

Flow-Cytometric Analysis of Human Basophil Degranulation

I. Quantification of Human Basophils and Their Degranulation by Flow-Cytometry

TAKEMASA NAKAGAWA, BEDA M. STADLER and ALAIN L. DE WECK

Institute of Clinical Immunology, University of Bern, Bern, Switzerland

Quantification of human basophils and their degranulation were performed using a flow-cytometric system. It was shown that the number of basophils among total leukocytes, before and after the effect of degranulating agents, can be obtained rapidly and reproducibly, following alcian blue staining. Dose-response curves of degranulation by anti-IgE and anti-IgG₄ were studied, and it was shown that these antibodies can induce basophil degranulation in a Ca⁺⁺ dependent manner. It was also shown that degranulation is completed within 10 min.

Values obtained by flow-cytometry and microscopic evaluation, as well as comparison made between percent basophil degranulation and percent histamine release, agreed well with each other in our system. Flow-cytometric analysis of human basophil degranulation provides a new reliable method to assess *in vitro* the morphological basis of immediate hypersensitivity.

Key words: basophil degranulation; flow-cytometry; histamine release; immediate hypersensitivity; IgE; IgG₄

Accepted for publication 3 July 1980

It has been well established that degranulation of basophils and/or tissue mast cells, followed by release of mediators, upon the interactions between cell-bound IgE and a specific antigen, plays an important role in inducing symptoms of immediate hypersensitivity (9-11). From this point of view, efforts have accumulated to determine the degree of alteration of the numbers of basophils after challenging with antigens and anti-IgE by microscopic observation (2, 7, 14), but it may be fair to say that microscopic quantification of basophils is rarely employed for clinical use, due to the difficulties in obtaining reliable values (5). Instead, the fluorometric determination of histamine release from human leukocytes has been utilized as the major *in vitro* assessment of immediate hypersensitivity (12, 15, 16).

Flow-cytometric analysis of cells, which has been developed recently, enables handling large numbers of cells in a rapid, accurate and reproducible manner (1, 8, 22). And we have shown in a previous study that flow-cytometry is also applicable to quantify rat mast cells and their degranulation, following metachromatic staining. This paper also showed that flow-cytometry can be substituted for microscopic evaluation, since values obtained by flow-cytometry and microscopic observation yielded good agreement with each other (17). Consequently, the present investigation was carried out using a flow-cytometric system to quantify human basophils and their response to anti-IgE as the degranulating agent.

In addition, since recent data indicate the presence of Fc receptors for human IgG on baso-

phils (13) and the possibility that histamine is to be liberated from leukocytes upon exposure not only to anti-IgG (11, 15) or Protein A, which combines with the Fc part of IgG (20), but also to antibodies against the IgG₄ subclass (27), experiments were undertaken to ascertain whether anti-IgG₄ has an effect on basophil degranulation. The relationship between basophil degranulation and histamine release from leukocytes was also studied.

MATERIAL AND METHODS

Instrumentation

The flow-cytometric technique described in this paper has been performed on a cytograf Model 6300A, manufactured by Bio/Physics Systems Inc., Mahopac, N.Y. The display oscilloscope used was a Type 603, manufactured by Tektronix Inc., Portland, OR. Measurement of histamine was performed using Technicon Auto-analyzer II, Technicon Instruments Co., Tarrytown, N.Y.

Material

The Tris buffer contained 0.025 M Tris, 0.12 M NaCl, 0.005 M KCl, and 0.03% human serum albumin (Fluka AG, Buchs SG, Switzerland), adjusted to pH 7.6, and was designated Tris-A; Tris-ACM was Tris-A supplemented with 0.6 mM CaCl₂ and 1 mM MgCl₂; Tris-EDTA was Tris-A containing 0.01 M EDTA. The Tris-NH₄Cl buffer contained 0.015 M Tris and 0.747% NH₄Cl. Goat anti-human IgE, Fc specific, was purchased from Nordic Immunological Laboratories, Tilburg, The Netherlands. Sheep anti-human IgG₄ (gamma globulin fraction) and purified sheep and swine anti-human IgG₄ (purified by adsorption on a IgG₄ immunosorbent, followed by elution through Sephadex G-200 column to remove eventual complexes and aggregates) were kindly donated by Dr. F. Skvaril, Bern, Switzerland, and Dr. D. C. Heiner, Torrance, CA.

