Membrane Bound IgE: The Key Receptor to Restrict High IgE Levels

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Abstract: The membrane form of immunoglobulins (mIg) is expressed on the surface of B lymphocytes from a very early developmental stage in the bone marrow (pre B cell) until the cell finally differentiates into a plasma cell. mIgs associate with other transmembrane proteins to form the B cell antigen receptor complex (BCR). Numerous studies have confirmed that signaling through the BCR not only steers the B cell through development, but also ensures its survival in the periphery as a fully matured cell. During development in the bone marrow, mlg is restricted to the IgM isotype, but as soon as the B cell leaves the bone marrow to populate peripheral lymphoid organs like spleen, lymph nodes, or intestinal mucosae tissue, it starts to express IgD and in later stages of maturation IgG, IgE and IgA. Engagement of the B cell receptor triggers signals that control affinity maturation, memory induction, differentiation and other physiological processes in B cells. We showed that truncation of the cytoplasmic tail of mlgE in vivo results in lower serum IgE levels, decreased numbers of IgE-secreting plasma cells, and abrogation of specific secondary responses correlating with a defect in the selection of high-affinity antibodies during the germinal centre reaction. We conclude that the BCR is necessary at all times during antibody responses not only for the maturation process, but also for the expansion of antigen specific B cells. As a result of these findings it is obvious that the IgE antigen receptor could be a useful target molecule for therapeutic intervention.

INTRODUCTION

Compared with the other immunoglobulin classes, which are present in serum in concentrations of up to milligrams per millilitre, the titre of IgE in the nano- to microgram/ml range is very low in the plasma of normal healthy individuals and laboratory mouse strains. Beside in plasma, IgE is found in epithelia and mucosae where it is bound to specific receptors on potent effector cells like eosinophilic granulocytes and mast cells. Cell bound IgE has a long half-life time (weeks to months), compared to the short half-life of free plasma IgE (~6 hrs.), suggesting that IgE plays a role in local immune reactions. Although the biological function of IgE is still a matter of speculations, to date IgE is best known for its strong, unwanted effector functions manifesting in allergic symptoms [1]. These can range from harmless local symptoms like hay fever, to life-threatening systemic reactions culminating in anaphylactic shocks, underlining the potential hazard of high systemic IgE titres. In the recent past several B-cell specific control mechanisms indicating a tight control of the IgE response have been described. Beside the reduced IL-4 dependent class switch recombination to the IgE locus [2], the short half life time of free IgE in serum [3], and the negative feed-back function of CD23, the "low" affinity receptor for IgE [4], the key regulator which finally decides about the quantity and quality of the IgE antibodies produced during an immune response is the IgE antigen receptor itself [5-7]. These observations indicate possible mechanisms to restrain potentially dangerous but apparently necessary IgE responses occurring at many different levels during the B cell differentiation process. Here we want to address the biological function of the B cell receptor in general, of the mlgE antigen receptor in particular, and the therewith connected regulatory function on the IgE production.

THE B CELL ANTIGEN RECEPTOR AND ITS FUNCTION DURING B CELL DEVELOPMENT

Diversification of the antibody repertoire starts with the largely random recombination of V, D and J segments on the heavy chain locus and V and J segments on the light chain locus. These recombination events result in the random generation of functional Ig genes and their subsequent transcription and translation in the pre-B cell stage of development. Finally, the mature, fully recombined mlg protein consists of at least 2 light chains and 2 heavy chains followed by the mlg transmembrane segments, which are about 25 amino acids long [8], and by the cytoplasmic domains which differ in length. They range from only three amino acid residues (KVK) in the case of mlgM and mlgD to 14 -28 residues for the other mlg subclasses. The cytoplasmic tail of human and mouse IgE is less conserved when compared to the other mlg subclasses. The cytoplasmic domain of IgE which differ in length. They range from only three amino acid residues (KVK) in the case of mlgM and mlgD to 14 -28 residues for the other mlg subclasses. The cytoplasmic domain of IgE which differ in length. They range from only three amino acid residues (KVK) in the case of mlgM and mlgD to 14 -28 residues for the other mlg subclasses. The cytoplasmic tail of human and mouse IgE is less conserved when compared to the other Ig classes (Fig. 1). In its membrane-bound form mlgE is obligatorily associated with two other membrane proteins, CD79-a and CD79-b (Ig-α and Ig-β, respectively [9]). These proteins not only function as a chaperone to facilitate membrane expression but they also couple the antigen receptor to membrane-proximal signaling elements. Numerous studies have confirmed that signaling through the BCR not only steers the B cell through development, but also secures its survival in the periphery as a fully matured cell (reviewed in [10]).

