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Antigen Valency as a Determinant of the Responsiveness of IgE-Sensitised Rat Basophil Leukemia Cells

Key Words

Allergy
Mast cell
RBL
IgE antibodies
Valency

Abstract

Rat basophil leukemia (RBL) cells were sensitised with varying proportions of monoclonal IgE anti-ovalbumin (OVA) and anti-DNP antibodies, and serotonin release was measured after challenge with aggregated OVA or dinitrophenylated human serum albumin (DNP-HSA). Highly aggregated OVA was shown to provoke the degranulation of RBL cells that had been sensitised with an IgE preparation containing 2% IgE anti-OVA antibodies. Highly substituted DNP₃₂-HSA induced degranulation of RBL cells sensitised with just 0.5% antigen-specific IgE. When cells were sensitised with high percentages of specific IgE, maximum degranulation was seen at concentrations of 2 µg/ml (aggregated OVA) and 50 ng/ml (DNP-HSA), while moderate degranulation was still seen at antigen concentrations as low as 50 and 2 ng/ml, respectively. Low-molecular weight aggregates of OVA and low-valency DNP₄-HSA only stimulated degranulation when high percentages of RBL Fcε receptor were occupied by antigen-specific IgE. The sensitising abilities of two anti-DNP monoclonal antibodies of differing affinities were compared. When challenged with low-valency antigen, only cells sensitised with the higher-affinity monoclonal antibody exhibited moderate levels of degranulation. Degranulation required exposure to high antigen challenge doses (5 µg/ml). Cells sensitised with either monoclonal antibody responded strongly when challenged with a wide range of concentrations (1–250 ng/ml) of high-valency DNP₃₂-HSA, although greater sensitivity was always seen with the higher-affinity antibody. These results suggest that antigen valency is a critical parameter for mast cell function, and that low-affinity antibody may be capable of sensitising mast cells to high-valency antigen.

Introduction

Regional lymph nodes have long been recognised as sites of IgE production [1], and have more recently been shown to be sites of accumulation of mast cell progenitors [2]. It has therefore been suggested that mast cells may be

sensitised by locally produced IgE antibodies in regional lymph nodes [3]. Mast cells are now known to be important sources of cytokines [4], including IL-4 [5] which is essential for IgE class switching. Furthermore, in an in vitro system, in the presence of IL-4, mast cells are capable of directing B cells to IgE production [6], leading to the hypothesis

Received:
September 6, 1994
Accepted after revision:
January 17, 1995

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1018-2438/95/1074-0547
\$8.00/0

that mast cell-B cell association may lead to increased IgE synthesis and local sensitisation of mast cells at sites distant from the lymph nodes [6]. Nevertheless, the prevailing model of mast cell function suggests that mast cell sensitisation is not a local phenomenon, but one in which cells resident throughout the body become similarly sensitised with serum-derived IgE.

Since the percentages of specific serum IgE in even highly allergic individuals is low, mast cell-bound IgE, if serum-derived, would be directed towards a multitude of antigens. Whether or not such a process of sensitisation could lead to reactivity to a particular antigen would therefore depend upon the proportion of serum IgE directed against that antigen, and upon the sensitivity mast cells acquire when varying percentages of cell surface Fcε receptors are occupied by IgE antibodies directed against any antigen.

It has been reported that the levels of allergen-specific IgE in the serum of allergic patients may be as high as 20%, and as low as 0.1% of total serum IgE [7]. It has also been estimated that approximately 300,000 high affinity receptors (FcεRI) for IgE are typically expressed on the surface of each mast cell [8]. Although FcεRI expression by mast cells and basophils is known to be variable [9], if the sensitisation of mast cells in allergic patients is a product of serum-derived IgE, these studies suggest that perhaps as few as 300 receptors carrying allergen-specific IgE could be sufficient for cell sensitisation to that allergen.

Evidence has been published that only 100 receptors require cross-linking to trigger degranulation of mast cells [10], and that maximum degranulation of rat basophil leukemia (RBL) cells may be attained with the cross-linkage of 10% of surface IgE [11]. While these reports are consistent with the possibility of serum IgE-mediated sensitisation, we have undertaken experiments to investigate more closely the relationship between the percentage of cell-bound antigen-specific IgE and antigen responsiveness. We have conducted these experiments using the RBL-2H3 cell line, which is a well-established mast cell model, and which is widely used to study the process of signal transduction and secretion after ligand binding to cell surface receptors [12, 13].

Our studies allow us to describe the 'threshold percentage', or the percentage of total cell-bound IgE that must be specific for an antigen if RBL cells are to show significant degranulation upon exposure to a particular concentration of that antigen. The availability of two monoclonal IgE anti-DNP antibodies has allowed us to examine the relationship between antibody affinity and the threshold percentage. By challenging cells with aggregated antigens fractionated according to molecular weight, or with hapten-carrier prepa-

arations having varying levels of hapten substitution, we have also been able to investigate the role of antigen valency on cell sensitivity. The results presented here suggest that antigen valency is of fundamental importance to mast cell sensitivity, and that even relatively low-affinity antibodies may be able to promote degranulation upon challenge with high-valency antigen.

