The Journal of Immunology

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J Immunol 2005; 174:4461-4464; ;
doi: 10.4049/jimmunol.174.8.4461
http://www.jimmunol.org/content/174/8/4461

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Death of a Dogma or Enforcing the Artificial: Monomeric IgE Binding May Initiate Mast Cell Response by Inducing Its Receptor Aggregation

Reinhard Schweitzer-Stenner and Israel Pecht[1†]*

***Several recent reports have suggested that binding monomeric IgE (mIgE) to its type 1 receptor, FcεRI, on mast cells induces important responses. These observations contradict the notion that it is the aggregation of this receptor that is essential for initiating mast cell response. In the present study, we suggest that the most probable causes for the reported observations are the experimental protocol used combined with the high expression levels of the FcεRI by mast cells. Specifically, we suggest using the published data and physicochemical calculations that the exceptionally high number of cell surface FcεRI-bound monoclonal IgE yields, in the two-dimensions of the cells' membranes, a situation where even a low affinity of these mIgE for epitopes on their own structure or on another cell surface component may lead to their aggregation. Hence, we hypothesize that the reported response to mIgE binding is a result of such an FcεRI-IgE induced aggregation. The Journal of Immunology,* 2005, 174: 4461–4464.**

The long-standing immunochemical dogma states that only upon FcεRI clustering is the cascade coupling it to mast cells' response triggered (1, 2). IgE binding was generally considered to just endow the FcεRI with specific Ag recognition capacity or affect its cell surface expression levels. However, this model has been challenged recently by a series of reports suggesting that monomeric IgE (mIgE)[2] binding to the FcεRI does initiate its stimulus-response cascade, resulting in several important cellular activities (3–8). The first two independent studies (3, 4) reported that mIgE binding prolonged survival of mouse mast cells under growth factor-limiting conditions. However, these studies differed in some crucial details of the observed biochemical responses and hence also in the mechanisms proposed for rationalizing their finding: Kalesnikoff et al. (4) found that mIgE binding to the FcεRI induces tyrosine phosphorylation of several intracellular components, as well as activation of PI3K, Akt, and MAPKs, followed by an increased de novo synthesis of cytokines, which were suggested to be responsible for the observed cell survival in an autocrine-dependent fashion. In contrast, Asai et al. (3) did not detect any signaling events or significant cytokine secretion. Several more recent articles attracted additional interest as they reported that mIgE binding even induces secretion of granule-stored mediators by the rat mast cells of the RBL-2H3 line, as well as by bone marrow-derived cultured mast cells (BMCMCs) (7, 8). Moreover, Kitaura et al. (5, 6) reported that binding of different monoclonal mIgE induces a spectrum of cell activation events that varied widely depending on the particular IgE mAb used and provided some evidence that these events are a result of FcεRI aggregation.

Mast cells' response to the binding of mIgE, if proven to occur, has significant implications by itself, as well as for our understanding the requirements for cell stimulation by other immunoreceptors, such as those of B cells. In addition, upon critically reading the reports published so far, some important differences are observed in the experimental protocols, results, and conclusions. Hence, the above phenomenon clearly deserves additional evaluation. As detailed in *Results and Discussion*, we suggest that certain nonphysiological elements of the experimental protocols used may be the actual cause for the observation, rather than a novel-unknown mechanism.

Probably the first observation suggesting that mIgE binding to the FcεRI is capable of inducing a biological response was made by Furuichi et al. (9), who have shown that exposure of RBL-2H3 cells to IgE up-regulates the FcεRI expression. This was later expanded by results of Yamaguchi et al. (10, 11), showing that the FcεRI expression levels on peritoneal mast cells from IgE-deficient ($IgE^{-/-}$) mice are reduced dramatically compared with those on cells from the corresponding normal mice. However, these studies have established that the enhanced FcεRI expression is in fact due to the increased lifetime of the surface-resident receptors rather than to its increased synthesis and/or transport to the plasma membrane (9, 12, 13). Thus, this effect of mIgE binding to the FcεRI is of a different nature.