Summarizing, BCR signaling controls: (a) the expansion of only those B cells that have undergone functional V(D)J rearrangements during early development; (b) the deletion of
self-reactive B-cell clones; (c) the survival of B cells by representing a maintenance signal in the periphery; (d) upon antigen encounter a general activation of the B cell, or, in the absence of appropriate co-stimulation, a specific deactivation, either resulting in apoptosis or in a state called "anergy"; (e) induction of the differentiation into a memory cell; and (f) the terminal differentiation into an antibody-secreting plasma cell, closely linked with the down regulation of the membrane expression of Ig [10].

The exact mechanism of the initiation of BCR signaling is still a matter of speculations and several models have been proposed. According to the most accepted model, a signal is initiated upon oligomerisation of two isolated BCR com-

**Fig. (1).** (a) Each Ig molecule can be expressed in two isoforms as result of an alternative splicing event. The constant exons for the secreted Ig molecule are followed by a poly(A) site for secreted antibody, followed again by exons M1 and M2 coding for transmembrane and cytoplasmic domains of immunoglobuline different isotypes. The mlg transmembrane segments are about 25 amino acids long and have the potential for interaction with other polypeptides. The cytoplasmic domains of mIgs are different in length and range from only three amino acid residues (KVK) in the case of mIgM and mIgD to 14-28 residues for the other mIg subclasses. One striking difference between mouse and man is the existence of two mIgE isoforms in the humans with respect to the extra membrane proximal domain. The EMPD region is of critical importance for mobilizing intracellular Ca\(^{2+}\), with the EMPD’s length apparently being the ‘sensor’ of caspase independent apoptosis sensitivity.
plexes through engagement by antigen (crosslinking of the receptors). Alternative models explain the initiation of signaling by a conformational change within the receptor upon antigen encounter, or by the selective shuttling of engaged receptors into lipid rafts which are specific domains of the plasma membrane, optimised for the initiation of signals (reviewed in [11, 12]).

Upon binding to antigen, the receptor is linked to the cytoskeleton and rapidly internalised. However, internalisation is not necessary for signal transduction and B cell activation. Rather, the route of internalisation is crucial for antigen processing and subsequent presentation to T cells to initiate an immune response [13].

Despite different opinions [11, 12], it is largely accepted that the different Ig isotypes initiate signaling in a similar way. Differences in the amino acid composition of the transmembrane domains and cytoplasmic tails of the different isotypes may, however, account for the recruitment of distinct signaling components in addition to Ig-α and Ig-β[11].

During development in the bone marrow, mlg is restricted to the IgM isotype, but as soon as the B cell leaves the bone marrow to populate peripheral lymphoid organs like spleen, lymph nodes, or intestinal mucosal tissue, it starts to express a second isotype on the surface, IgD. From this time point onwards these cells are called mature naïve B cells. On antigen contact, linked with T cell help through engagement of the T cell receptor, mature B cells are able to diversify the constant Ig regions by class switch recombination (CSR). In CSR the isotype of the Ig is changed without affecting the antigenic specificity by a deletion mechanism that combines a specific region S' of the IgM constant region gene (switch region) with a homologous switch region S' of another constant region gene, thereby excising the interceding DNA. The recombination events generate new B cells producing Ig isotypes belonging to the γ, ε or α class depending on the switched region.

