

Regulation of IgE-receptor expression, IgE occupancy and secretory capacity of mast cells

Review article

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Mast cells play an important role in initiating and modulating allergic and inflammatory reactions. Their responsiveness is determined by three important factors: the expression of IgE receptors on the cell surface, the IgE occupancy of these receptors, and the intrinsic secretory capacity of the cells. In this review, we will summarise some findings relevant to these three aspects of mast cell function, and discuss possible regulatory mechanisms. It appears that the genetic background as well as environmental factors influence all three of these components of the response. T cells appear to play an important role in regulating the IgE-receptor expression and also, independently, the intrinsic secretory capacity of mast cells via an unidentified route, possibly involving the secretory signal transduction chain directly. IgE itself appears to have an important role in the regulation of IgE-receptor expression, as indicated by the upregulation of receptors *in vitro* in the presence of IgE, and the absence of IgE-binding capacity of mast cells in IL-4 gene knockout mice, lacking IgE production. The IgE-receptors of mast cells are saturated to a high degree under different normal conditions, without an obvious relation to antigenic stimulation, also in athymic animals. We have suggested that this basal IgE content on mast cells may be the result of an antigen-independent production of IgE directed by the mast cells themselves and serving regulatory purposes, modifying the secretory response and preventing a massive possibly harmful degranulation.

Key words: Mast cells; IgE, receptors; mast cell secretion; histamine; cytofluorometry; cytokines.

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Mast cells, which express receptors (FcεRI) with a high affinity for IgE molecules and c-kit tyrosine kinase receptors on their surface, are mediator-containing cells involved in allergic and inflammatory responses. Their functional activities are initiated through the cross-linkage of the IgE-receptor complexes by specific antigen, triggering an intracellular signal transduction system, which initiates the release of a variety of preformed and newly generated inflammatory mediators such as histamine and eicosanoids. We now know that mast cells not only release such classical mediators of in-

flammation but also generate and secrete a variety of cytokines when the IgE-receptor complexes are cross-linked (1–3). Of these, IL-3 is essential for regulation of the proliferation of mast cells (4, 5), thus suggesting that the stimulation of mast cells via FcεRI could induce an autocrine self-sustained growth. IL-4 is an essential cytokine in inducing the IgE-isotype switch of B lymphocytes and enhancing IgE production (6–8). IL-5 is essential for the proliferation and differentiation of eosinophils (9, 10). In addition, bone-marrow-derived mouse mast cells have been found to be able to express

MHC II antigen and to present antigen to immune T cells in vitro (11). The mast cell-IgE system therefore may also be involved in the regulation of immune responses.

T-CELL-DEPENDENT AND T-CELL-INDEPENDENT IgE PRODUCTION: THE ROLE OF MAST CELLS

Cytophilic antibodies of the immunoglobulin E class, synthesised and secreted by B lymphocytes bearing IgE (sIgE⁺) on their surface after antigen stimulation, play two distinctive roles in the immunological triggering of mast cells. First, they bind to Fc receptors on the plasma membrane, and secondly, they transmit extracellular stimuli to the sensitised cell through the effects of their subsequent interaction with specific antigen (allergen).

IgE production may be induced along T-cell-dependent or T-cell-independent pathways. It can also be classified into antigen-specific or antigen-unspecific responses depending on whether the IgE produced is against the antigen(s) introduced or against unknown antigen(s). An IgE response may be initiated by antigen-driven or non-antigen-driven mechanisms (12). All types of IgE response should be reflected in the total IgE concentration in the serum as well as in the IgE occupancy on mast cells and basophils.