Materials and Methods

Antibody and Antigen Preparations

Dinitrophenyl coupling reagents, DNP-lysine, human serum albumin (HSA), ovalbumin (OVA) and purified SPE-7 IgE anti-DNP antibodies were obtained from Sigma Chemical Co., St. Louis, Mo. DNP-HSA was prepared at varying levels of substitution and characterised spectrophotometrically using standard techniques [14]. OVA was aggregated using glutaraldehyde, and aggregates of various sizes were separated by gel filtration chromatography on a Sephacryl S300 Superfine column (Pharmacia LKB Biotechnology, Uppsala, Sweden). Purified monoclonal B1E3 rat anti-mouse IgE and H1-ε-DNP murine IgE anti-DNP antibodies were kindly provided by Dr. Dan Conrad, Virginia Commonwealth University, Virginia, USA. IgE anti-OVA antibodies were raised as ascites using the OVA6B cell line, kindly provided by Mr. G. Georgiou, Royal Children's Hospital, Melbourne. Antibody concentrations were quantified by RBL degranulation assay. RBL cells were cultured in the presence of doubling dilutions of the various monoclonal IgE antibodies, and degranulation was induced by further incubation of cells with B1E3 rat monoclonal anti-mouse IgE antibodies. It was first confirmed that the maximum levels of degranulation and the slopes of the titration curves were identical for all antibodies. Preparations of unknown IgE concentration were then quantified by reference to the degranulation resulting from the sensitisation of cells with known concentrations of purified SPE-7 IgE anti-DNP antibodies.

ELISA Assays

An indirect ELISA assay was developed for the measurement of monoclonal IgE antibodies. Briefly, 96-well microtitre plates (Nunc A/S, Roskilde, Denmark) were coated with DNP₃₂-HSA (10 µg/ml in 0.01 M carbonate buffer, pH 9.6) by overnight incubation at 4°C, and blocked with 1% skim milk/0.01 M carbonate buffer for 1 h at 37°C. Samples were diluted in PBS/1% skim milk/0.05% Tween-20 and incubated for 1 h at 37°C. B1E3 monoclonal rat anti-mouse IgE was added to plates at a concentration of 10 µg/ml, and incubated for 1 h at 37°C. Plate-bound rat IgG antibodies were then detected by the addition of 1:1,000 peroxidase-labelled goat anti-rat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, USA) and incubation for a further hour. Finally, tetramethylbenzidine substrate (Sigma) was added to the wells and incubated at room temperature for 10 min. The reaction was stopped using 2 M H₂SO₄. Between steps of the assay, plates were washed with PBS/0.05% Tween 20. Optical densities were read at 450 nm using a Bio-Rad 3550 Microplate reader (Bio-Rad Laboratories, Richmond, Calif.).

The affinity of anti-DNP antibodies was determined by an ELISA inhibition assay, a modification of the assay described above, which was developed by reference to the radioimmunoassay of Varga [15]. Near-saturation concentrations of the two anti-DNP antibodies were

first determined by the IgE anti-DNP ELISA assay. Aliquots of antibodies at this concentration were then incubated for 45 min at 37°C in the presence of concentrations of free monovalent antigen (DNP-lysine) ranging from 10^{-3} to 10^{-9} M. Antibody/antigen preparations were then added to the plates and the assay completed as previously described. The affinity of the antibodies was expressed as the molar concentration [the 'I(50)'] of DNP-lysine necessary for 50% inhibition of antibody binding to the DNP-HSA-coated plate.

Determination of Serotonin Release

RBL-2H3 cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal calf serum (FCS: Cytosystems, Castle Hill, Australia) in 75-cm² culture flasks (Greiner GmbH, Frickenhausen, Germany). Cells were harvested, counted and resuspended in DMEM/FCS containing 2 µCi/ml ³H-5-hydroxytryptamine creatinine sulphate (Du Pont, Boston, Mass.), then seeded into 96-well Nunclon microtitre plates (Nunc A/S, Roskilde, Denmark) at a concentration of 5×10^4 cells/well, and incubated overnight. ³H-serotonin-pulsed RBL cells were sensitised with monoclonal IgE antibodies at percentages of specific IgE ranging from 0.5 to 100%. This was achieved by the incubation of RBL cells in the presence of saturating concentrations (1 µg/ml) of mixtures of anti-DNP antibodies and anti-OVA IgE (e.g. 25% or 0.25 µg/ml anti-DNP + 75% or 0.75 µg/ml anti-OVA). Cells were cultured for a further 3.5 h before being challenged with a cross-linking agent. After 30 min, 20 µl supernatant samples were transferred to a Wallac 1450 PET 96-well microtitre plate (Wallac Oy, Turku, Finland) containing 200 µl Hisafe III scintillant (Wallac) per well. The RBL cells were then lysed with 1% Triton X-100 (Sigma) and 20 µl lysate samples transferred to a second Wallac microtitre plate containing 200 µl scintillant per well, for the measurement of un-released ³H-serotonin. Plates were then read in a Wallac Microbeta Plus microplate liquid scintillation counter. The percentage release of serotonin upon antigen challenge was then calculated. Initial experiments used BIE3 monoclonal anti-mouse IgE antibodies (10 µg/ml to 1 ng/ml) as the cross-linking agent. Later, specific antigen (DNP-HSA or aggregated OVA) was used at concentrations varying from 2 ng/ml to 5 µg/ml. Negative control wells set up on each plate included IgE-free wells and antigen-free wells. Each plate also included positive control wells containing cells sensitised with 10 ng/ml or 100 ng/ml H1-ε-DNP IgE, and then challenged with 100 ng/ml high valency DNP₃₂-HSA or with 1 µM calcium ionophore A23187 (Sigma). Four-fold replicates were set up for each antibody and antigen concentration. In one series of experiments, sensitised RBL cells were challenged with consecutive fractions of aggregated OVA eluted from the S300 gel filtration column. In later experiments, sensitised cells were challenged with DNP₃₂-HSA, DNP₈-HSA or DNP₄-HSA.