**THE MECHANISM OF CLASS SWITCH RECOMBINATION**

After the induction of switch recombination a gene segment between two switch regions is removed, causing a double strand break in the S regions, followed by repair (Fig. 2). These results in a new, hybrid switch region which is made up of the 5' and the 3' end of the S regions involved and which can be used again in a further switch recombination process. Switch recombination is dependent on transcription starting from a promoter site upstream of the S region. The transcript includes a non-functional exon (I exon), located 5’ of the S region. Successful switch recombination apparently depends on processing (i.e. splicing) of this transcript. Switch recombination is normally dependent on the close interaction with helper T-cells (Th cells) [14, 15]. Upon priming, Th cells secrete cytokines like interleukin (IL)-4, IL-13, interferon (INF)-γ or Transforming Growth Factor (TGF)-β [16]. The cytokines initiate or suppress transcription from the promoters upstream of I exons therewith determining the isotype that will be involved in switch recombination. Priming of T-cells is not dependent on antigen presentation by B cells, switch recombination, however, is dependent on class II MHC expression by B cells [17]. Primed T cells express the CD40 ligand which binds and crosslinks the CD40 antigen on B cells [18]. Primed T cells express the CD40 ligand which binds and crosslinks the CD40 antigen on B cells [18]. This interaction, and others like the CD28-CD80/CD86 interaction [19], take place in the germinal centre [20-22] and are essential for the initiation of the switch recombination. Switch to IgE is induced by IL-4 and IL-13 [23], and inhibited by IFN-γ [24]. Interaction of IL-4 with its receptor causes the activation of STAT6 and its translocation to the nucleus, where it binds to an IL-4-responsive element in the promoter region S' of the ε gene [25].

Switch to IgE in vitro is also induced by lipopolysaccharides (LPS) and IL-4 [14]. Recently, the receptor for LPS on B cells has been identified. It is the Toll-like receptor-4 (Tlr4), which belongs to the IL-1-receptor/Toll-like receptor family [26]. CD40 and the Toll receptor family share a sig-

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**Fig. (2).** The VDJ re-arranged segments are followed by eight (mouse) or nine (human) constant regions of different isotypes. Each isotype (except IgD) consists of a switch region, regulated by an upstream promoter/I exon region. So far identified transcription factor binding sites are indicated for the promoter region of the ε region.
nal transduction pathway: they both induce the translocation to the nucleus of NFkB [27]. Among other activities NFkB can cooperate with STAT6 in the induction of germ line epsilon transcripts [28].

In conclusion, switch recombination depends on direct T-B cell interaction, the type of cytokine produced, transcriptional activation, RNA processing, DNA excision, and efficient DNA repair. The effects of some of these factors on the switch to IgE are well documented like the effect of cytokines; others are poorly or not studied at all (RNA processing, DNA excision, and efficient repair). Yet, they are of essential importance in the number of cells that will be recruited in an IgE-mediated immune response.

THE BIOLOGY OF THE IgE ANTIGEN RECEPTOR AND ITS IMPACT ON IgE SYNTHESIS

From an evolutionary point of view, IgE is conserved and can be found in all mammals, including monotremata [29]. It therefore originated at least 160 million years ago, possibly even more than 300 million years ago [30], from a gene duplication of IgY, in which the anaphylactic and opsonic activities of IgY were separated, giving rise to IgE and IgG, respectively [31]. Apparently, in an evolutionary sense, anaphylactic defence mechanisms are needed, but can be of potentially high risk for the organism. The segregation of anaphylactic and opsonic activities in separate genes allowed principally a tighter and more specific control of both immune responses.

