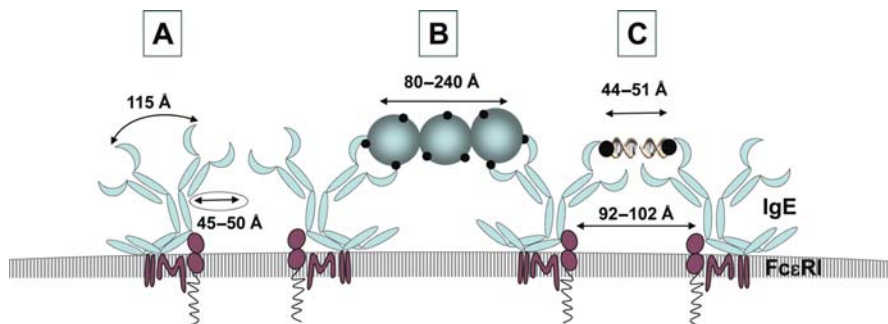


Fig. 10.3 Inter-molecular distances optimal to stimulate degranulation of effector cells via IgE bound to its high affinity receptor, Fc ϵ RI. (A) The estimated intra-molecular distance between two Fab binding sites is at least 115 Å [88] and thus fits to the calculated upper limit for the radius of Fc ϵ RI-bound IgE in the range of 45–50 Å [79]. (B) Cellular degranulation through antigen-mediated IgE crosslinking was optimally triggered by dimers or polymers from the length of 80 to 240 Å [79], but also (C) by smaller proteins using dsDNA as rigid model spacer with a distance of 44–51 Å [80]. The minimal distance between two IgE molecules cross-linked by antigen was estimated in the range of 92–102 Å [79]

Fab arms are not free to undergo extensive “wagging” motions [79] also because of the bent shape of IgE [81], both in solution and on Fc ϵ RI [82–84]. This means that the IgE antibody itself cannot adapt the fit to the allergen.

Allergic individuals have a small number of IgE antibodies on the basophil surface (about 40,000 molecules per cell) evenly distributed in non-activated condition [85]. It was speculated by Siraganian et al. that short bivalent haptens are capable of eliciting histamine release because the second Fc ϵ RI-bound IgE molecule would move closer through lateral diffusion within the cell membrane [86], today more precisely defined as movements into and within the lipid rafts of detergent-resistant membranes [87]. Resonance energy transfer measurements suggested a minimal distance of 92–102 Å between two cross-linked IgE molecules and an upper limit for the receptor radius of 45–50 Å [79].

On the other hand, the two Fab-binding sites *within one IgE molecule* were calculated to be at least 115 Å apart [88] (Fig. 10.3). It was furthermore shown in this study that flexible bivalent ligands longer than 130 Å form cyclic monomers with IgE in solution, that is intra-molecular bridging, and thereby rather block than induce histamine release. This would mean that, for instance, a small globular allergen dimer like Bet v 1, the major birch pollen allergen, with an estimated diameter of 38 Å could not form intramolecular bridging of Fab sites of the same IgE and is actually also below the optimal distance for cross-link of two separate IgE antibodies.

10.4 AAMPs: Allergen-Associated Molecular Patterns

Motifs recognized by PRRs may be called pathogen-associated molecular patterns (PAMPs) when associated with membranes of infectious agents [89]. We

suggest here that also allergens display repetitive motifs which we accordingly designate allergen-associated molecular patterns (AAMPs). Our suggestion is based on the observation that in many cases allergenic molecules occur as dimers or even multimers, a list growing longer over time (Table 10.1).