The Threshold Percentage

The extent of degranulation of antigen-challenged cells sensitised with various percentages of specific IgE was compared with results for fourteen negative control wells set up on each microtitre plate. Results were analysed using the unpaired T test and statistical significance was accepted at the 5% level. The threshold percentage for each antigen preparation was defined as the lowest percentage of specific IgE necessary to sensitise cells for significant degranulation.

Results

Serotonin Release vs. Antigen Concentration

Preliminary experiments were conducted to determine suitable antigen concentration ranges for the investigation of RBL cells sensitised with the various antibodies, for degranulation of sensitised cells may be reduced by saturation at high antigen concentrations. It was shown that degranulation declined at concentrations of high-valency DNP-HSA above 250 ng/ml (fig. 1). Similarly, we established that a suitable concentration of aggregated OVA for the challenge of anti-OVA sensitised cells was 2 µg/ml (data not shown).

The Affinity of IgE Anti-DNP Antibodies

Near-saturating concentrations of H1-ε-DNP and SPE-7 antibodies were incubated at 37°C for 60 min in the presence of DNP-lysine at concentrations from 10^{-3} to 10^{-9} M. The antigen/antibody preparations were then added to a DNP-BSA-coated plate, and the percentage inhibition of antibody binding determined by reference to a series of DNP-lysine-free wells. The H1-ε-DNP IgE was shown to have an I(50) of 1.0×10^{-7} M, while SPE-7 IgE had an I(50) of 1.0×10^{-6} M (fig. 2).

Investigations of the Threshold Percentage

Aggregated OVA was fractionated by gel filtration. The relationship between elution volume and molecular weight was established using a series of purified protein standards, and the predominant molecular weights of the various aggregated OVA fractions were then calculated. Each fraction certainly contained a mixture of aggregate sizes, but nevertheless the fractions could be used as a series to study the effect of antigen valency on the threshold percentage. Fractions used in the following experiments were numbered from 32 to 70. Fraction 70 contained dimeric OVA. Trimeric OVA eluted as fraction 62, tetrameric OVA as fraction 56 and heptameric OVA as fraction 42. With respect to their binding to the monoclonal anti-OVA antibody, these antigen aggregates can be described as having valencies of 2, 3, 4 and 7, respectively.

RBL cells were cultured in the presence of mixtures of H1-ε-DNP and OVA6B IgE antibodies with anti-OVA antibodies present at proportions varying from 0.5 to 100%. Degranulation of sensitised RBL cells was induced by challenge with aggregated OVA fractions at a concentration of 2 µg/ml. Results are presented in figure 3a for cells sensitised with 100, 25, 5 and 1% anti-OVA IgE. All experiments additionally examined degranulation from cells sensitised with 50, 10, 2 and 0.5% specific IgE. Complete results ex-

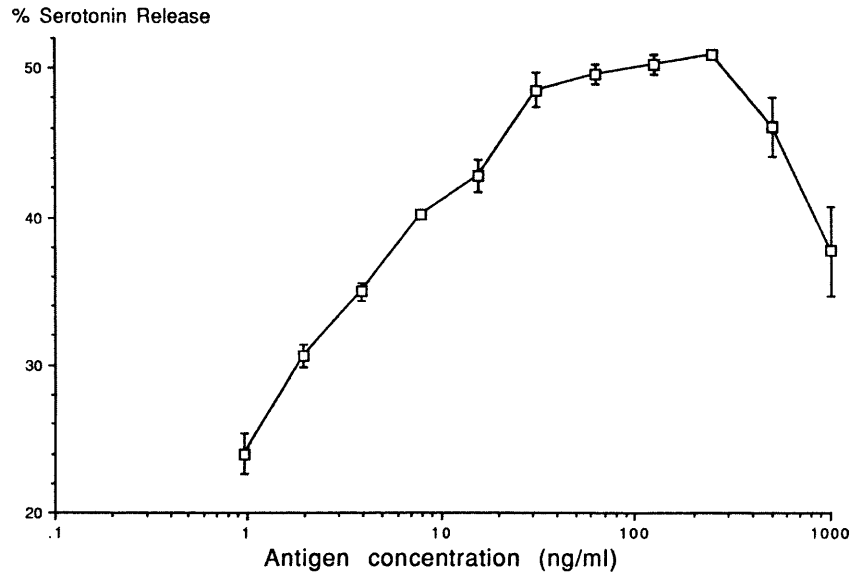


Fig. 1. ^3H -serotonin release from RBL cells sensitised with $2\text{ }\mu\text{g/ml}$ H1- ϵ -DNP IgE anti-DNP antibodies and challenged with DNP₃₂-HSA at concentrations ranging from 1,000 to 1 ng/ml. Data shown are means of four-fold replicates. Error bars are not indicated when graphically too small.