Specificity of the anti-IgG₄ preparations was demonstrated by radioimmunoassays, with and without the addition of 100 ng of purified mye-

loma proteins to the system. Only IgG₄ inhibited the binding of ¹²⁵I-anti-IgG₄ to paper disc coated with IgG₄. IgG₁, IgG₂, IgG₃, IgA₁, IgA₂, IgM, IgD and IgE had no effect (6). Specificity for IgG₄ and absence of cross-reacting with the remaining three subclasses of IgG was also demonstrated by double immunodiffusion (25) and passive sensitization experiments using IgG₂ and IgG₄ as described in detail elsewhere (18). Clinical dextran (approximate mol. wt.: 234,000) and alcian blue were purchased from Sigma Chemical Co., St. Louis, Mo., and Fluka AG, Buchs AG, Switzerland, respectively.

Preparation of cells

For each experiment, 10 ml of venous blood was drawn from allergic individuals who had seasonal rhinitis, insect allergy or drug hypersensitivity. In some experiments, blood from normal donors was also examined. After collecting the blood in 5-ml siliconized plastic tubes containing 25 units heparin and 0.1 ml of 0.2 M EDTA (Laboratoire des Stallergènes, Paris, France), blood was transferred to another tube, mixed with 5 ml of a 6% clinical dextran in isotonic saline, and was allowed to sediment at room temperature for 45–60 min. The plasma supernatant containing leukocytes was centrifuged at 900 r.p.m. (110 g) for 8 min at 4°C. The cells at the bottom of the tube were then resuspended in Tris-NH₄Cl buffer for complete erythrolysis. After washing once with Tris-A, the cells were resuspended in 6–7 ml of Tris-ACM in a concentration of approximately 5×10^6 leukocytes/ml.

In some experiments designed to assess the reliability of flow-cytometric counting of basophils, basophils were further enriched by differential centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden, and Winthrop Laboratories, New York, N. Y., respectively: density of the mixture was 1.077) layer according to the method described by Ishizaka et al. (11). Basophil-enriched leukocyte populations contained 2–3% basophils (Fig. 2).