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Received for publication December 6, 2004. Accepted for publication February 2, 2005.

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[2] Abbreviations used in this paper: mIgE, monomeric IgE; BMCMC, bone-marrow derived cultured mast cell.

0022-1767/05/$02.00

The simplest explanation for the observed surprising cellular responses to mIgE could be that the supposed mIgE still contains traces of IgE oligomers, which upon binding mediate FcεRI clustering. However, great efforts were made in all these studies to exclude this trivial cause. Hence, there is a clear need for another rationale for these interesting and challenging observations.

An important insight into the events induced upon mIgE binding was that addition of monovalent haptens (for which the mIgE used is specific) abrogated the cells' responses (4, 8, 14). This additionally supported the intriguing possibility that FcεRI-bound mIgEs may undergo aggregation by binding to their Ag recognition site of an epitope on their own structure or of another cell surface component. Indeed, the marked dependence of the cells' response on specific properties of the monoclonal IgE class Ab used strongly suggested that some FcεRI-IgE complexes undergo aggregation (Refs. 6 and 14, see also Ref. 4).

In the present study, we suggest that this FcεRI-IgE aggregation can be the outcome of the nonphysiological experimental conditions used in all these studies: the exceptionally high FcεRI cell surface expression levels ($3–6 \times 10^5$ copies/cell for RBL-2H3 and $4–10 \times 10^4$ copies/cell for BMCMCs (Ref. 15 and J. Rivera, unpublished observation), i.e., 5- to 100-fold higher than those of the TCR for Ag) are all occupied by one and the same monoclonal IgE (in contrast to the in vivo situation where the whole specificities repertoire of IgEs will be displayed). The concentration of the FcεRI-IgE complexes in the two dimensions of the cell surface is, as illustrated in *Results*, dramatically higher than its molar equivalent in solution. Thus, we suggest that it is rather plausible that under such nonphysiological conditions, even a low affinity of the used monoclonal IgE for a given epitope can lead to either homo- or heterotypic association and induce clustering of the cell-resident FcεRI-IgEs.

Materials and Methods

The enhancement in the apparent affinity of binding an IgE Ag-combining site to an epitope on another IgE in the two-dimensional surface of a cell can be estimated by the following simple statistical consideration: as shown earlier (16), the probability of finding as nearest neighbor a distinct receptor at distances between r and dr on a cell surface is given by:

$$w(r)dr = 2\pi r\sigma_{rec}e^{-\pi r^2\sigma_{rec}}\,dr \qquad (1)$$

where σ_{rec} is the cell surface density of the component in question, i.e., in our case a monomeric FcεRI-IgE complex. The corresponding probability function for a three-dimensional isotropic system representing reactions in solution can be obtained from Chandrasekar (17):

$$w(r) = 4\pi r^2 n e^{-\frac{4}{3}\pi r^3 n} \qquad (2)$$

where n is the average number of molecules (i.e., IgEs) per unit volume. For treating the current problem, we used the normalized probability for a segment $r_0+\Delta r$, where $r_0 = 75$Å is the assumed sterically allowed minimal distance between two FcεRI-IgE complexes. The corresponding equation is written as:

$$P(r_0) = \frac{\int_{r_0}^{r_0+\Delta r} w(r)dr}{\int_{r_0}^{r_{lim}} w(r)dr} \qquad (3)$$

where r_{lim} is the radius of a circular shell representing the total area of the cell surface, or, for the three-dimensional system, the radius of a sphere representing the unit volume.