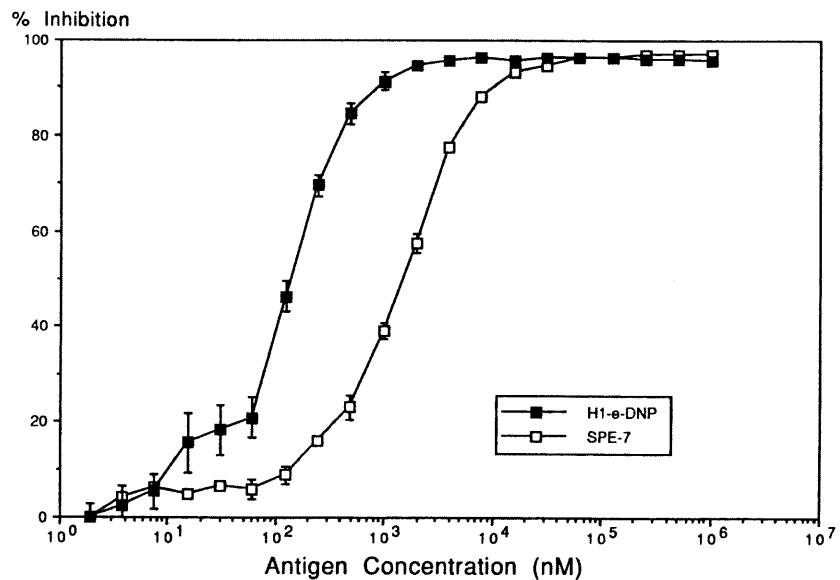


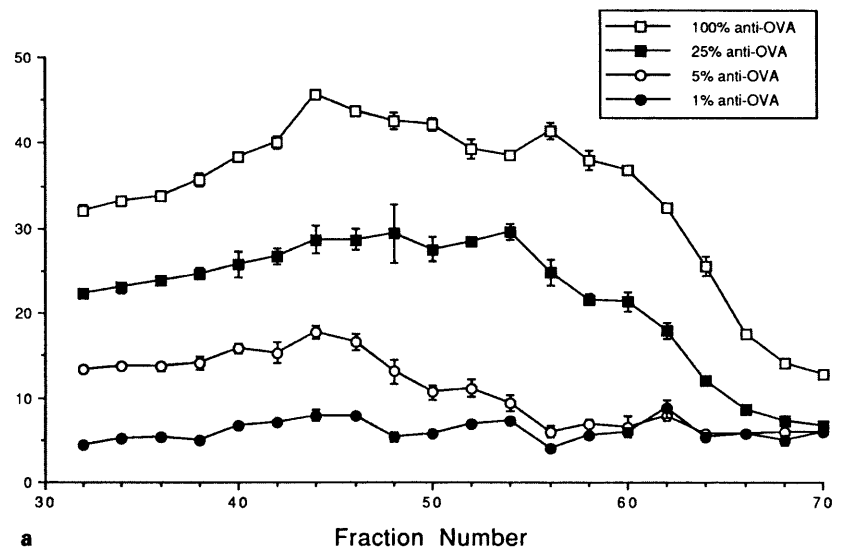
Fig. 2. Inhibition of binding of H1- ϵ -DNP and SPE-7 antibodies to solid-phase bound DNP₃₂-HSA in the presence of concentrations of monovalent DNP-lysine ranging from 10^{-3} to 10^{-9} M. Data shown are means of duplicate samples. Error bars are not indicated when graphically too small.

pressed as percent serotonin release vs. percent occupancy RBL Fc ϵ R by specific IgE are shown for selected fractions (fractions 38, 50, 60, 64 and 68) in figure 3b.

Significant degranulation was only seen for low valency antigen when high percentages of specific IgE were cell-bound. Maximum degranulation was seen with 100% OVA-specific IgE as the sensitising antibody, and a complex relationship between degranulation and fraction number is evident from figure 3a. The strongest degranulation was seen

upon challenge with the heptameric fraction 44 (46% serotonin release). Higher molecular weight fractions showed declining levels of degranulation, perhaps as a result of steric hindrance. The percentage release also declined slowly from fraction 44 to fraction 60, and then dropped sharply from fraction 62 to fraction 70. The same general trends were apparent for cells sensitised with lower proportions of specific IgE. At 5% specific IgE, degranulation increased gradually, then decreased, with declining aggregate size.

% Serotonin Release



% Serotonin Release

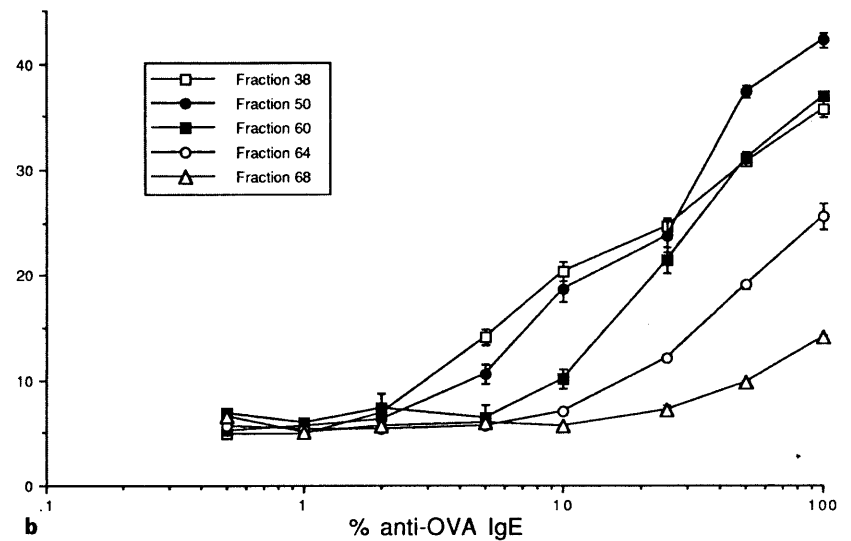


Fig. 3.a, b ^3H -serotonin release from RBL cells sensitised with various percentages of specific IgE anti-OVA antibodies, and challenged with 2 µg/ml aggregated OVA fractions separated according to molecular weight by gel filtration. Data shown are means of four-fold replicates. Error bars are not indicated when graphically too small.

