Organisation of B-cell receptors on the cell membrane

D. Iber and T. Gruhn

Abstract: B-cell receptors (BCRs) have been reported to organise into oligomeric clusters on the B-cell surface, and mutations, that are likely to interfere with such clustering, result in B-cell unresponsiveness. This has led to the suggestion that pre-formed BCR clusters may be crucial for B-cell signalling. However, neither the size nor the fraction of BCRs organised in such clusters have yet been determined in experiments. Hence, the authors use a statistical approach to predict the membrane organisation of BCRs, based on available experimental data. For physiological parameters, most BCRs will organise into supramolecular polymers that comprise about five receptors where the non-covalent interactions are mediated by the IgH transmembrane helix. A reduction in the density of IgM to 2–5% of the normal density, a characteristic of anergic MD4 B cells, strongly reduces IgM polymerisation, and it is suggested that impaired BCR clustering may be responsible for the unresponsiveness of anergic B cells.

1 Introduction

B-cell receptors (BCRs) are unique in that they can recognise a large number of different ligands (antigens) and can signal according to the affinity of binding over a wide range of affinities [1, 2]. This is even more impressive when considering that the cytoplasmic tail of the receptor comprises only three amino acids. Signalling is initiated at the co-receptors Igα/Igβ that bind to the transmembrane domain of the IgM. Although some evidence suggests that the initiation of BCR signalling involves receptor clustering [3, 4], such that kinases binding to the resting BCR can cross-phosphorylate each other [5], the mechanism of receptor clustering as well as the structure and dynamics of such clusters have remained elusive.

More recent studies reveal that membrane IgM organises into small oligomeric clusters, even before antigen binding [6]. The necessary BCR–BCR interactions appear to be mediated by one side of the IgH transmembrane–domain helix that contains isotype-specific, conserved amino acids [6, 7]. In analogy with structures formed by secreted IgM, it has been proposed that the observed IgM clusters form a ring-like structure that comprises 5–6 BCRs [6–8]. However, secreted IgMs are covalently linked by a polypeptide, the J chain [9], and it is therefore unclear whether these results apply to membrane IgM. It also remains unclear what fraction of the BCR would organise into such clusters and what impact BCR density would have on such organisation. An insight into these questions is required to understand why these pre-formed clusters do not result in B-cell signalling before the antigen binds to the receptors and how an affinity-dependent response is enabled.

We use a statistical approach to estimate the organisation of BCR on the B-cell surface and, we find that for physiological parameters, the majority of BCRs are organised in supramolecular polymers, most of which comprise five BCRs under physiological conditions. We further find that a 20–50-fold reduction in IgM density strongly reduces IgM polymerisation. Such reduction in IgM density has been observed for anergic MD4 B cells that are specific for hen egg lysozyme [10, 11]. Anergic B cells are characterised by a lower responsiveness towards an immunogenic antigen challenge and arise when B cells are exposed to a relatively weak, co-stimulatory-deficient antigenic stimulus for a sufficiently long time [10–12]. The basis of their unresponsiveness has so far not been understood because proteins important for BCR-induced signal transduction, such as Syk, Lyn, CD45 and SHP-1, are expressed at a normal level and with normal basal activity [13]. We propose that impaired BCR clustering may be responsible for the unresponsiveness and we discuss the potential of BCR clustering in regulating B-cell signalling.