In a T-cell-dependent and antigen-driven line, the antigens presented by antigen-presenting cells (APC) stimulate T lymphocytes to differentiate into antigen-specific suppressor and antigen-specific helper T cells. Interleukin-4 secreted by T-helper type 2 cells (Th2) induces an IgE-isotype switch of resting B cells to sIgE⁺ B cells, which are the precursors of IgE-forming cells. The suppression pathway of IgE synthesis is due to the action of interferon γ (IFN- γ) secreted by Th1 cells (6, 7). Cytokines are, meanwhile, not the only factors necessary for inducing IgE production. When T and B cells were separated by a Millipore membrane permitting molecules but not cells to pass, IL-4 failed to induce IgE synthesis. It succeeded when T and B cells were co-cultured in the same chamber (13, 14). This indicates that a physical interaction between T and B cells is necessary

for the synthesis of IgE in addition to IL-4. The interaction between T and B cells has been shown to involve CD40 ligand expressed on CD4⁺ T-helper cells and CD40 receptor on B cells (15–18).

IgE synthesis can also be obtained in vitro, without the assistance of T cells, by stimulating B cells with monoclonal anti-CD40 or by co-culturing B cells with a cell line expressing CD40 ligand in the presence of excess IL-4 (15, 19, 20). It thus appears that the regulation of IgE production requires two signals, IL-4 and the activation of CD40 receptors on B cells. The question which arises from these results is whether T-cell-independent IgE production also occurs in vivo. This possibility has been highlighted by the finding that tissue mast cells and non-B, non-T cells (Fc ϵ RI⁺) located in the spleen and bone marrow are able to generate and release IL-4 when their IgE receptors are cross-linked (1, 2, 21, 22). Human mast cells and basophils can express CD40 ligand as well (23). The entire machinery necessary for T-cell-independent IgE production is thus available. IgE production was successfully induced when B lymphocytes were co-cultured with human mast cells or basophils in an excess of IL-4 without the involvement of antigen in vitro (23). However, the possibility and significance of T-cell-independent IgE production in vivo is an open question.

CELL-BOUND IgE AND SERUM IgE: WHERE DO MAST CELLS ACQUIRE IgE?

IgE antibody exists in two compartments in the body. One is the free IgE in serum and other body fluids, while the other is the cell-bound IgE on mast cells, basophils and, to some extent, a few other cell types (24–26). A positive correlation has been found between the basophil-bound IgE and the serum IgE concentration in both atopic and non-atopic individuals (27–29). A correlation between human serum IgE levels and tissue mast cell fluorescence titres has also been reported (24, 30).

Ishizaka et al. (31) did not find a positive correlation between serum IgE and basophil-bound IgE. On the contrary, they found that the cell-bound IgE was even higher in subjects with a low serum IgE level than in those with high