Degranulation rose from the highest molecular weight fractions (13%) to peak at fraction 44 (18%), then dropped sharply to baseline levels (6%) at fraction 56. No degranulation was seen above background levels from cells sensitised with less than 2% anti-OVA IgE, and considerably higher percentages of specific IgE were required to sensitise cells to low valency antigen. Threshold percentages are presented in table 1.

Experiments were repeated using anti-DNP-sensitized RBL cells and various DNP-HSA antigens. RBL cells were again cultured in the presence of mixtures of monoclonal

IgE anti-DNP and anti-OVA antibodies. Experiments were performed using both SPE-7 and H1-ε-DNP IgE anti-DNP antibodies at proportions varying from 0.5 to 100%. Degranulation was induced by incubation of sensitized cells with DNP₃₂-HSA at concentrations ranging from 2 to 250 ng/ml. Results are presented in figures 4a, b. Degranulation reached a maximum of 42% using H1-ε-DNP IgE, and 33% using SPE-7 IgE. No significant increase in degranulation was seen using antigen concentrations above 50 ng/ml. Other than in experiments where cells were challenged with very low concentrations of DNP₃₂-HSA (2 ng/

ml), degranulation tended to peak at 25% specific IgE occupancy of cell surface FcεR. This was observed even when cells were challenged with 10 ng/ml antigen, and is most evident in the results presented for SPE-7 sensitised cells (fig. 4a), where degranulation reached a plateau at 22% serotonin release with 25% occupancy.

Sensitised cells were then challenged with low valency antigen (DNP₄-HSA) at concentrations of 5 µg/ml, 1 µg/ml, 250 ng/ml and 50 ng/ml (fig. 5a, b). Significant degranulation was seen when cells were challenged with 5 µg/ml antigen, but not at lower concentrations. Even with a 5 µg/ml challenge, SPE-7-sensitised cells were only responsive when very high proportions of the sensitising antibodies were DNP-specific. SPE-7 sensitised cells were also challenged with DNP-HSA having a mean valency of 8. Results are presented as figure 6. The maximum degranulation seen was intermediate between that induced by the DNP₄-HSA and the DNP₃₂-HSA. No degranulation followed challenge with less than 250 ng/ml DNP₈-HSA.

Threshold percentages were calculated for H1-ε-DNP (table 2) and SPE-7 sensitising antibodies (table 3) as has previously been described. While SPE-7-sensitised cells were barely responsive to low-valency antigen, the threshold percentage for this antibody using DNP₃₂-HSA at a concentration of 50 ng/ml was just 1%. Threshold percentages were even lower for the H1-ε-DNP antibody. At the lowest DNP₃₂-HSA concentration tested (2 ng/ml), the threshold percentage was 5%, and at higher antigen concentrations (50 ng/ml) degranulation was seen at the lowest percent occupancy tested (0.5%). Low-valency DNP₄-HSA provoked degranulation from cells sensitised with at least 10%-H1-ε-DNP antibody.

Discussion

We have shown that the percentage of specific IgE that must be cell-bound if RBL cells are to acquire sensitivity to a particular concentration of antigen is dependent upon the valency of the challenge antigen. Sensitised cells were refractory to challenge with low-valency antigen. Very little degranulation was seen upon exposure to DNP₄-HSA, even when 100% of cell-bound IgE was DNP-specific. Similarly, challenge of sensitised cells with dimeric and trimeric aggregated OVA resulted in serotonin release which was only slightly above background levels.

Differences seen in the threshold percentages of two IgE anti-DNP antibodies, and differences in the maximum levels of degranulation between the anti-DNP and anti-OVA IgE antibodies may be a reflection of different affinities of

Table 1. The minimum (threshold) percentage of antigen-specific sensitising IgE necessary for degranulation of RBL cells upon exposure to 2 µg/ml aggregated OVA

Fraction No.	Threshold %	Degranulation %	Background degranulation
32	2	6.6±0.5	3.4±0.2
36	2	6.9±0.3	4.2±0.3
40	2	8.7±0.3	6.3±0.2
44	2	9.5±0.5	8.1±0.2
48	5	13.1±1.4	6.3±0.2
52	5	11.1±1.1	6.0±0.2
56	10	12.7±1.8	5.6±0.2
60	10	10.1±0.9	6.0±0.5
64	25	12.1±0.1	6.4±0.2
66	25	8.5±0.3	6.4±0.2
68	50	9.8±0.2	7.0±0.2
70	100	8.8±0.1	7.0±0.2

Results are shown for aggregated OVA fractionated by gel filtration. Heptameric OVA eluted at fraction 42, trimeric OVA at fraction 62, and dimeric OVA at fraction 70.

the various antibodies. Certainly the higher-affinity H1-ε-DNP consistently outperformed the SPE-7 anti-DNP antibody. No differences were seen between the antibodies when rat monoclonal anti-mouse IgE antibodies, rather than antigen preparations, were used as the cross-linking agent (data not shown).