Membrane IgE (mIgE), in contrast to its soluble form, contains three additional structural features encoded by exon M1 and M2, namely the EMPD (extracellular membrane-proximal domain) domain, the transmembrane domain anchoring the receptor and being involved in the assembly with the CD79α/β sheath [32, 33] and the cytoplasmic domain. The EMPD regions of the five isotypes differ in length and amino acid composition, e.g. the two functional forms of human εEMPD, namely εEMPDshort and εEMPDlong are composed of 14 and 66 amino acids [34-36], respectively. According to a study of Poggianella et al., carried out in the mature murine B-cell line A20, the presence of the EMPD region is of critical importance for mobilizing intracellular Ca2+, with the EMPD’s length apparently being the ‘sensor’ of caspase independent apoptosis sensitivity. A similar phenomenon of proliferation inhibition has been reported in murine WEHI-231 cells transfected with the shorter human version of mlgE [37]. This study also showed that the rate of transport by which the two forms are brought to the cell surface as well as the association with CD79-a, and the kinetics of protein tyrosine phosphorylation in response to receptor cross-linking, differ between the short and the long version. Thus, the lenght of the EMPD region might have an essential function in shaping the repertoire of mlgE plasmablasts selected towards the long lived plasma cell fate.

The fourth constant exon of IgE, which is located 5-prime of the transmembrane and the cytoplasmic exons, is a composite exon: it contains an internal splice donor site, which is used when mRNA for membrane bound Ig is generated. It is also followed by an “internal” polyadenylation-addition site that is used when mRNA for secreted Ig is made. A 3’ “external” polyadenylation-addition site is found downstream of the membrane exon. Except for the “internal” and the “external” polyadenylation signals of IgE, the consensus sequence AATAAA is used. However, the ε membrane locus uses three cryptic “external” polyadenylation signals. These signals (AGTAAA, AAGAAA and AT-TAAA) are in considerable disagreement with the consensus sequence [38]. The ratio of transcripts for the secreted and the membrane form of immunoglobulin reflects the usage of either polyadenylation signal. The choice depends normally on the developmental stage of the B lymphocyte. Therefore, the production of the two types of mRNA are determined by alternative splicing or rather, alternative polyadenylation [39]. The ratio between the amount of secreted versus membrane-bound Ig that is produced by a single cell is therefore determined by the efficiency with which the internal or external polyadenylation sites are used and by the stability of the resulting mRNA’s.

Our data [38] unambiguously show that a fundamental difference between IgE and other immunoglobulins lies in the pattern of expression of the mRNA for the secreted and membrane form. Responsible for this difference is poor expression of the mRNA coding for the membrane form of IgE. Deletion of the “internal” polyadenylation signal results in a shift towards the expression of the mRNA for the membrane form of IgE, which indicates that the “internal” polyadenylation signal is preferentially used. It also shows that the mRNA for the membrane form is not particularly liable to degradation. The exchange of the 3’UTR of the ε HC gene with that of the μ HC gene also induces a shift towards the expression of mRNA coding for the membrane form, indicating poor processing of the mRNA for the membrane form derived from the altered polyadenylation signals in the 3’UTR of the ε HC gene.

We conclude that low mlgE expression in human and in mouse is caused by a poor processing of the pre-mRNA of IgE due to deviant polyadenylation signal sequences. This distinct mechanism appears to set a high transcriptional threshold for IgE expression. Because expression of the membrane form of IgE is necessary for the survival or recruitment of IgE-secreting cells, low expression of the mRNA for mlgE most likely limits the number of IgE-secreting cells and thereby limits the magnitude of an IgE-mediated immune response.

ANIMAL MODELS

A step forward in understanding the role of mIgs was achieved with two mouse lines with mutations in the ε heavy chain gene. In the first mouse line, the intracellular domain of IgE was removed except for three amino acids (Lys, Val, Lys) (KVKtail line). The cytoplasmic domain of IgE in these mice is the same as that of mlgM and mlgD. In the second mouse line both the intracellular and transmembrane domains of IgE (ΔM1M2 line) are lacking [6, 40] (Fig. 3). In serum of ΔM1M2 mice IgE is reduced to less than 10% of normal mice, while serum of KVKtail mice show a reduction of 50%, reflecting a serious impairment of the IgE-mediated immune response. Class switch to IgE was not impaired by the targeting event. Upon stimulation of isolated spleen cells of wild type, ΔM1M2 and KVKtail mice with LPS and IL-4 in vitro [14, 41, 42], concentrations of IgE and IgG1 in the culture supernatants were comparable in wild type and mutant mice. These results imply that the reduced
IgE titres found in both mutant lines are solely a reflection of the loss of biological activities associated with the transmembrane and cytoplasmic domains of IgE.