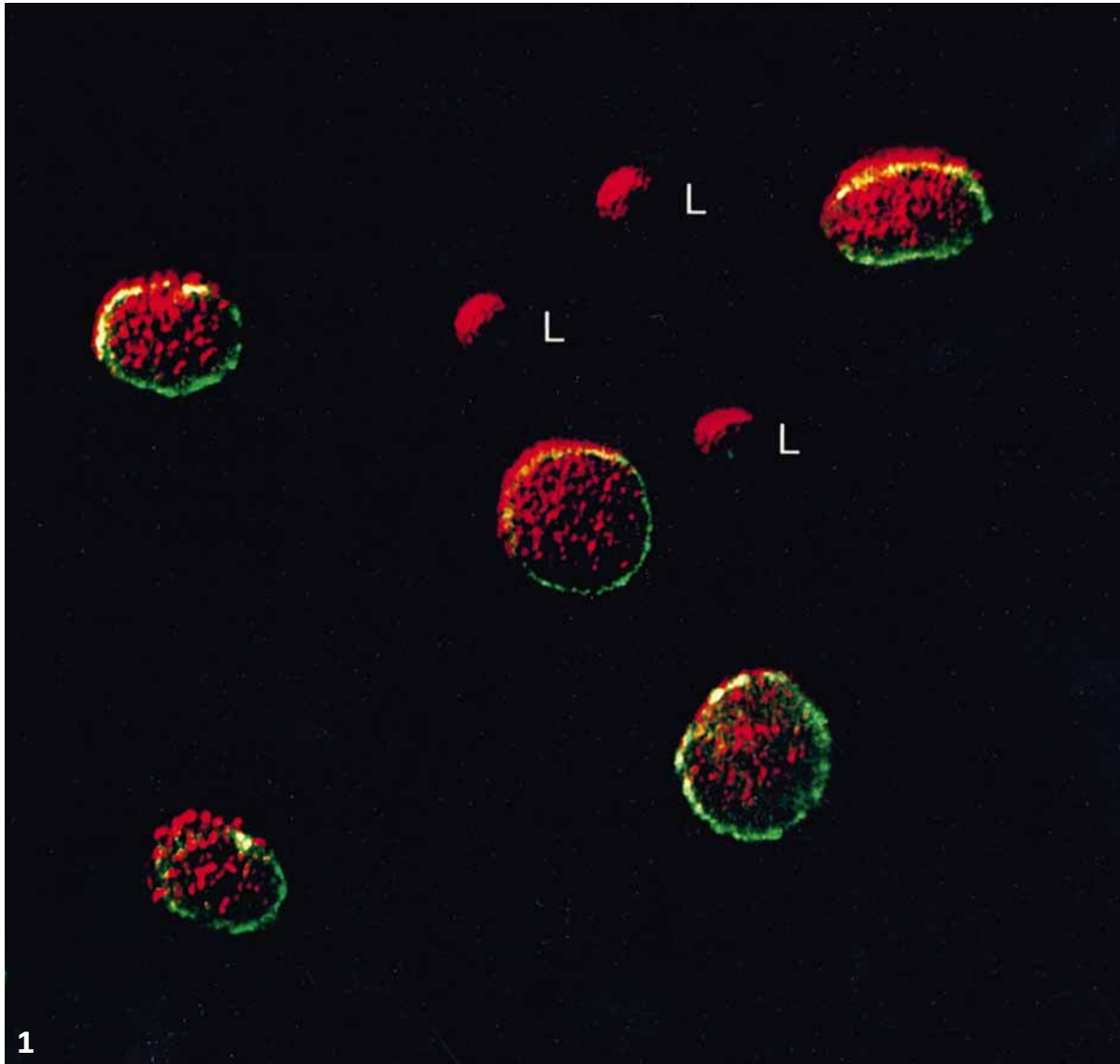


Fig. 1. Peritoneal cells immunolabelled with goat anti-rat IgE/FITC and examined using laser scanning confocal microscopy. Fluorescence image overlaid on differential interference contrast (DIC) image of the same cells. Note five mast cells with a granular surface image showing a distinct green discontinuous fluorescence rim representing IgE-receptor complexes labelled with anti-IgE/FITC together with small lymphocytes (L) lacking fluorescence label. A plan-Neofluar 40/1.3 oil lens was used together with an electronic zoom factor of 2.5. An Ar 488 nm laser was used for both fluorescence excitation and DIC.

serum IgE and ascribed this to differences in receptor affinity for IgE. In canine mast cells, the number of cell-bound IgE molecules did not correlate consistently with the levels of serum IgE (32).

The relation between serum IgE and cell-bound IgE depends to a large extent on where the cells acquire IgE. IgE production is a local event that takes place in peripheral lymphoid tissues and requires a direct contact between the

antigen-producing B-cells and Th2 cells in addition to antigen-presenting cells. Newly formed IgE that is not used up locally will be drained into the circulation via the lymphatics. Thus, mast cells and basophils could be armed with IgE either locally, at the IgE-producing site, or from the peripheral circulation. In the case of basophils, which spend most of, if not their entire life span in the circulation, the latter alternative appears to be most likely.

It is not known where mast cells acquire IgE. A local acquisition of IgE would perhaps appear to be more plausible since mast cells are local tissue inhabitants having the potential capacity for both amplifying and restricting the extent of the tissue responses. In the case of mucosal mast cells, it was suggested that they are armed with IgE locally rather than from the blood. This assumption was based on the temporal relationship between the increase in mucosal mast cell numbers and serum IgE during the course of infection by, and expulsion of the nematode *N. brasiliensis* (33).