Some, but not all, antibody/antigen combinations elicited maximum levels of degranulation when less than 100% of cell-surface IgE was antigen-specific. High valency DNP₃₂-HSA provoked maximum degranulation with 25% occupancy, while degranulation with DNP₄-HSA and aggregated OVA did not plateau. Experiments using a range of additional high-valency DNP-HSA confirmed the pattern of responsiveness seen with DNP₃₂-HSA (data not shown). A possible explanation for these observations is that epitopes are more tightly clustered on higher-valency antigens. Steric hindrance could limit the ability of closely spaced epitopes to aggregate additional specific IgE. An early report of the cross-linking of IgE on sensitised rabbit basophils observed no such relationship between epitope spacing and histamine release, but only short bivalent antigens were used in the study [16].

Ours is not the first study to suggest that valency is important for the degranulation of RBL cells, for valency has been a focus for studies investigating cell signalling and FcεR function for many years [17, 18]. Typically these studies have utilised dimeric, trimeric and oligomeric IgE at

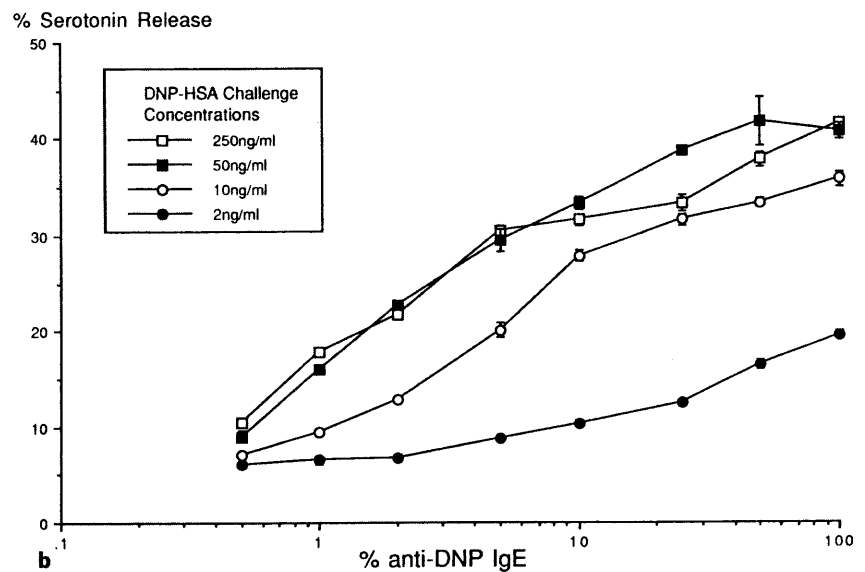
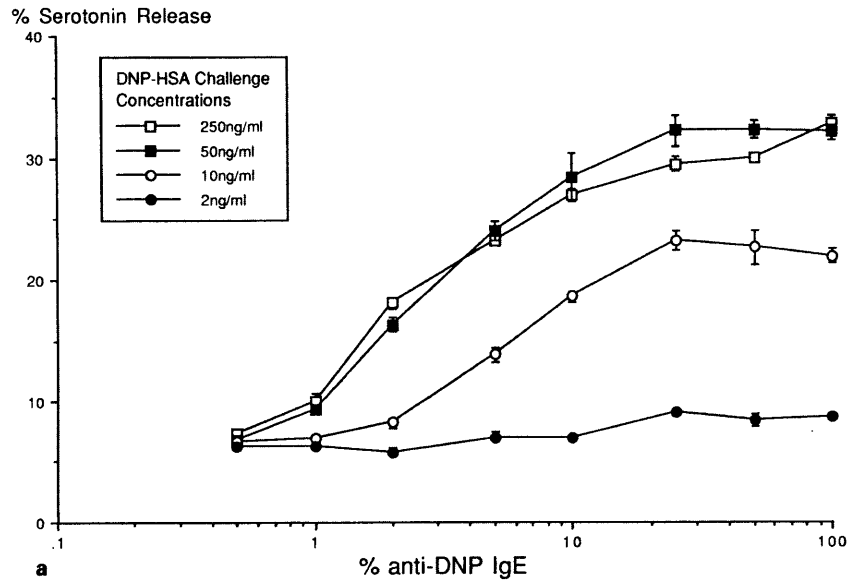


Fig. 4. ^3H -serotonin release from RBL cells sensitised with various percentages of SPE-7 IgE anti-DNP antibodies (**a**), and H1- ϵ -DNP anti-DNP IgE antibodies (**b**). Cells were later challenged with concentrations of DNP₃₂-HSA ranging from 2 to 250 ng/ml. Data shown are means of four-fold replicates. Error bars are not indicated when graphically too small.

high concentrations to initiate signalling [18, 19], and although they have demonstrated degranulation using dimeric IgE, these results do not contradict our own. We have shown that dimeric OVA is a poor stimulator of RBL degranulation, but the mean *effective* valency of these aggregates, by virtue of their size and random orientation, is certainly less than two. A study using DNP-HSA, IgE anti-DNP and rat peritoneal mast cells demonstrated that increasing the valency of challenge antigen beyond seven did not lead to further increases in cell sensitivity [20]. A similar upper limit to the amplification of sensitivity was seen in our experi-

ments with aggregated OVA, where maximum sensitivity was also seen with antigen having a valency of seven.