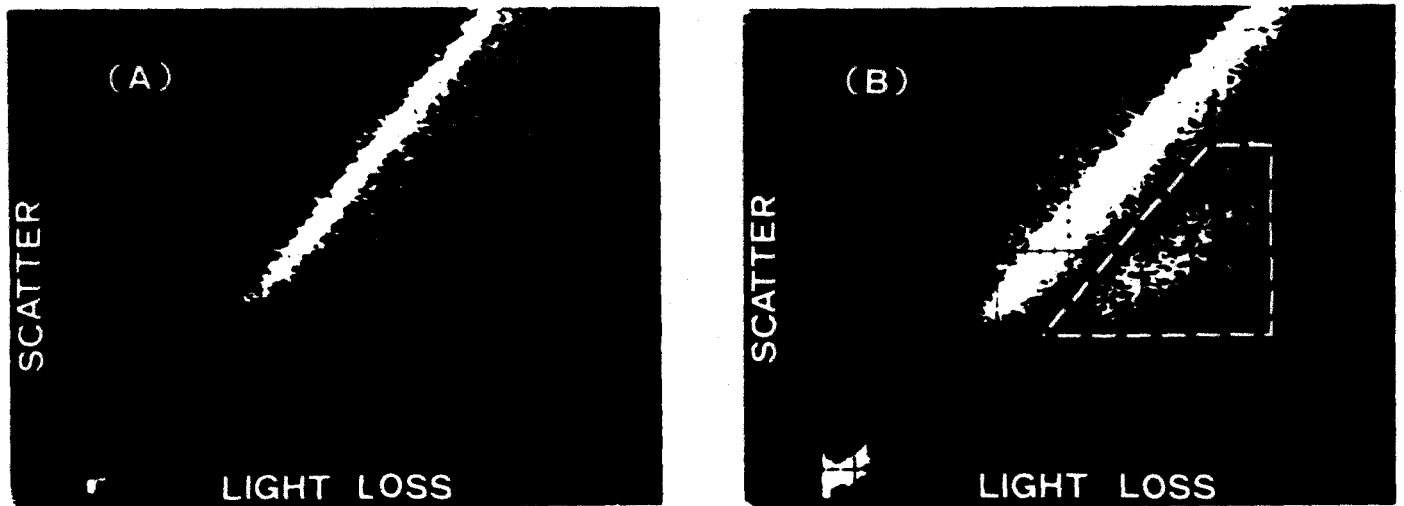


Fig. 1. Differential counting of basophils. Pictures are taken from the cathode ray tube of a cell sample without (A) or with (B) alcian blue staining. Each dot represents one cell characterized by two measurements: light scatter and axial light loss. Cells falling in the selected 'electronic window', outlined by white lines, appear after alcian blue staining only and are presumed to be basophils.

Degranulation of basophils

Degranulation of basophils was carried out in a volume of 0.5 ml, consisting of 0.45 ml cell suspension and 0.05 ml of an appropriate dilution of degranulating agents in Tris-ACM, or of 0.05 ml buffer alone as control. Unless specified, the cells were incubated at 37°C for 20–30 min. The cells were then washed twice with Tris-EDTA, resuspended in 0.2 ml of the same buffer, and kept on ice until counting. Basophil counts remained stable at least for 6 h under these conditions. In the kinetic experiments, degranulation was terminated at the various time points by putting on ice, followed by adding 0.5 ml Tris-EDTA.

Histamine release

In some experiments, histamine measurement was simultaneously performed. After incubation with degranulating agents at 37°C for 45–60 min, the supernatants were obtained by centrifugation at 1,600 r.p.m. (450g) for 15 min at 4°C. The amount of histamine released into the supernatant was measured by automated fluorometric histamine analysis as described by Siraganian (24). The spontaneous histamine release was approx. 10%, and these values were subtracted from the total histamine, when percent histamine release was calculated.

Staining and counting of basophils

Basophils were stained by adding 0.9 ml of 0.15% alcian blue solution, pH 3.5 (composed of 39 ml saline, 1 ml glacial acetic acid, 10 ml 40% formaldehyde and 50 ml ethanol) to 0.1 ml of the cell suspension. The staining solution was filtered through millipore filter (pore size, 0.22 μ) before use.

After a 10-min incubation at room temperature, the samples were applied to the cytograf which automatically yielded the percentage of stained cells among the total cell population by electronic discrimination. The principles of flow-cytometry are described elsewhere (1, 8, 22). In brief, cells in liquid suspension are carried in single file through a glass capillary where they interact with a focused laser beam. Information derived from these interactions (light scatter and axial light loss) are then electronically displayed as a scatter diagram on a cathode ray tube. The 'electronic window' used for counting basophils was set to fill the space indicated in Fig. 1. For each determination, usually triplicate countings of 100,000 leukocytes were performed. One ml sample with a normal basophil concentration (approximately 2×10^3 /sample) enabled to count triplicates of percent basophils per 100,000 cells as well as the total number of cells per 0.1 ml volume. The coefficient of variation calculated for each determination was

usually less than 7.5%. Reproducibility of the duplicate or triplicate determinations on the same cell sample was determined to be $\pm 1\text{--}5\%$, when mean values were compared. The time needed to run one sample in triplicate is about 4 min. Including previous manipulations and leukocyte preparations in the described form, approximately 50–60 samples can be handled on 1 day by a single person. In experiments dealing with basophil degranulation, the percent values of degranulation were calculated according to the formula:

$$\% \times \frac{\text{Basophil (\%) control} - \text{Basophil (\%) test}}{\text{Basophil (\%) control}} \times 100$$

Throughout the experiments described here, spontaneous degranulation, measured in samples without degranulating agents and serum, was occasionally as high as 10%. Throughout the whole procedure, including preparation and handling of cells, plastic materials were utilized.

For comparison, microscopic quantification of the percent values of basophils among total leukocytes, following alcian blue staining, was also performed on 40 samples with a 40×10 magnification. More than 1,000 leukocytes were counted for each determination. Reproducibility of microscopic counting by the same observer on the same cell preparation was determined to be $\pm 20\%$.

Statistical analysis

The correlation coefficient, coefficient of variation, and the linear regression line were calculated by routine methods.

