Human basophil degranulation triggered by very dilute antiserum against IgE

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When, human polymorphonuclear basophils, a type of white blood cell with antibodies of the immunoglobulin E (IgE) type on its surface, are exposed to anti-IgE antibodies, they release histamine from their intracellular granules and change their staining properties. The latter can be demonstrated at dilutions of anti-lgE that range from 1 x 10^{12} *to 1* x 10^{120}*; over that range, there are successive peaks of degranulation from 40 to 60% of the basophils, despite the calculated absence of any anti-lgE molecules at the highest dilutions. Since dilutions need to be accompanied by vigorous shaking for the effects to be observed, transmission of the biological information could be related to the molecular organization of water.*

THE antibodies responsible for human immediate hypersensitivity belong to the IgE isotype[1]. The most salient feature of IgE is its capacity to bind to mast cell and polymorphonuclear basophil membranes through receptors with high affinity". Human basophils are specifically challenged by immunological stimuli such as allergens or anti-lgE antiserum that can bridge IgE molecules in membrane'. This process triggers transmembrane and intracellular signals followed by granule exocytosis with the release of histamine and loss of metachromatic staining of basophil granules by a basic dye such as toluidine blue. Optical basophil degranulation is well correlated with other *in vitro* and *in vivo* procedures for the diagnosis of allergy[4-7].

In preliminary experiments, degranulation of human basophils contained in leukocyte suspensions was induced not only by the usual concentration of anti-lgE antibody (1 x 10^{3} dilution of anti-lgE antiserum, corresponding to 2.2 x 10^{-9} M anti-lgE antibody in the assay), but also by very low concentrations of this antibody (2.2 x $10^{-16/18}$ M), where the number of IgG anti-lgE; molecules in the assay is supposedly too low to trigger the process. We then further explored this phenomenon.

Serial tenfold dilutions of goat anti-human IgE (Fc) antiserum (1 mg specific antibody per ml) were prepared in HEPES-buffered Tyrode's solution containing human serum albumin (USA) down to 1 x 10^{60} dilution, corresponding to a 2.2 x 10^{-66} M theoretical concentration (th) in the assay (see Fig. 1 legend for methods). The expected basophil degranulation, which was assessed by counting cells with metachromatical properties, was observed after exposure of leukocyte preparations to low antiserum dilutions with a maximum at ~ 1 x 10^{3} dilution. Successive peaks of degranulation varying between 40 and 60% were then found down to 1 x 10^{60} dilution, with periods of 6 to 9 tenfold dilutions (Fig. 1a). In other experiments, the antiserum was serially diluted a hundred-fold down to 1 x 10^{120} (to give 2.2 x 10^{-126} M th in the assay) and similar results were obtained (Fig. 16). Degranulation induced by high dilutions of anti-lgE antiserum was observed in ten experiments on the full range of dilutions down to 1 x 10^{60}, when at least 70 similar results were obtained at one or the other part of the high dilution scale in the participating laboratories (Toronto, preliminary results). As controls, goat antihuman IgG (Fc) antiserum (Fig. 1*b*, n = 4) or Tyrode's solution containing HSA (n = 5) were diluted down to 1 x 10^{120} and 1 x 10^{30}, respectively. Cells incubated in conditions identical to those with anti-lgE antiserum gave no significant degranulation. The repetitive waves of anti-IgE-induced degranulation were reproducible, but the peaks of degranulation could shift by one or two dilutions with every fresh sequential dilution of anti-lgE and depended on the blood sample. The waves of basophil degranulation were also seen with substances other than anti-lgE anti-serum at high and low dilutions, such as monoclonal anti-human IgE antibodies, specific antigen in allergic patients or in peroxidase-immunized rabbits, phospholipase A2 from bee venom or porcine pancreas, the Na^{+} ionophore monensin (up to 90% degranulation at 1 x 10^{-30} M th) and the Ca^{2+} ionophores A23187 and monomycin (1 x 10^{-38} M th). The specificity of the observed effects at high dilutions (already noted when comparing antiserum against IgE with antiserum against IgG) was further strikingly illustrated in the ionophore experiments, because removing the corresponding ion from the cellular environment blunted basophil

Table 1 Basophil counts after exposure to anti-IgE antiserum at low and high dilutions

Samples	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Tyrode's-HSA*	81.3±1.2†	89.0±3.1	81.7±2.2	106.7±1.8
Tyrode's-HSA	81.6±1.4	87.7±1.4	83.0±1.0	105.0±1.2
Tyrode's-HSA	80.0±1.5	88.0±2.3	81.7±1.8	105.7±0.9
aIgE 1 × 10^{31}*	35.5±1.8 (56)‡	42.3±4.8 (53)	27.7±0.7 (66)	40.0±1.5 (62)
aIgE 2 × 10^{32}	77.6±0.8 (4)	87.3±1.2 (3)	66.3±2.3 (18)	93.7±1.9 (12)
aIgE 1 × 10^{33}	76.0±1.1 (6)	88.7±1.8 (1)	77.7±1.8 (4)	74.7±2.8 (30)
aIgE 1 × 10^{34}	53.6±1.4 (33)	52.7±1.4 (41)	38.0±0.6 (53)	48.3±2.4 (55)
aIgE 1 × 10^{35}	45.0±0.5 (44)	35.0±1.0 (61)	41.3±1.8 (49)	49.3±1.2 (54)
aIgE 1 × 10^{36}	49.0±1.7 (40)	50.3±0.7 (44)	55.0±2.1 (32)	74.3±2.3 (31)
aIgE 1 × 10^{37}	79.0±2.3 (2)	85.3±0.7 (5)	73.3±1.7 (10)	105.3±0.7 (0)

Blind experiments: test tubes were randomly coded twice by two independent pairs of observers and assayed. The codes were simultaneously broken at the end of all experiments. Dilutions of anti-lgE antiserum were performed as described in legend to Fig. 1.
* Uncpded additional tubes for negative (Tyrode's-HSA) or positive (aIgE 1 x 10 ~*) controls, t Data represent the mean 1 s.e. of basophil number actually counted in triplicate (see legend to Fig. 1 for methods). % Number in parenthesis indicates percentage degranulation compared with Tyrode's-HSA.