There are difficulties relating our measured threshold percentages to normal *in vivo* mast cell function, for uncertainty must surround the level of degranulation indicative of physiologically relevant mast cell function. In our studies, degranulation at the threshold percentage was barely above background, while the explosive potential of mast cell degranulation encourages the belief that normal mast cell function must include the possibility of substantial degranulation. Uncertainty also surrounds the antigen concen-

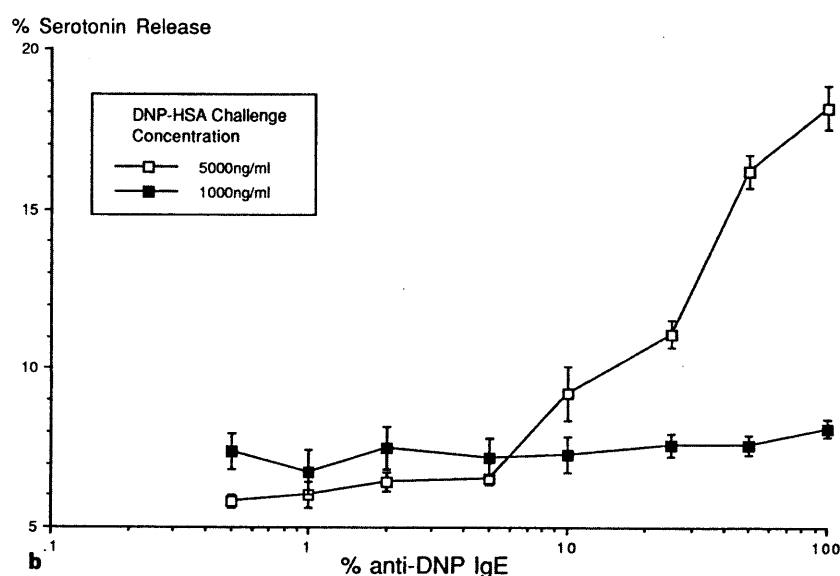
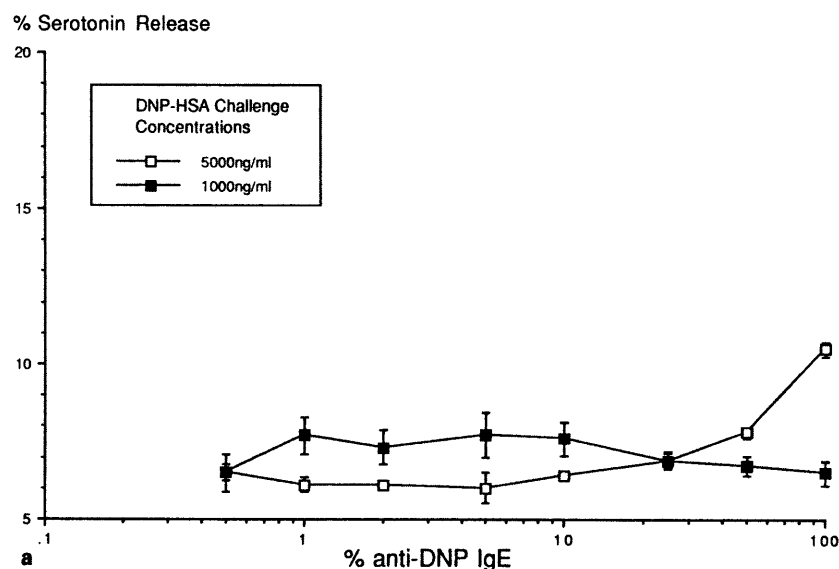


Fig. 5. ^3H -serotonin release from RBL cells sensitised with percentages of SPE-7 (**a**) or H1-e-DNP IgE anti-DNP antibodies (**b**) ranging from 0.5 to 100%. Cells were later challenged with DNP₄-HSA at concentrations of 5,000 and 1,000 ng/ml. Data shown are means of four-fold replicates. Error bars are not indicated when graphically too small.

trations to which 'sensitive' mast cells must be able to respond. Many in vitro studies of mast cell function have challenged sensitised cells with antigen at concentrations in the 1–100 $\mu\text{g}/\text{ml}$ range [e.g. 15]. Although in these circumstances mast cell degranulation may occur, the concentration of antigen to which mast cells are exposed in vivo is likely to be considerably lower, for it has been estimated that the *annual* human exposure to important aeroallergens is of the order of 1 μg [21].

We believe the study of antigen valency, IgE antibody affinity, and the avidity of antigen/antibody binding at cell

surfaces may provide important insights into the phenomenon of allergic cross-reactivity. Our results suggests that if relatively low-affinity antibodies on the surface of a mast cell are directed against sufficient determinants on an allergen, they may together function as high avidity receptors for that allergen. The mechanism we propose for the increased avidity of cell-bound as opposed to free antibody parallels that recognised to operate in the case of IgM antibodies. Despite the generally low affinity of IgM antibodies, they represent an effective first line of defence because their avidity is enhanced manyfold by the pentameric form