RESULTS

In flow-cytometric analysis of human basophils, our first purpose was to quantify the percentage of basophils among total leukocytes. After staining with alcian blue, the cell suspensions were applied to the cytograf. As shown in Fig. 1, the cytogram clearly demonstrated a demarcation of stained cells from other leukocytes, with the percentage of stained cells obtained simulta-

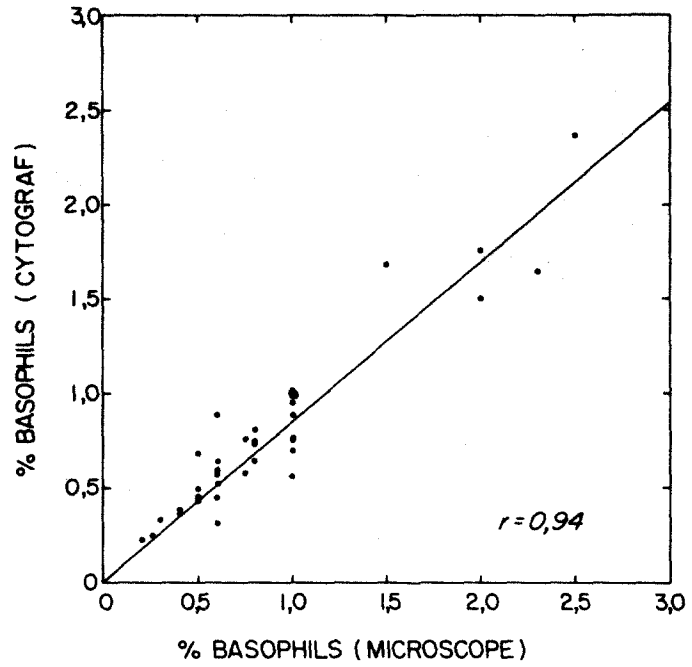


Fig. 2. Correlation between flow-cytometric and microscopic quantifications of basophil counting. Forty samples of washed leukocytes were evaluated. The linear regression line was calculated by the least square method ($Y = mX + b$) where the slope of the line was $m = 0.817$ and the Y-axis intercept $b = 0.088$. The linear correlation coefficient was $r = 0.94$.

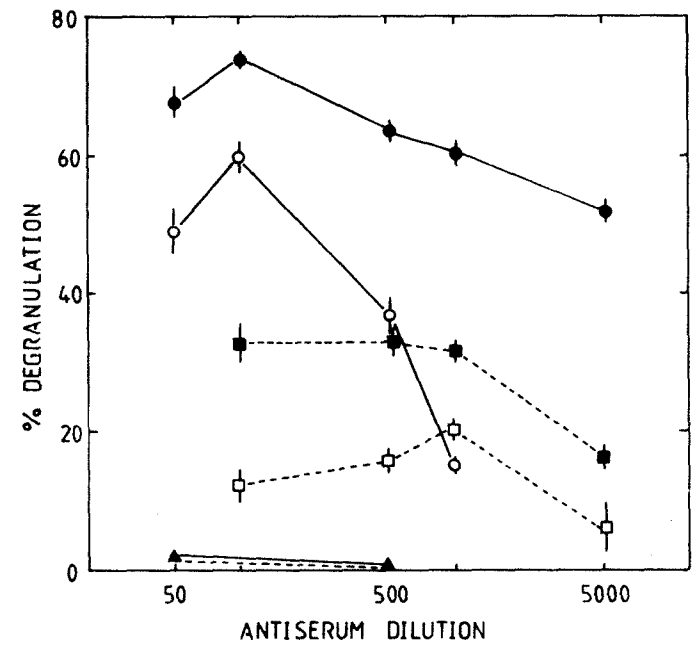


Fig. 3. Dose-response curves of basophil degranulation induced by anti-IgE (●, ■), anti-IgG4 (○, □), and by normal sheep serum (Δ). Washed leukocytes obtained from two donors (—, ----) were studied. Anti-IgE and anti-IgG4 were diluted with Tris-ACM, and 0.05 ml of these agents was added to 0.45 ml of the leukocyte suspension, followed by incubation at 37°C for 20 min. Final concentration of antibodies is indicated in the figure. Each symbol represents mean of percent degranulation $\pm$ s.d. calculated from triplicate counting of 100,000 leukocytes.