Results and Discussion

Fig. 1, *a* and *b*, illustrates $P(r_0)$ as a function of the IgE solution concentration and the corresponding number of FcεRI-bound

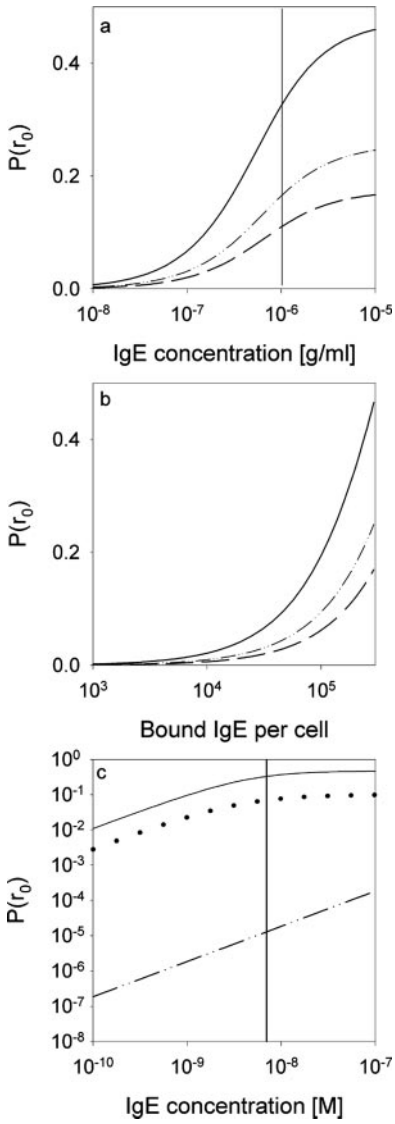

FIGURE 1. The probability $P(r_0)$ to IgE-IgE interaction to occur in a distance interval $r_0+\Delta r$ as a function of IgE-concentration. *a*, $P(r_0)$ was calculated as a function of mIgE bulk concentration of mIgEs bound to type 1 FcεRs on a two-dimensional (cell) surface with $\Delta r = 30$ Å (solid line), 15Å (dash-dot-dot line), and 10Å (medium dash), assuming 3×10^5 FcεRI/cell. *b*, $P(r_0)$ was calculated as a function of mIgE bound to type 1 FcεRs on a two-dimensional (cell) surface with $\Delta r = 30$ Å (solid line), 15Å (dash-dot-dot line), and 10Å (medium dash), assuming 3×10^5 FcεRI/cell. *c*, $P(r_0)$ was calculated as a function of mIgE bulk concentration for mIgEs bound to type 1 FcεRs on a two-dimensional (cell) surface (solid line) and for free mIgE in solution (medium dash), assuming 3×10^5 FcεRI/cell, and for mIgEs bound to type 1 FcεRs on a two-dimensional (cell) surface, assuming 5×10^4 FcεRI/cell (dotted line). All three calculations were conducted using $\Delta r = 30$ Å. The binding of IgE to FcεRI was modeled with a intrinsic binding constant of $K_i = 3 \times 10^{-8}$ M obtained by Ortega et al. (18) The conversion from bulk to cell surface concentration was achieved by a considering a sample with a cell concentration of 5×10^9 cells/L. A value of 2.5 μm was assumed for the cell radius.

IgE, respectively. Binding of mIgE to FcεRI was calculated as described by Ortega et al. (15, 18). Fig. 1*a* reveals that $P(r_0)$ reaches significant values (> 0.4) at total mIgE concentrations > 1 μg/ml ($\sim 7 \times 10^{-9}$ M). This corresponds nicely to the mIgE concentration where onset of β-hexosaminidase secretion has been observed for RBL-2H3 cells from three different sources. The vertical line in Fig. 1*a* represents the mIgE (mAb SPE-7) concentration (1 μg/ml) for which these authors observed secretion between 40 and 60% of the granule contents (8). As shown in Fig. 1*c*, the corresponding $P(r_0)$ for a three-dimensional system (i.e., mIgE in solution) is several orders of magnitude lower in the investigated concentration range: at 7×10^{-9} M (~ 1 μg/ml) our simulation suggests an enhancement factor of $\sim 10^4$ for the reaction on the cell surface. This is somewhat larger than the value derived from the equilibrium constants determined for the reaction of the DNP-specific IgE (mAb-H1) with the divalent hapten $(DCT)_2$-Cys in solution and on the surface of RBL-2H3 cells, which are indicative of a 10^3-fold increase in the apparent equilibrium constant for IgE dimerization by the respective divalent haptens on the cell surface (19). Still, in view of the simplicity of our model used, the agreement with these experimental data may be considered as sufficient. It is noteworthy that our simulation does not take into account restrictions of the orientational mobility and the effect of volume exclusion on the cell surface, which may both further increase the enhancement (20).