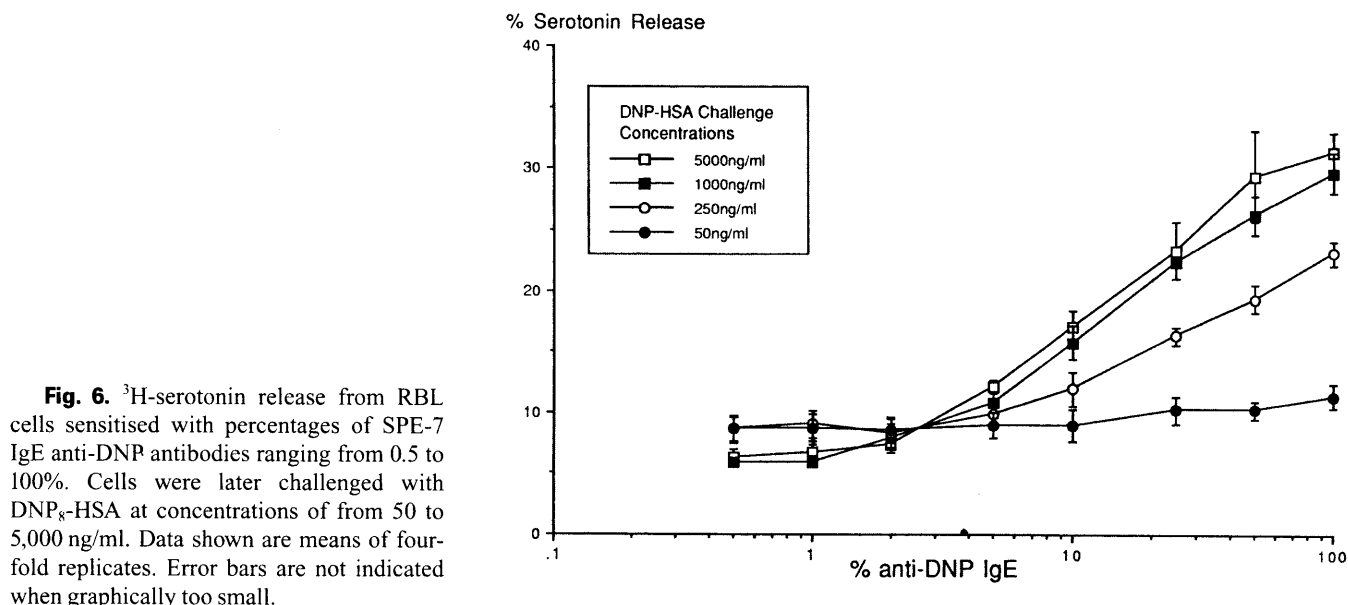


Fig. 6. ^3H -serotonin release from RBL cells sensitised with percentages of SPE-7 IgE anti-DNP antibodies ranging from 0.5 to 100%. Cells were later challenged with DNP₈-HSA at concentrations of from 50 to 5,000 ng/ml. Data shown are means of four-fold replicates. Error bars are not indicated when graphically too small.

Table 2. The minimum (threshold) percentage of H1- ϵ -DNP sensitising IgE necessary for degranulation of RBL cells upon exposure to various concentrations of DNP-HSA of mean valency 4 and 32

Estimated valency	Antigen concentration ng/ml	Threshold % (H1- ϵ -DNP)	Degranulation %	Background degranulation
4	5,000	10	9.0 $\pm$ 1.0	6.3 $\pm$ 0.4
	1,000	—	ND	
	250	—	ND	
	50	—	ND	
32	250	0.5	10.5 $\pm$ 0.3	7.2 $\pm$ 0.2
	50	0.5	9.0 $\pm$ 0.6	6.6 $\pm$ 0.2
	10	1	9.4 $\pm$ 0.3	7.2 $\pm$ 0.3
	2	5	8.8 $\pm$ 0.2	6.8 $\pm$ 0.3

Results show mean percentages of degranulation, and standard errors of the means. ND = Not detected above background levels.

of the IgM molecule. The relationship between antibody valency and antibody avidity is well demonstrated by a recent report that hexameric IgM has an avidity that is 20-fold higher than its corresponding pentameric form [22]. While IgM attains its high avidity by self-aggregation, IgE antibodies may acquire a functionally equivalent 'high avidity' by their association on the surface of mast cells.

Although the ultimate cause of allergic disease is undoubtedly multifactorial, our work leads us to hypothesise that the immune response of allergic individuals could be

Table 3. The minimum (threshold) percentage of SPE-7 sensitising IgE necessary for degranulation of RBL cells upon exposure to various concentrations of DNP-HSA of mean valency 4, 8 and 32

Estimated valency	Antigen concentration ng/ml	Threshold % (SPE-7)	Degranulation %	Background degranulation
4	5,000	50	7.8 $\pm$ 0.2	6.3 $\pm$ 0.4
	1,000	—	ND	
	250	—	ND	
	50	—	ND	
8	5,000	5	12.1 $\pm$ 0.2	6.7 $\pm$ 0.3
	1,000	5	10.7 $\pm$ 0.5	6.7 $\pm$ 0.4
	250	5	9.9 $\pm$ 0.6	7.3 $\pm$ 0.3
	50	25	10.2 $\pm$ 0.6	6.7 $\pm$ 0.7
32	250	1	10.0 $\pm$ 0.7	7.2 $\pm$ 0.2
	50	1	9.3 $\pm$ 0.2	6.6 $\pm$ 0.2
	10	5	13.8 $\pm$ 0.5	7.2 $\pm$ 0.3
	2	25	9.0 $\pm$ 0.1	6.8 $\pm$ 0.3

Results show mean percentages of degranulation, and standard errors of the means. ND = Not detected above background levels.

characterised by responsiveness towards a higher number of allergen epitopes than is seen in non-allergics. A high-valency response would not only account for the exquisite sensitivity of mast cells for specific antigen in allergic individuals. The enhancement of antibody avidity to multivalent antigen might also allow binding to a multitude of relat-

ed antigens, and so promote the phenomenon of allergic cross-reactivity. Investigations are now underway in our laboratory to determine the number of epitopes on common environmental allergens against which allergic and non-allergic individuals respond.

Acknowledgements

This study was supported by a grant from the National Health and Medical Research Council.

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