If the membrane domains are a prerequisite to recruit cells for the production of secreted IgE, how can we explain that residual serum IgE is present in M1M2 mice? Interestingly, the isotype switch to IgE induced by IL-4 and the polyclonal B-cell activator LPS is not impaired in M1M2 mice. The LPS-receptor Tlr4 belongs to the Toll-like receptor family, a conserved family of proteins that is involved in innate immunity. It uses the same signal transduction pathway as CD40 to achieve a change in nuclear metabolism: NFκB is activated and translocated to the nucleus. It is conceivable that any activation which involves NFκB and STAT6 can induce switch recombination to IgE, even independent of the anatomical site. The residual IgE in M1M2 mice could therefore stem from a polyclonal activation involving NFκB in the presence of sufficient amounts of IL-4. A real-life example of such a scenario could be the encounter with the helminth Nippostrongylus brasiliensis. N. brasiliensis induces a robust IgG1 and IgE production, both through a dominant activation of Th2 cells, and a strong, T-cell independent activation of B cells [43, 44]. We measured the serum levels of IgE after infestation with N. brasiliensis: wild type mice showed a 50 fold increase in serum levels, as did the mutant mice, however, these mice started with a much lower serum IgE titer. Following secondary infestation, 11 weeks after the first challenge with N. brasiliensis, a strong and fast IgE-response was seen in the wild type mice, indicative of a memory response. The IgE response in the KVKΔtail was also substantial, but reached only 55% of the wild type response, and in ΔM1M2 mice IgE reached measurable levels. Interestingly, when the IgE titres of control and mutant mouse lines are compared to the titres in infested animals, a rather constant ratio is observed (20: 10: 1, comparing wild type to KVKΔtail and ΔM1M2, respectively). The results indicate that the IgE response to N. brasiliensis also needs a specific interaction with the IgE antigen receptor complex on the B cell, accompanied by strong Th2 helper activity. The measurable level of IgE secretion in the ΔM1M2 mice after the secondary challenge with N. brasiliensis can therefore be explained by an even stronger Th2 memory response together with a direct stimulation of B cells, leading to the activation of NFκB, switch recombination and IgE secretion, similar to the response to LPS + IL-4 obtained in vitro. An alternative explanation would be the relatively rare occurrence of an indirect isotype switch from IgM to IgE via IgG1. The last pathway would rely on the control function of mIgG1, and therefore be mIgE independent.

Our data clearly show that the transmembrane domain of IgE is indispensable for a T-cell-dependent IgE-mediated immune response, and that the cytoplasmic tail not only determines the absolute amount of IgE produced, but also the quality of the antibodies.

Two hypotheses can be brought forward. The first implies that signals generated via membrane-bound Ig are needed at all times, not only for the maturation process, but also for the expansion of antigen-specific cells. The second hypothesis postulates that antigen presentation to T-helper cells is necessary at all times during an antibody response and that the antigen receptor is the only device for an effective antigen presentation. The hypotheses are not necessarily mutually exclusive. All Ig classes can associate with the Ig-α/Ig-β heterodimer [45], the signal-transducing unit of the B-cell receptor. Recent experiments have shown that an intact antigen receptor on B cells is required for their survival.
could speculate that anti-apoptotic signaling might enhance the maintenance of memory B cells expressing class-switched BCRs. As shown by self-reacting immature B cells, cross linking of the IgE receptor without further T cell support should directly induce apoptosis. Second, as shown during the induction of peripheral tolerance of mature B cells in answer to monovalent (self) antigen, receptor blockade of IgE by, for example, a Fab fragment should result in an anergic state of the IgE population. In contrast to normal mature B cells, which have a half life of 4 to 5 weeks, anergic B cells were found to last for only 3 to 4 days. Nevertheless the fate of these anergic B cells finally turns out to be cell death.