We measured the IgE occupancy on peritoneal mast cells using cytofluorometry and serum IgE using an ELISA during the nematode infection (34). In the absence of infection, IgE was detectable on the mast cells but not in the serum of both athymic and euthymic Lewis rats. During the course of the infection, an enhanced IgE production in athymic rats was reflected by an early increase in the number of IgE molecules on the mast cells, when the IgE level in serum was still below the detection limit of the ELISA system. In euthymic rats, the IgE occupancy on the mast cells increased to a higher level than in athymic rats after 2 weeks of infection. The serum IgE in euthymic rats increased above the detection limit during 2 weeks of infection and remained at a high level until 3 weeks of infection. These results suggest that the mast cells acquire IgE locally rather than from the blood. It also shows that the quantification of IgE on mast cells may be a more sensitive means of

early detection of a low-level IgE response than measuring IgE in serum.

IgE-RECEPTOR EXPRESSION AND IgE OCCUPANCY OF THE RECEPTORS: RELATIONSHIPS TO THE SECRETORY CAPACITY OF MAST CELLS

The number of IgE molecules per basophil in non-atopic subjects was reported to be about 40 to 50% of that in atopic subjects (27, 31, 35). Ishizaka et al. (31) found that the number of cell-bound IgE molecules per basophil increased two to seven times when basophils from non-atopic individuals were incubated in 100 $\mu\text{g/ml}$ of IgE myeloma protein, while no further increase was found after incubation in 1 mg/ml of IgE myeloma protein. This means that the IgE receptors on basophils were partly occupied under non-atopic conditions and that receptor-bound IgE can exchange with serum IgE. Malveaux et al. reported that less than half the IgE receptors on basophil leukocytes were occupied by IgE when the donor serum total IgE was less than 40 ng/ml (36). At a concentration of 15 nM IgE, the receptors were saturated. However, Stallman et al. and van Toorenbergen et al. found that IgE-related fluorescence on basophils from normal non-atopic subjects did not increase after incubation with atopic serum, suggesting that all the receptors were occupied (37, 38).

Cytofluorometric methods were employed to measure the IgE content on individual mast cells based on the fluorescence intensity resulting from the binding of FITC-labelled anti-IgE (39). The specificity of the binding can be checked using laser scanning confocal microscopy as shown in Fig 1. By measuring the fluorescence intensity of the mast cells before and after the saturation of IgE receptors in an excess of rat myeloma IgE, the IgE occupancy as well as the total number of IgE receptors available for binding could be estimated. It was found that about 70 to 80% of the IgE receptors on the mast cells of rats were occupied by IgE molecules with unknown specificity. In addition, the IgE occupancy and IgE-receptor expression level appear to be age-independent in adult animals (40, 41), but increase during the neonatal period (42). On the other hand, the dry mass

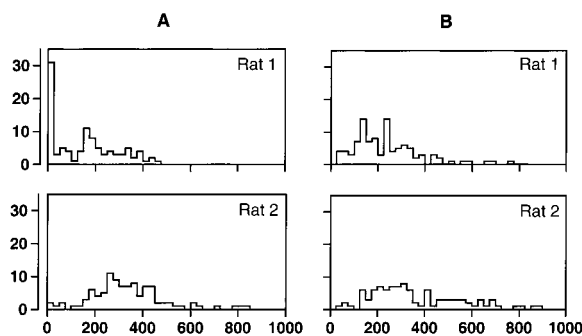


Fig. 2. Fluorescence intensity related to IgE occupancy (A) and IgE-receptor expression level (B) of 100 mast cells from two Sprague-Dawley rats. Frequency distributions, where the x-axis represents fluorescence intensities and the y-axis the number of cells. The cells were labelled with GARaIgE(Fc)/FITC at a dilution of 1/40.

of mast cells reflected by protein content, the granularity reflected by heparin content and the mediator content (histamine or serotonin) increase in an age-dependent fashion in the animals (40, 42–44). This indicates that the elimination of IgE receptors and the expression of newly assembled receptors are controlled independently of cell growth. The density of IgE receptors therefore appears to decrease gradually as the volume and surface area of mast cells increase during the process of cell maturation.