2 Model and results

2.1 IgM cluster size distribution

We model the experimentally observed IgM clusters as supramolecular polymers where the non-covalent interactions are mediated by the IgH transmembrane helix [7]. While only one side of the helix appears to mediate direct IgM–IgM interactions, the other side has been found to be responsible for interactions with Igα/Igβ, which may be shared between two IgMs [7]. It is thus assumed that each IgM is able to interact non-covalently with two other IgMs, on one side directly and on the other side in the form IgM–Igα/Igβ–Igα/Igβ–IgM. In order to facilitate the analysis, we will initially ignore the details of the IgM–IgM interaction and, instead, consider IgM monomers that can each interact with two other IgM monomers directly, with equal binding probability p. If, in a first step, we also ignore any ring formation, then, according to Flory [14] and van der Gucht [15], we can estimate the cluster length distribution as

$$c(n) = \varrho(1-p)^2 p^{n-1} \quad (1)$$

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where $c(n)$ is the density (number/area) of clusters of length n, ϱ is the total IgM density (IgM/area) and p is the probability of forming a bond between two monomers. The number density, $\langle n\rangle$, of clusters follows as

$$\langle n\rangle = \frac{\sum_{n=1}^{\infty} nc(n)}{\sum_{n=1}^{\infty} c(n)} = \frac{\varrho(1-p)^2\sum_{n=1}^{\infty} np^{n-1}}{\varrho(1-p)^2\sum_{n=1}^{\infty} p^{n-1}} = \frac{1/(1-p)^2}{1/(1-p)} = \frac{1}{1-p} \quad (2)$$

and $nc(n)$ attains a maximum at $n = -1/\ln(p)$, which is the polymer length into which the majority of monomers organises. A plot of the $nc(n)$ curve for two different values of p is shown in Fig. 1.

The binding probability p can be related to the binding constant K [15] by observing that the total density of all bonds is given as $[B] = p\varrho$, and the density of all free sites on the monomers is $[F] = 2(1-p)\varrho$, such that

$$K = \frac{k_+}{k_-} = \frac{[B]}{[F]^2} = \frac{p\varrho}{(2(1-p)\varrho)^2} = \frac{p}{4(1-p)^2\varrho} \quad (3)$$

Three-dimensional protein–protein on-rates are in the range 10^6–10^7 M^{-1}. The two-dimensional counterpart can therefore be expected to be of the order $k_{on} \in [0.1-1]$ μm^2 (using $K_d^{2d} = \eta K_d^{3d}$ and $\eta = 1.2 \times 10^{19}$ m^{-2} M^{-1} [16, 17]). Due to diffusion limitations, we, in general, expect $k_+ \neq k_{on}$. According to Keizer [18], the effect of diffusion limitations on the two-dimensional on-rate can be estimated as

$$k_+ = \frac{4\pi D k_{on}}{k_{on}K_0(\sqrt{(k_+\varrho/D)}d) + 4\pi D} \quad (4)$$

where $K_0(x)$ is the zero-order modified Bessel function of the second kind. $D \sim 0.1$ μm^2 s^{-1} represents the T cell receptor (TCR)-based estimate for the diffusion constant [19] and $d \sim 0.02$ μm the lateral distance at which receptors can interact. The implicit equation (4) can be solved numerically (Fig. 2); this shows that for $k_{on} \in [0.1-1]$ μm^2 s^{-1} and $\varrho \in [50, 500]$ μm^{-2}, $k_+ \sim 0.1$ μm^2 s^{-1}. In order to determine $n\rangle$, we need to estimate the physiological values of the employed parameters. The approximate IgM density on B-cell surfaces is 500 μm^{-2}. Although the affinity for BCR–BCR interactions has not yet been determined, it is likely to be rather low (as observed for self-interactions between other large membrane proteins [20]), such that the off-rate k_- will be high ($k_- \sim 1$ s^{-1}) and $K \sim 0.1$ μm^2.

For sufficiently large ϱ, it follows from (3) that $p = 1 - (4K\varrho)^{-0.5} = 1 - (4 \times 0.1 \times 500)^{-0.5} = 0.93$ [15].