This simplified scenario is restricted to a very special lymphocyte elimination process. However, T and B lymphocytes undergo apoptosis in many instances in their development, homeostasis, and activation. Lymphocytes die during development if they fail to locate or compete for cytokines, fail to properly rearrange an antigen receptor, fail to be positively selected, or are negatively selected. In the periphery, resting lymphocytes are eliminated from, or unable to enter the long-lived recirculating pool if they fail to locate or compete extrinsic signals. For example B cells that bind and present antigenic peptides on MHC II to T cells can also undergo receptor induced apoptosis. Germinal centre B cells that lose affinity for selecting antigen or gain specificities for self antigens are also eliminated. Furthermore, lymphocytes that fail to become memory cells after antigen clearance are also eliminated (reviewed in [56]).

Our knowledge about the regulation of the expression of membrane-bound IgE is at best limited. Unfortunately, a similar statement can be made regarding the function of the transmembrane and the cytoplasmic domain of IgE. We,
however, know that the production of IgE is tightly regulated on the level of DNA recombination (switch), transcription, and RNA processing. Transgenic mouse experiments [6] clearly showed that the transmembrane domain of mIgE is indispensable for T-cell dependent IgE secretion and that the cytoplasmic domain not only determines the absolute amount of IgE produced, but also influences the quality of the immunoglobulins. Thus, if mIgE seems to be the prerequisite for the later production of secreted IgE, targeting mIgE bearing B cells with anti-mIgE specific antibodies could be a therapeutic option. A possible target domain is represented by the extra membrane proximal domain (EMPD), coded as part of the transmembrane domain M1 (Fig. 4). The EMPD could be used as target sequence for generating anti mIgE antibodies with the capacity to inhibit IgE synthesis. First results with this approach were published by Chen et al., which examined the ability of an anti-spacer specific antibody on targeting and lysing mIgE expressing B cells [57]. Thus, the pharmacological targets of this approach are memory B cells involved in secondary immune responses.

Summarizing, if anti-mIgE antibodies indeed inhibit or down-regulate IgE synthesis in vivo, these antibodies may be used to treat allergic patients with very high IgE levels. The advantage of this second generation anti-IgE therapy would be the ability of inhibiting IgE secretion before secreted IgE production starts.

CONCLUSIONS

The BCR plays a central role in almost all processes along the developmental pathway of B cells, i.e. the generation, maturation, survival, and activation of B cells. Obviously, between Ig subclasses, there should be no differences in the transduction of signals regulating the central features of the immune system such as the control of allelic exclusion, early cellular transitions and in the function of the membrane-bound immunoglobulin as a receptor for antigen capture and presentation. However, recent experiments indicate that, apart from the signal transduction pathways via Ig-α/Ig-β cytoplasmic tails, the mIgE itself have the capacity to engage in the signal transduction pathway, influencing the quantity and quality of the immune response.

The synthesis of sIgE by plasma cells is comparable to that of other Ig classes. However, alternative polyadenylation of the mIgE transcript [38], together with a negative feed-back regulatory mechanism [4], the low half-life of sIgE [3], and the low frequency of switch recombination [2, 5] leads to a more than 1000-fold lower IgE than IgG1 serum level in the mouse. This is in agreement with the notion that IgE is needed for immune defence, but only localised and at very low level. It is, however, not known whether the functional recruitment of B cells after a switch to IgE is a stochastic process, selecting only those cells that managed a sufficient expression of mIgE, or whether other factors, only expressed in a fraction of ε-switched B cells, facilitate mIgE expression and recruitment.

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