The advantage of the cytofluorometric quantification is that the measurement is based on individual cells. The frequency distribution of the individual cells in a population can therefore be presented as a histogram. Marked heterogeneity of mast cells in a cell population in terms of the surface expression of IgE receptors and IgE occupancy has been found in our studies (39, 40, 45) (Fig. 2). This phenomenon of heterogeneity in both IgE receptors and IgE occupancy on mast cells was also reflected in the variations in response to anti-IgE challenge between individual mast cells when examined in the electron microscope (45).

Ishizaka et al. (31) found a roughly inverse relationship between the number of cell-bound IgE molecules and the optimum concentration of anti-IgE for maximum histamine release from basophils. Conroy et al. (27), meanwhile, found that the basophil sensitivity to anti-IgE appeared to be independent of the number of IgE molecules per cell.

These findings suggest that the number of IgE molecules bound on mast cells or basophils may not be the only factor that determines the release capacity of the cells. When different strains of rat were compared, the IgE occupancy and IgE-receptor expression level on mast cells were higher in Wistar Kyoto (WKY) and Hooded Lister (HL) rats than in Sprague-Dawley (SD) rats, regardless of the breeding and living conditions. In the conventional SD rats, the mast cells acquired a higher level of IgE occupancy than the mast cells obtained from rats kept under barrier-maintained conditions, but they expressed a similar number of IgE receptors. A close correlation was obtained between IgE-mediated histamine release and IgE occupancy or IgE-receptor density on the mast cells. The difference in IgE levels on the mast cells was reflected by the higher IgE-mediated his-

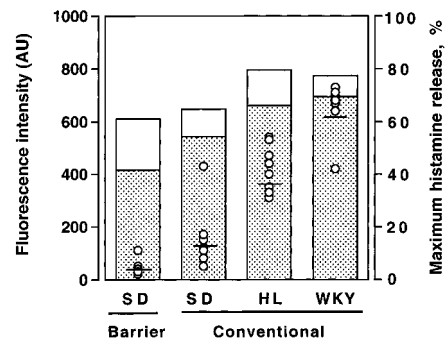


Fig. 3. Comparison of IgE occupancy (filled part of the columns) and surface expression of IgE receptors (filled plus open part of the columns) on peritoneal mast cells measured using microscopic fluorometry, and IgE-mediated histamine releasability of the mast cells, expressed as the percentage of histamine release (open circles), in different strains of rat kept under different conditions as denoted on the x-axis. The mean release values in each group of rats are denoted with horizontal bars.

amine release of the mast cells of SD rats kept under conventional conditions compared with those maintained under barrier conditions. However, in spite of the similar levels of both IgE receptors and IgE occupancy, the mast cells of WKY rats displayed a higher IgE-mediated histamine releasability than those of Hooded Lister rats (45) (Fig. 3). Both genetic and environmental factors thus affect IgE occupancy on mast cells, but genetic factors may determine the expression of IgE receptors to a greater extent. The IgE-mediated histamine releasability was largely, but not exclusively, a function of the number of IgE molecules on the mast-cell surface. The genetic control of the secretory capacity of mast cells appears to occur not only via the surface expression of IgE receptors and IgE production but also via some influence on the intracellular signal transduction system and other events following the cross-linkage of IgE-receptor complexes.