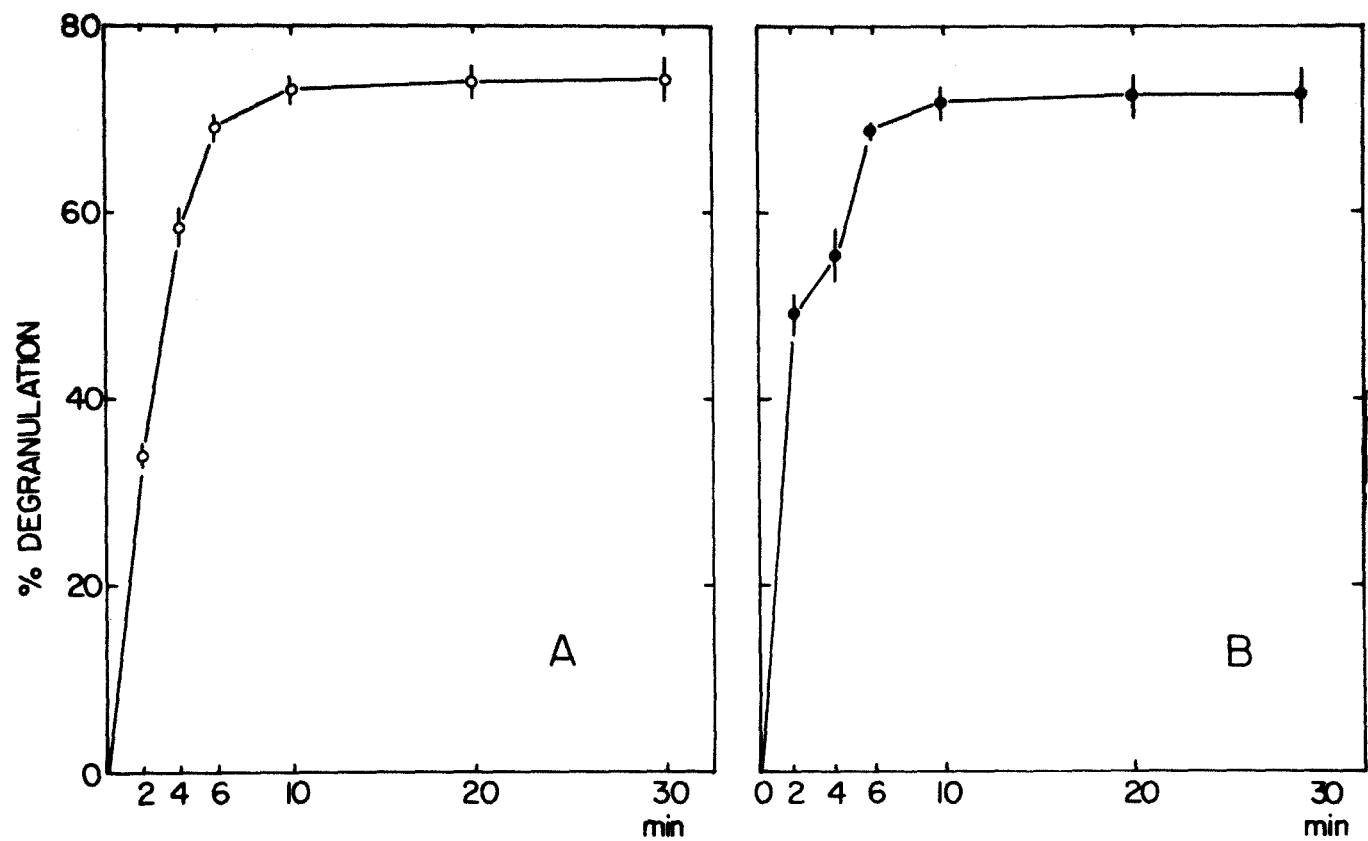


Fig. 4. Time-course of basophil degranulation induced by anti-IgE (A) and by anti-IgG4 (B). Leukocytes from the same donor were challenged by either anti-IgE or anti-IgG4. Final concentrations of the agents were 1:100 in each experiment. Each symbol represents mean of percent degranulation $\pm$ s.d., calculated from triplicate countings of 100,000 leukocytes.