The consequence of di- or multimerization is the repetitive display of individual epitopes within the molecular complex. As suggested previously, the repetitive epitope display is important for both the sensitization and the effector phase of type I allergy [69] and may represent the long-sought common characteristic of allergens. Some allergens, especially small proteins, may possess only few or even a single immunodominant B-cell epitope and therefore as a single molecular unit do not fulfill the requirements for crosslinking. Examples are Api m 4 from bee (*Apis mellifera*) venom with only 2.8 kDa (26 amino acid residues) [90] and Amb a 5 from ragweed (*Ambrosia artemisiifolia*) pollen, which shows one very dominant B-cell epitope for human IgG binding [91]; also for the major birch (*Betula verrucosa*) pollen allergen Bet v 1 a single immunodominant murine IgG epitope was identified [92, 26, 93], which was distinct from the epitope of human polyclonal IgE [94]. For such small proteins, multimerization or oligomerization may provide the necessary means for efficient IgE crosslinking. Indeed, for Amb m 4 the formation of tetramers in aqueous solution was confirmed [95, 96] and Bet v 1 also forms dimers and higher oligomers in physiological solution, which is a necessary pre-requisite for its biological allergenic activity [69]. In accordance, Ghosh et al. have recently demonstrated that protein T1, a non-allergenic Bet v 1-homologue, lacks the capacity to dimerize although sharing a high-sequence and structural homology with other members of the Bet v 1 family [97].

When a series of recombinant monoclonal antibodies equipped with a humanized Fc ϵ domain were tested for their efficacy to activate mast cells, the antibody concentration, affinity and clonality affected the degree of effector cell degranulation [98]. As this experiment was based on murine IgG monoclonals originally raised in mice it may, however, be not representative for the human setting. The impact of epitope presentations for the crosslinking of monoclonal IgE antibodies was further tested with synthetic B-epitope peptides of allergen Phl p 1 from timothy grass (*Phleum pratense*) pollen. The polymeric peptide resulted in the highest release of β -hexosaminidase (70% of total release) compared to monomeric peptide (no release) and dimeric peptide (41% of total release) in in vitro experiments [99]. Moreover, although bivalent DNP-antigen was sufficient to bind and crosslink IgE, the best degranulation results were obtained with multivalent DNP-antigen using a rigid DNA display system [80] (Fig. 10.3).

A limited number of epitopes has also been shown for the major allergen from house dust mite (*Dermatophagoides pteronyssinus*), Der p 1, and Amb a 1 from ragweed, where binding of polyclonal IgE from serum of allergic patients was studied with respect to binding affinities and heterogeneity of recognized epitopes [100]. It was observed that in highly sensitized donors only two to three different affinity levels in antibody-antigen binding studies were relevant. The authors conclude from their work that a large proportion of sensitized patients harbor high-affinity IgE antibodies toward the same epitopes and that therefore the final affinity is determined

Table 10.1 Examples of allergens able to form dimers, multimers and higher aggregates (sorted by year of publication)

Allergen (source) ^a	Year of publication	References
Gad c 1 (Baltic codfish)	1967	Aas and Jebsen [140]
Api m 4 (bee venom)	1982	Terwilliger et al. [96]
ABA 1 (<i>Ascaris</i> , nematodes)	1990	McGibbon et al. [142]
A2U (rat urinary allergen)	1992	Bocskei et al. [143]
Mus m 1 (MUP; mouse urinary protein)	1992	Bocskei et al. [143]
Gly m Bd 30 K (soy bean)	1993	Ogawa et al. [144]
Sol i II (fire ant)	1993	Hoffman [145]
Tropomyosin (e.g. fish)	1995	Gimona et al. [146]
Alt a 1 (<i>Alternaria</i> , fungi)	1996	De Vouge et al. [147]
Bet v 1 (birch pollen)	1996	Wellhausen et al. [148]
Bos d 2 (BDA20) and Bos d 5 (alpha-lactalbumin), bovine dander	1996	Rautiainen et al. [149]
Ara h 1 (peanut)	1998	Shin et al. [150]
Phl p 1 (grass pollen)	1998	Petersen et al. [151]
Aca s 13 (<i>Acarus siro</i> , dust mite)	1999	Eriksson et al. [152]
Equ c 1 (horse dander)	1999	Gregoire et al. [153]
Tropomyosin (seafood)	1999	Reese et al. [102]
ABA-1 (<i>Ascaris</i> , nematodes)	2000	Xia et al. [154]
Ara h 1 (peanut)	2000	Maleki et al. [155]
Equ c 1 (horse dander)	2000	Lascombe et al. [156]
Ves v 5 (wasp venom)	2000	Suck et al. [157]
Ole e 1 (olive pollen)	2001	Huecas et al. [141]
Beta-lactoglobulin (bovine milk)	2001	Sakurai et al. [158]
Ara h 2 (peanut)	2002	Sen et al. [159]
Gad m 1 (Atlantic codfish)	2002	Das Dorees et al. [160]
Phl p 5b (grass pollen)	2002	Rajashankar et al. [161]
Phl p 7 (grass pollen)	2002	Verdino et al. [162]
Profilin (panallergen)	2002	Wopfner et al. [163]
Phl p 1 (grass pollen)	2003	Ball et al., RCSB Protein Data Bank 1N10 [168]
Cav p 1 (guinea pig dander)	2003	Fahlbusch et al. [164]
Fel d 1 (cat dander)	2003	Gronlund et al. [165]
Bet v 1 (birch pollen)	2005	Schöll et al. [69]
Dac g 5 (grass pollen)	2005	van Oort et al. [166]
Ara h 1 (peanut)	2006	van Bostel et al. [105]
Der p 1 (house dust mite)	2006	de Halleux et al. [167]
Can f 1 and 2 (dog dander)	2007	Kamata et al. [169]
Bos d 4 (bovine α -lactalbumin), Bos d 5 (β -lactoglobulin) (bovine milk)	2008	Roth-Walter et al. [56]
Bla g 2 (cockroach)	2008	Li et al. [170]
Der p 21 (house dust mite)	2008	Weghofer et al. [171]
Per a 3 (cockroach)	2008	Bellinghausen et al. [103]
Per a 4 (cockroach)	2009	Tan et al. [172]