The significance of functional heterogeneity of T-helper cells in the regulation of IgE synthesis has been clarified and related to the different cytokines generated from Th1 and Th2 cells (6, 7, 46). The significance of T cells in the surface expression and occupancy of IgE receptors, as well as in the IgE-mediated histamine releasability of the mast cells, was investigated using athymic rats as an animal model (42, 47). Under normal conditions, the IgE-receptor ex-

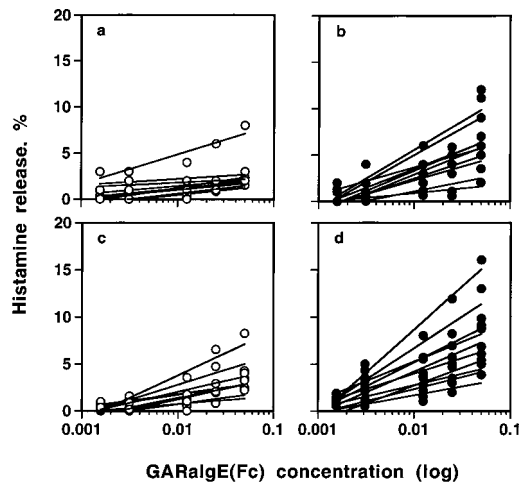


Fig. 4. IgE-mediated histamine release of mast cells as a function of anti-IgE concentration in athymic (a, $r=0.47$ to 0.99 , significant correlation in 8 of 11 rats) or euthymic rats (b, $r=0.86$ to 0.998 , significant in 10 of 11 rats) under barrier-maintained conditions, and of athymic (c, $r=0.70$ to 0.997 , significant in 8 of 10) or euthymic rats (d, $r=0.95$ to 0.99 , all significant) kept under conventional conditions during an additional period of 14 days. Isolated ($>95\%$) mast cells (0.5 to 1×10^5) were incubated with L- α -phosphatidyl-L-serine followed by goat anti-rat IgE in a water bath at 30°C . Histamine release expressed as a percentage of the total.

pression and IgE occupancy were similar in euthymic and athymic rats. In contrast, IgE-mediated histamine releasability was impaired in athymic rats (Fig. 4). During the nematode *N. brasiliensis* infection, the surface expression of IgE receptors and IgE occupancy on mast cells increased in both euthymic and athymic rats. It showed a further increase from one week of infection and reached a peak 2 weeks after infection in euthymic rats but not in athymic rats. The IgE-mediated histamine releasability of mast cells was significantly enhanced in euthymic rats, in particular after one week of infection. However, it showed no clear enhancement in athymic rats, suggesting that the lack of T cells in athymic rats impaired the IgE-mediated histamine release of the mast cells.

A parasite IgE immune response therefore consists not only of increased IgE production by B cells induced by antigen but also of the upregulated surface expression of IgE receptors, increased IgE occupancy and the enhanced secretory capacity of the mediator-containing cells. T lymphocytes appear to be essential in all

these events, in particular the secretory response of the mediator-containing cells.

FACTORS REGULATING IgE-RECEPTOR EXPRESSION

Since the functional activities of mast cells are initiated via the cross-linkage of IgE-receptor complexes, the level of IgE receptors expressed on mast cells at least partly reflects the functional state of the cell. Meanwhile, our knowledge of the way IgE-receptor expression on mast cells is regulated is still somewhat limited.

The cell cycle may be one of the factors that determine the number of IgE receptors on the cell surface. The receptors have been reported to accumulate in the G_1 phase, to be stabilised in the S and G_2 phases, and to be distributed equally to daughter cells during mitosis (48). When rat basophilic leukaemia (RBL) cells, an analogue of mucosal mast cells (49), were co-cultured with monomeric IgE, the number of IgE-binding sites increased (50). The authors suggested that this was the result of the reduced elimination of receptors from the cell surface, along with the continuing insertion of resynthesised receptors.