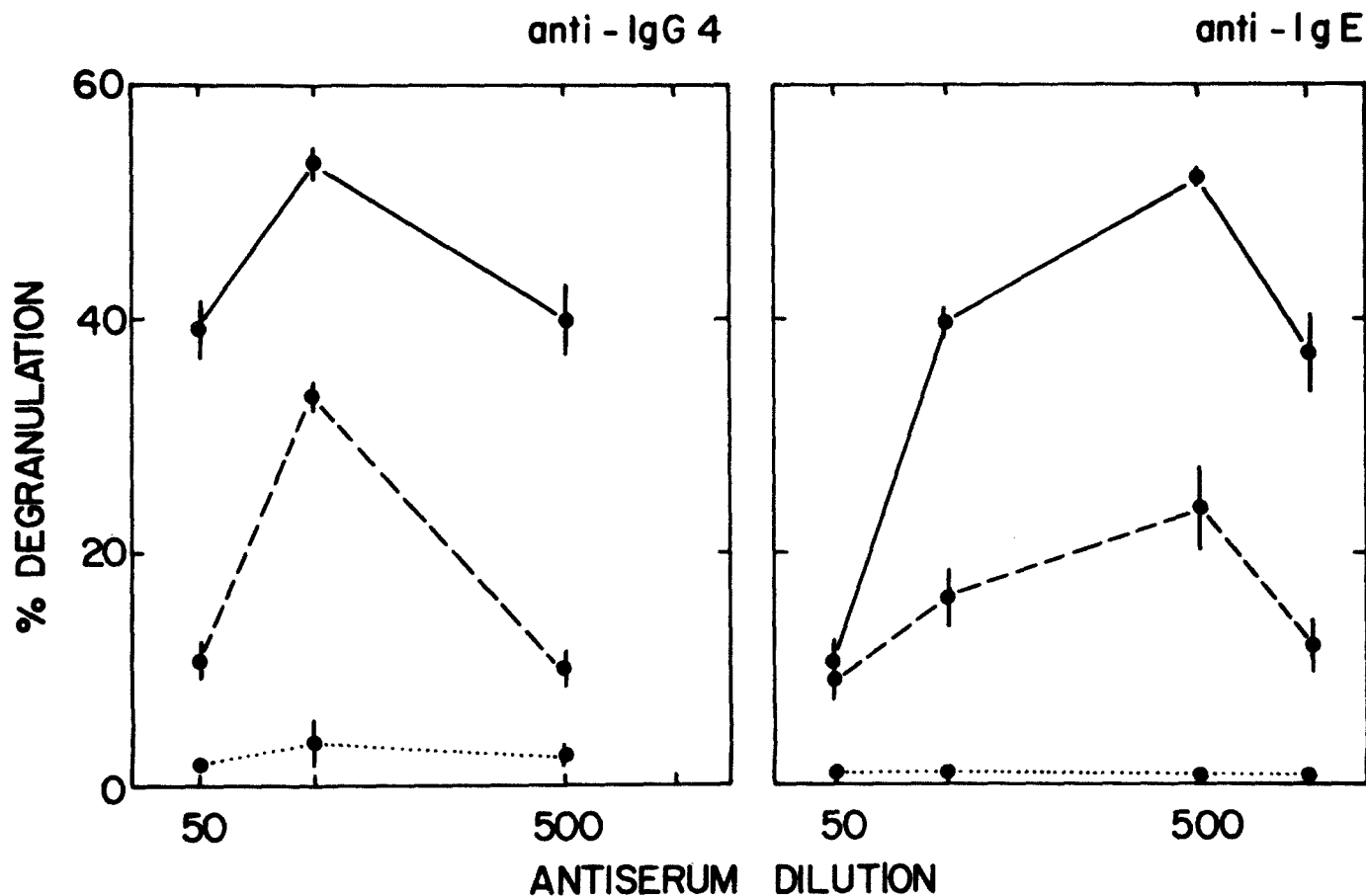


Fig. 5. Influence of Ca^{++} ions on basophil degranulation. Dose-response curves of degranulation induced by anti-IgE (right panel) and by anti-IgG4 (left panel), in buffers containing 0.6 m M Ca^{++} (—), 0.06 m M Ca^{++} (----) or 0.06 m M Ca^{++} + 0.01 M EDTA (.....) are shown. Leukocytes from the same donor were studied. Each symbol represents mean of percent degranulation $\pm$ s.d., calculated from triplicate countings of 100,000 leukocytes.