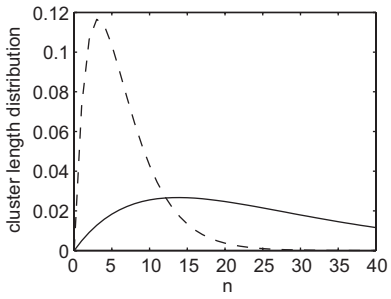

Fig. 1 *Cluster length distribution*

Cluster length distribution weighted by the length n and normalised with the IgM density ϱ, $(nc(n))/\varrho$, dependent on the cluster length n for $p = 0.93$ (continuous line) or $p = 0.73$ (broken line)

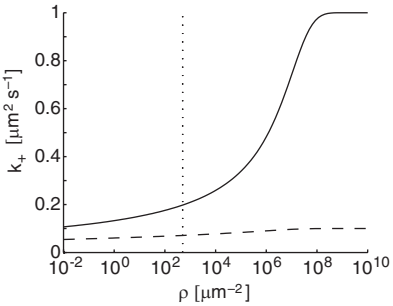

Fig. 2 *Estimate of diffusion effects on the BCR–BCR binding rate*

Receptor density (ϱ) dependent on-rate (k_+), if the on-rate without limitations is either $k_{on} = 1$ (solid line) or $k_{on} = 0.1$ (dashed line). The dotted line marks the estimated BCR density on the B-cell surface

Accordingly, the number density of IgM clusters on the B-cell surface in the absence of any ring formation would be $n\rangle = 1/(1-p) \simeq 2\sqrt{(K\varrho)} = 14.3$ and the distribution peaks at $n = 1/\ln(p) = 13.8$ (Fig. 1). At lower IgM density, as may be characteristic for anergic B cells [10, 11], p is much smaller and has to be determined from $p = 1+(1/8\rho K) - \sqrt{((1+(1/8\rho K)^2 - 1))}$. If IgM is reduced to 5% of its normal density, then for $K = 0.1$ μm^2, $p = 1 + (1/20) - \sqrt{((1+(1/20))^2 - 1)} = 0.73$, such that $n\rangle = 3.7$ and the distribution peaks at $n = -(1/\ln(p)) = 3.2$ (Fig. 1). The chains therefore shorten at lower receptor density approximately with the square root of ϱ.

2.2 Impact of ring formation on IgM cluster organisation

So far, we have neglected any ring formation, but IgM clusters on the B-cell membrane have been suggested to form similar structures, as secreted IgM that would include the formation of closed rings [7]. We can extend this model to include ring formation and we obtain for the length distribution of closed chains

$$c_c(n) = \varrho(1-p)^2 p^{n-1} p_r \quad (5)$$

and for the length distribution of open chains

$$c_o(n) = \varrho(1-p)^2 p^{n-1}(1-p_r) \quad (6)$$

where p_r is the probability that a chain closes into a ring. For small n, p_r can be expected to be very low. Experimental data are, however, not available to judge whether (and if how) p_r depends on n also at larger polymer length. We therefore only consider the simplest case, that p_r does not depend on n. We note by comparison with (2) that, in this case, the formation of rings does not affect the number density $\langle n\rangle$ of clusters, which is given by

$$\langle n\rangle = \frac{\sum_{n=1}^{\infty} n(c_c(n) + c_o(n))}{\sum_{n=1}^{\infty} c_c(n) + c_o(n)} = \frac{1}{1-p} \quad (7)$$

The probability p_r with which a chain closes, therefore, only determines the fraction, F, of chains that are closed, as can be seen from

$$F = \frac{\sum_{n=1}^{\infty} c_c(n)}{\sum_{n=1}^{\infty} c_c(n) + c_o(n)} = \frac{\sum_{n=1}^{\infty} \varrho(1-p)^2 p^{n-1} p_r}{\sum_{n=1}^{\infty} \varrho(1-p)^2 p^{n-1}} = p_r \quad (8)$$