Some 10 years after this finding was made (50), further investigations were conducted to understand the mechanisms of regulating IgE-

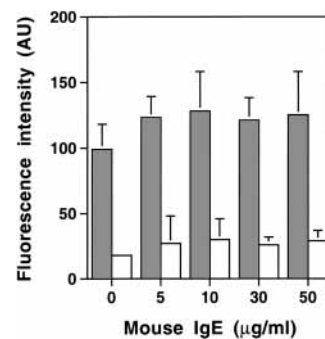


Fig. 5. Saturation of IgE receptors on the mast cells of IL-4 ($-/-$) (open symbols) and ($+/+$) (filled symbols) mice. Peritoneal mast cells were incubated with different concentrations of mouse IgE (x-axis) for 60 min at 4°C and then incubated with goat anti-mouse IgE/FITC for 90 min in an ice-bath. The anti-IgE fluorescence intensity on individual mast cells was recorded using microscope fluorimetry and expressed as arbitrary units (y-axis). Each symbol represents the mean $\pm$ one standard deviation of fluorescence intensity on the mast cells of four mice.

receptor expression on mast cells and basophils. The level of IgE receptors expressed on the mast cells of IgE-deficient mice was extremely low, but it could be markedly upregulated when the cells were incubated with IgE in vitro, or injected with IgE in vivo (51). Similar findings could also be observed on human mast cells and basophils (52–54). In IL-4-gene knockout mice, which lack a serum IgE response after immunisation with the nematode *N. brasiliensis* (55, 56), we found that the IgE occupancy and IgE-receptor expression on mast cells were very much lower in IL-4 (–/–) mice than in their wild-type controls (Fig. 5), indicating that a lack of IgE production in IL-4 (–/–) mice resulting from the absence of IL-4 is the critical factor in inducing IgE-receptor expression on mast cells. However, the mRNAs of the α , β and γ chains of IgE receptors could all be detected in the mast cells of both IL-4 (–/–) mice and IL-4 (+/+) controls (57). The transcription of the genes coding for the IgE-receptor subunits must therefore indeed take place in the mast cells of the IL-4 (–/–) mice. Despite clear differences at the IgE-receptor protein level, the gene transcription of IgE receptors in mast cells was similar in IL-4 (–/–) and IL-4 (+/+) mice. The loss of IgE-binding capacity must therefore be the result of the absence of or a defective translation of mRNA to receptor proteins on cell surfaces in IL-4-deficient mice.

These findings clearly indicate that IgE ligand-mediated upregulation appears to be the main mechanism in regulating IgE-receptor expression on mast cells and basophils. It may, nevertheless, not be the only way to regulate IgE-receptor expression. As discussed above, environmental and genetic factors appear to influence the level of IgE-receptor expression on mast cells as well.

DOES ANTIGEN-INDEPENDENT IgE-PRODUCTION TAKE PLACE IN VIVO AND DOES IT REGULATE MAST CELL RESPONSIVENESS?

In studies of different strains of rat (45), we observed that around 80% of IgE receptors on mast cells were occupied by IgE under normal conditions in vivo, independently of the IgE responsiveness of the strain. This high-degree IgE

occupancy was also observed on the immature mast cells of neonate rats (42), which have received little exposure to environmental antigens. A similar finding was obtained in germ-free animals bred under sterile conditions and breathing filtered air (unpublished). In athymic rats, without detectable serum IgE, a similar high occupancy of IgE receptors could be observed on the mast cells (41, 47). These findings suggest the existence of a non-antigen-driven, T-cell-independent IgE production in vivo. If non-specific IgE occupies the majority of IgE receptors under normal conditions, newly synthesised allergen-specific IgE will have to compete for binding sites with IgE already bound. Since mast cells possess the machinery necessary for the stimulation of IgE production by B cells, such IgE produced without the assistance of T cells and independently of antigen stimulation could serve an important function in the regulation and modification of the secretory responses of mast cells. We have therefore put forward the hypothesis that the basal IgE occupancy on mast cells may be the result of an IgE synthesis directed by the mast cells themselves. We have also suggested that it plays a regulatory role, modifying secretory responses when mast cells are exposed to specific allergens and preventing a massive potentially lethal mediator release.

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