Fig. 1*c* depicts the result of another simulation conducted for the situation of a significantly lower number of FcεRI on the cells (i.e., 5×10^{-4}), which accounts for BMCMCs. Even under this condition, $P(r_0)$ reaches values of ~ 0.1, certainly sufficient to trigger a substantial secretory response. Moreover, this simulation shows that cells with a higher number of receptors (notably RBL-2H3 cells) can be activated even in the presence of a mixture of IgE class mAbs if only 10% of them will react with each other on the cell surface. This point may have relevance to the in vivo situation where increased tissue levels of IgE were indeed found to induce activation (14).

Therefore, we can safely conclude that the apparent affinity driving the proposed mIgE clustering is several orders of magnitudes higher on the cell surface than in solution. Thus, even a low intrinsic IgE affinity for an epitope present on the cell surface would be sufficient for inducing substantial clustering FcεRI-IgE. In this context, it should be further emphasized that optimal secretion of granule-stored mediators by RBL-2H3 cells generally requires the clustering of only $\sim$10% of the FcεRI. Moreover, an even lower degree of aggregation is required for inducing the de novo synthesis and secretion of cytokines (21). This may explain the marked differences in response reported for RBL-2H3 and BMCMCs by different labs applying distinct IgE class mAbs. For example, in the latter cells, mAb SPE-7 induces significant cytokine secretion causing strong antiapoptotic effects, whereas other mAbs (such as H1) display a lower (or undetectable) capacity to induce cytokines secretion and exert less robust survival effects (5).

The recent and rather detailed structure-function relation studies of mAb SPE-7 provide a compelling illustration of how the Ag binding site can provide the affinity leading by the above enhancement to the cell surface FcεRI-IgE aggregation (22, 23). This study demonstrated that the SPE-7 binding site may exist in at least two distinct (unbound) conformations, which markedly differ in their epitope-binding specificities and affinities. One specificity is for a protein (thioredoxin peptide derivative) epitope, which may probably cross-react with a different one present on the cells' surfaces, albeit with low affinity.

Finally, it should be emphasized that in the present study we addressed a different problem from that of the classical study of DeLisi (24). The latter focused on elucidating the lifetime of encounter complexes of FcεRI-IgE in the absence of any aggregating agent. He has shown that although the high FcεRI density allows for frequent encounters, receptor aggregates do not persist long enough to allow for triggering the biochemical cascade, which culminates in the cells' secretion. In the present study, we consider affinity-driven aggregate formations, i.e., longer lived FcεRI-IgE clusters, that, as reported, induce cell response. Furthermore, we did not address the effect of IgE binding on FcεRI expression levels, which is due to rather different mechanism (9, 12, 13).

In conclusion, our above analysis illustrates the drastic differences between cell surface concentrations and those of bulk solution. It has a particular significance in the case of the mast cells' surface resident FcεRI-IgE. Still, it may also be encountered when ever a relatively high copy-number of a cell membrane component comes into play (24)

Acknowledgments

We wish to express our deep gratitude to Jakub Abramson and Dr. R. P. Siraganian for their helpful comments and discussions.

Disclosures

The authors have no financial conflict of interest.

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