2.3 Impact of co-receptors on IgM clustering

We have so far neglected co-receptors that have been found to interact with the resting BCR. For example, CD22, an inhibitory receptor, has been noted to interact with membrane IgM, both at the receptor stem as well as at the 'arms' [21]. If CD22 binds to the arms, it is unlikely to affect BCR clustering. In the case that it interacts with the stem, it may either intercalate and become integrated into the polymer, or it may block interactions between IgMs and thus prohibit IgM clustering. If CD22 blocks receptor interactions, it may do so either on one site or on both. Given that ring formation does not affect the number density $\langle n \rangle$, we neglect ring formation in the following and modify (1) to

$$c(n) = \varrho(1 - p(1 - p_\mathrm{I})^i)^2 (p(1 - p_\mathrm{I})^i)^{n-1} \tag{9}$$

where p_I is the probability for an inhibitor to bind to an IgM site and i is the number of inhibitor interaction sites per IgM. The number density in the presence of co-receptors follows as

$$\langle n \rangle = \frac{\sum_{n=1}^{\infty} nc(n)}{\sum_{n=1}^{\infty} c(n)} = \frac{1}{1 - p(1 - p_\mathrm{I})^i} \tag{10}$$

We therefore see that for $p_\mathrm{I} \to 1$, the cluster size will be reduced substantially. Experiments find that 15% of all IgMs are bound by CD22 [21], and $(1 - p_\mathrm{I})^i = 0.85$ is, therefore, a reasonable estimate; other co-receptors that bind with a lower probability will make up for the fraction of CD22 that binds to the antibody arms. Using the same parameter values as used earlier, we then have $\langle n \rangle = 1/(1 - p(1 - p_\mathrm{I})^i) = 4.8$. We conclude that the model predicts IgM clusters of size similar to that found in experiments, if the IgM–IgM affinity is $K = 0.1\ \mu\mathrm{m}^2$.

If the receptor density is reduced, then the ratio of inhibitor to IgM increases and so will the fraction of IgM that is bound by the inhibitor. As a consequence, the cluster size will shrink even further.

2.4 Igα/Igβ co-clustering

Antigen-bound IgMs translate antigen binding into a cellular signalling via Igα/Igβ, and co-precipitation studies show that each IgM binds on average one Igα/Igβ complex [6]. It has been found in experiments that a large fraction of IgMs (50–80%) on anergic B cells do not firmly interact with Igα/Igβ [22]. We can use our modelling approach to judge whether this is a consequence of IgM downregulation. Monomeric interactions between IgM (m) and Igα/Igβ (a) can be modelled as

$$\dot{b} = l_+ ma - l_- b \tag{11}$$

where b denotes the density of IgM–Igα/Igβ complexes and l_+ and l_- are the rate constants for binding and unbinding reactions, respectively. As IgMs and Igα/Igβ are transported together to the cell surface [23], the total Igα/Igβ density is taken to be equal to the receptor density, ϱ. We can then rewrite (11) as

$$\frac{\dot{b}}{\varrho} = l_+ \left(1 - \frac{b}{\varrho}\right)^2 - l_- \frac{b}{\varrho} \tag{12}$$

In equilibrium ($\dot{b}/\varrho = 0$), we then have

$$\frac{b}{\varrho} = 1 + \frac{K_d}{2\varrho} - \sqrt{\left(1 + \frac{K_d}{2\varrho}\right)^2 - 1} \tag{13}$$

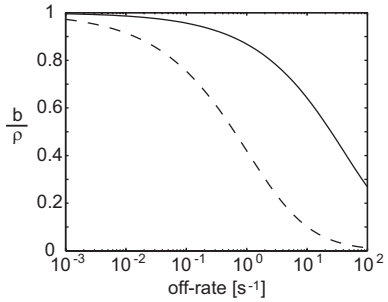

Fig. 3 *Fraction of IgM bound to Igα–Igβ*

Fraction of IgM bound to Igα–Igβ (b/ϱ) dependent on the off-rate l_- of the interaction if the IgM density is normal ($\varrho = 500\ \mu\mathrm{m}^{-2}$, continuous line) or reduced to 5% ($\varrho = 25\ \mu\mathrm{m}^{-2}$, broken line)