^aCodes for allergen molecules are given according to the international allergen nomenclature (<http://www.allergen.org/Allergen.aspx>).

by the structure of the allergenic epitope rather than by variations of the immune response of the individual. In accordance, our own surface plasmon resonance affinity determinations with purified human polyclonal IgE rendered reactivity profiles like monoclonal antibodies, indicating that the repertoire of allergen-specific IgE antibodies within a single patient may be restricted to a handful high-affinity clones [101].

The IgE crosslinking process – already discussed above – is not only important in the effector phase of type I allergy but also for sensitization with allergens. A much better secondary antibody response is produced when allergens display their epitopes repetitively through multimerization [69] (Fig. 10.1A). Accordingly, dimerization was shown to have a positive effect on the immunogenicity of Dau c 1, a major allergen of carrot (*Daucus carota*) [102]. In these immunization studies of BALB/c mice, the highest IgG titers were reached in serum when the dimer of Dau c 1 was applied compared to monomers or mutated proteins. As professional antigen-presenting cells, like DCs, may be the first to handle allergens, they are in focus in several recent studies. For example, monomeric Per a 3 from American cockroach (*Periplaneta americana*) was demonstrated to be less immunogenic and allergenic than its naturally occurring hexamer structure [103]. When monocyte-derived autologous DCs of sensitized and non-sensitized individuals were pulsed with monomeric Per a 3, the release of mainly Th1 cytokines like IFN- γ was observed; in contrast, hexameric Per a 3 induced higher Th2-cytokine levels only in sensitized patients. Also the leukotriene release from leukocytes of allergic patients was higher with the Per a 3 hexamers. Similarly, without touching the question of multimericity, stimulation of allergic DCs from allergic donors with Bet v 1 and its homologues from food rendered Th2 cytokine production [104].

A number of allergens form even larger oligomers or multimers, like Ara h 1 from peanut (*Arachis hypogea*), which has been thought to be a trimer, but it was revealed that it might even form larger oligomers with a molecular weight of approximately 700 kDa, independent of the salt concentration and therefore of the ionic strength [105]. As mentioned above, the aggregation state of proteins is substantially affected by food processing and other manufacturing processes, as well as environmental pollution.