where we set $K_\mathrm{d} = l_-/l_+$. For the forward rate constant, the estimate by Keizer can be used again, such that $l_+ = k_+ = 0.1\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$. No experimental estimate exists for the off-rate, but plotting b/ϱ against the off-rate l_- reveals that, as long as the receptor density is high ($\varrho = 500\ \mu\mathrm{m}^{-2}$), for the physiological low-affinity range ($l_- \sim 1\ \mathrm{s}^{-1}$), $b/\varrho > 0.8$ (Fig. 3, continuous line), which means that >80% of all monomeric IgM is bound by Igα/Igβ. The physiological figure may even be higher if conformational constraints in the IgM polymer further reduce IgM–Igα/Igβ unbinding. On the other hand, for $\varrho = 25\ \mu\mathrm{m}^{-2}$, we find that, in agreement with experiments [22], <50% of all IgM are bound by Igα/Igβ, and, given that at lower IgM density there is substantially less IgM polymer formation, further constraints are unlikely to increase this figure.

A reduction in the IgM density therefore reduces both IgM–IgM and IgM–Igα/Igβ interactions. The latter not only reduces the number of signalling proteins in the vicinity of the receptor but also reduces the fraction of IgM that can interact with other IgMs on both sides. This will further reduce IgM clustering, below the value estimated in the previous section. Together, this may explain the experimentally observed lower responsiveness of anergic B cells.

3 Conclusion

Based on recent experiments, BCRs have been suggested to organise into small oligomeric clusters on the B-cell surface [6, 7]. Despite their likely importance for B-cell signalling, little is known about the structure, size and dynamics of these clusters. By employing mathematical modelling based on available values for parameter (ranges), we find that the majority of IgM will organise into clusters of about five BCRs if the binding of co-receptors is also taken into account. This is similar to a suggestion that was based on the structure of secreted IgM complexes [6, 7]. In deriving this result, it was not necessary to assume an upper size for IgM clusters, and the result is independent of whether or not these polymers form closed rings. Pre-clustering of the BCR in the absence of antigen may explain the basal level of signalling observed in experiments [24].

The polymeric organisation of IgMs postulated here can explain the contradictory results obtained at first sight by biochemical [6] and fluorescence [25] studies. Contrary to biochemical studies, fluorescence resonance energy transfer (FRET) experiments reveal no interactions between Igαs in the absence of antigen, which have previously been expected to exist in IgM clusters. However, if as proposed here, the IgM polymer was connected by two Igα/Igβ

heterodimers per segment that each interacted with two IgMs, then one would expect the distance between any two Igαs to be too large to be detected by FRET.

A number of studies suggest that BCR clustering is required for B-cell signalling [3–5]. In that case, the lower responsiveness of anergic B cells may be accounted for by impaired clustering observed in the mathematical model when the IgM density is reduced to 2–5% of the normal density. Such reduced density has been observed on anergic MD4 B cells [10, 11] as a consequence of a selective block in transport from the endoplasmic reticulum [26]. The normal expression levels of IgD will not facilitate IgM clustering because the receptor clustering is isotype-specific [6]. That tolerance can be broken by membrane antigen [27] and cross-linking substances can be explained with the higher local IgM density (30-fold in experiments with lipid bilayers [28]) and the enforced cluster formation. Control of B-cell reactivity on the level of receptor densities is an elegant mechanism, as it is simple and prevents any difficult signal integration at later steps. Moreover, IgM downregulation is a rapid process that can also be quickly reversed.

Other studies account the unresponsiveness of anergic B cells to the dissociation of IgM from Igα/Igβ [22, 29]. The mathematical model suggests that such dissociation can also be the consequence of IgM downregulation.

Anergic B cells that are specific for single-stranded DNA have been found to express almost normal IgM levels [30, 31], and it will be interesting to find out whether in this case IgM still forms clusters or whether alternative mechanisms exist to destabilise BCRs.

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