In addition to the formation of multimeric protein aggregates, the occurrence of repetitive epitopes on the native molecule might enhance the allergenic capacity (Fig. 10.2). An example is Hev b 5 from latex (*Hevea brasiliensis*) [106], where an immunodominant B-cell epitope is repeated three times within the protein sequence and, therefore, makes the allergen multivalent. Also, Bla g 1 from German cockroach (*Blattella germanica*) shows tandem repeats, forming two duplexes within the natural allergen [107], and tropomyosin, for example, from shrimps has repetitive motifs [108]. Molecular motifs as such seem to play an important part in the allergenicity of an antigen [109, 110]. A consensus sequence was recently created from different tropomyosin motifs (tropomyosins from house dust mite, cockroaches, shrimp and other). When it was expressed in *E. coli* and tested for IgE binding with sera of shellfish-allergic patients, binding of IgE to the consensus motif correlated well with binding to the full-length allergen, suggesting that the motif represents the allergenic part of the protein [109, 110].

Refining this approach, Kong et al. used an algorithm combining motifs of known allergens. Using motif length of 35 amino acids and motif number of 500, the algorithm can achieve both a high sensitivity and a high specificity for prediction of allergenicity [111]. In the light of novel proteins continuously introduced by the genetic engineering into our plants and diet, *in silico* approaches may provide useful means to predict allergenicity and protect sensitized patients.

In summary, the number and spacing of B-cell epitopes and the display of repetitive epitopes determine the induction and outcome of allergic reactions in the sensitized patient and, therefore, may be called AAMPs.

10.5 TAMPs: Tumor-Associated Molecular Patterns

From above it is clear that correct epitope spacing is a marker for foreignness and thus a key event in B-cell activation. Consequently, highly repetitive epitope display like by particulate antigens, but not monovalent-soluble proteins can break B-cell tolerance [112]. This basic principle represents a safety measure against autoimmunity by several means. Soluble antigens which may be secreted or shedded off by cells do rarely elicit a significant immunity. Several tumor types are shedding off their antigens to escape from eventual immune recognition. Among them, soluble forms for the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2 (HER-2) and carcinoembryonic antigen (CEA; CEACAM5) have been described and are already used or suggested for diagnostic purposes [113–115]. In experimental conditions, soluble antigens require the addition of adjuvants to form immunogenic aggregates and even then may induce insufficient antibody titers only. Further, some of the body's own molecules which are displaying repetitive motifs are safely locked within the cells and thus are not encountered by B lymphocytes in healthy conditions. Only when exposed through inflammation elicited through physical damage or infections, self-molecules with repetitive epitope display, including proteins (actin, myosin, collagen) or DNA, will become accessible TI antigens and lead to strong IgM autoantibody production, followed by T-cell activation and IgG synthesis. Moreover, inflammatory oxidative stress may modify self-antigens making them even more antigenic, and thereby supporting the breaking of tolerance [116], or supporting upregulation of stress proteins. One example is HSP70, which is overexpressed in colon cancer and promotes antitumor immunity [117].

Thus, repetitive display of self- and modified epitopes is possible in mammals, and B lymphocytes are indeed infiltrating tumor tissues [118]. Since Taylor and coworkers made the observation that the distribution of membrane immunoglobulins on a B lymphocyte is dynamic [119], one may speculate that low-affinity interactions between autoantigens on tumor cell membranes and the specific BCR may be too weak to allow receptor aggregation and crosslinking. As a result, similarly as with TI-2 antigens, only minimal immunoglobulin production may take place at least in the absence of exogenously added cytokines [120]. However, in settings of antigen overexpression by a tumor cell, the epitope density increases dramatically

and should favor productive B-lymphocyte activation. Indeed, natural antibodies of the IgM, IgG and IgE class toward tumor antigens have been detected, indicating that the epitope display reaches sufficient density to induce B-lymphocyte activation [121].

For instance, the four members of the EGFR family (EGFR/HER-1, HER-2, HER-3, HER-4) lead to signal transduction by various homo- and heterodimerizations and by large-scale clustering within lipid rafts, impacting internalization and signaling processes [122, 123]. There are only a few published attempts to precisely define the intermolecular distances between these tumor antigens, which decrease with the absolute numbers of molecules overexpressed per cell. Moreover, the distances between various EGFR family members depend on the molar ratio to each other and to lipid-raft-specific mono-sialo-ganglioside GM1 [124]. In an early approach, the number of HER-2 molecules was calculated as 10^4 – 10^6 molecules per cell, depending on the type of tumor [125]. Due to low degree of expression, exact determinations sometimes fail [126]. In addition, the more members of the EGFR family are overexpressed, the more heterodimers could be observed by fluorescence resonance energy transfer (FRET) technology [127], making the composition of antigens on the cell membrane more complex. The authors state that the ratios of EGFR : HER-2 produced "...subtle changes in the average (molecular) distance. ..." More precisely, the intermolecular distances were recently calculated for EGFR homodimers, which on A431 cells membranes reside in 8 or 5.5 nm distance, as recently revealed by single-molecule imaging combined with fluorescence lifetime imaging [128]. This distance exactly fulfills the minimal requirements for B-cell activation (see above).

A mechanistic problem, however, may be the relatively loose, low-affinity interaction between homo- or heterodimer partners within the lipid rafts resulting in easy dissociation. Nagy et al. have also shown previously that crosslinking of raft lipid GM1 by Cholera toxin subunit B resulted in decrease of HER-2 cluster density as well as of HER-2/HER-3 heteroassociations [124]. Which conditions could, therefore, stabilize the tumor antigen display within the lipid rafts and render higher immunogenicity? As one interesting example, it has been shown that cholesterol, an important constituent of mammalian cell membranes, supports clustering and heterodimerization of EGFR and HER-2 within lipid rafts [129]. Cholesterol contents in tumor versus normal tissues are enhanced [130], and furthermore, the cholesterol content of tumor cell membranes was associated with increased metastatic potential [131]. In contrast, cholesterol depletion impairs microviscosity and thus receptor activation and downstream signaling [132], except EGF receptor [133]. When carcinoma cells were treated with cholesterol or cholesterol esters, their immunogenicity upon transplantation in animal models increased [134, 131]. Although the immunological mechanism was not examined in these pioneer studies, the improved display of tumor-associated antigens in cholesterol-enriched lipid rafts could contribute to the enhanced specific immunogenicity. Recently, IgE antibodies (but no other antibody classes) against a 50-kDa pancreatic cancer antigen were detected in sera of patients suffering from pancreatic cancer. The isolated IgE indeed elicited tumoricidal effects via ADCC [22] (Fig. 10.2). In accordance, it has been shown

that statins through inhibition of cholesterol synthesis impair the attack of CD20-overexpressing lymphoma cells by monoclonal antibody rituximab [135]. Even though the authors suggest a conformational change of CD20 to occur upon cholesterol depletion, the reduced recruitment of CD20 into decholesterinated lipid rafts could contribute to the observed effect [136, 137].

Also the glycosylation pattern on tumor cell membrane changes during the disease development, affecting adhesivity and metastatic potential of malignant cells. In principal, the resulting high-molecular glycoproteins could be targets of IgE antibodies. For instance, MUC4 exposes a rigid tandem repeat structure possibly mimicking EGFR-like domains, which could support interaction with HER-2 [138, 139]. Whether natural IgE against heavily glycosylated tumor antigens can be formed in humans and be implicated in the surveillance of cancer has to the best of our knowledge not been investigated to this date.

10.6 Conclusion

Today, mostly parasite antigens and allergens are known as targets of IgE antibodies. However, accumulating evidence indicates that also tumor antigens are recognized by IgE antibodies. Especially against cancer cells, IgE could be beneficial for the following reasons: (i) IgE binds efficiently to Fc ϵ RI-bearing tumoricidal effector cells and may exploit them as shuttles into tumor tissues, (ii) IgE recognizes preferentially 3D epitopes and can, therefore, target viable tumor cells and (iii) IgE may exert surveillance functions against tumor stem cells, as due to high-affinity recognition of the antigens it may trace and kill low numbers of malignant cells. An important molecular requirement for IgE synthesis by B lymphocytes is the recognition of epitope patterns. Molecular patterns can be achieved when allergens display the epitopes repetitively such as in aggregated state or when self-antigens are highly overexpressed like on tumor cell membranes. Repetitive epitope display could also largely facilitate the crosslinking of IgE bound by its Fc ϵ Rs on effector cells and thus support the triggering of immediate inflammation. From the collected evidence we suggest that molecular patterns displayed by allergens or tumor antigens, which we here designated as AAMPs or TAMPs, respectively, are decisive for the synthesis and triggering of IgE antibodies.

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