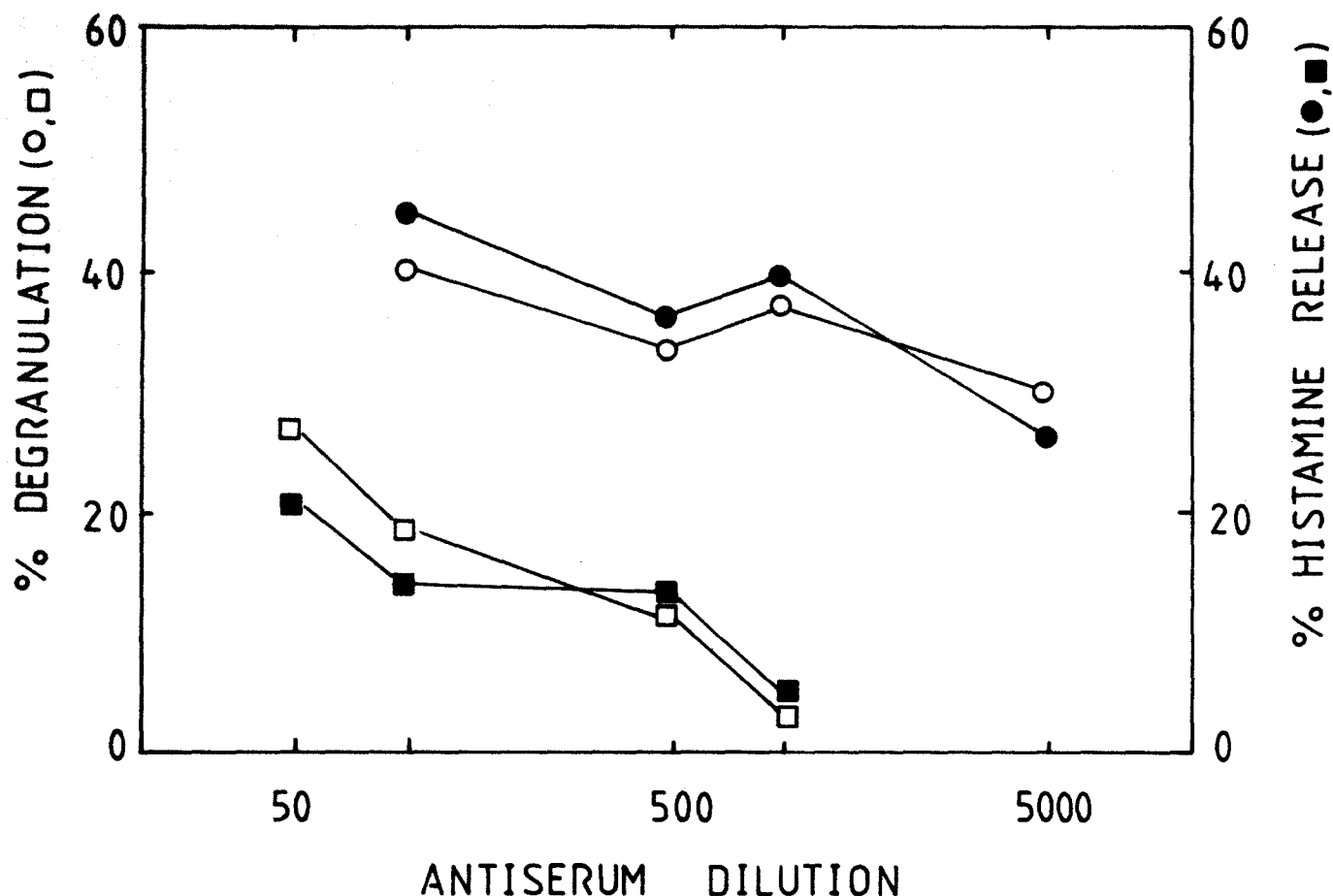


Fig. 6. Dose-response curves of basophil degranulation (open symbols) and histamine release (closed symbols). Leukocytes from the same donor were challenged by either anti-IgE (○, ●) or anti-IgG₄ (□, ■). After incubation at 37°C for 60 min, supernatants were analyzed for histamine content, while the remaining cells were studied for basophil counting.

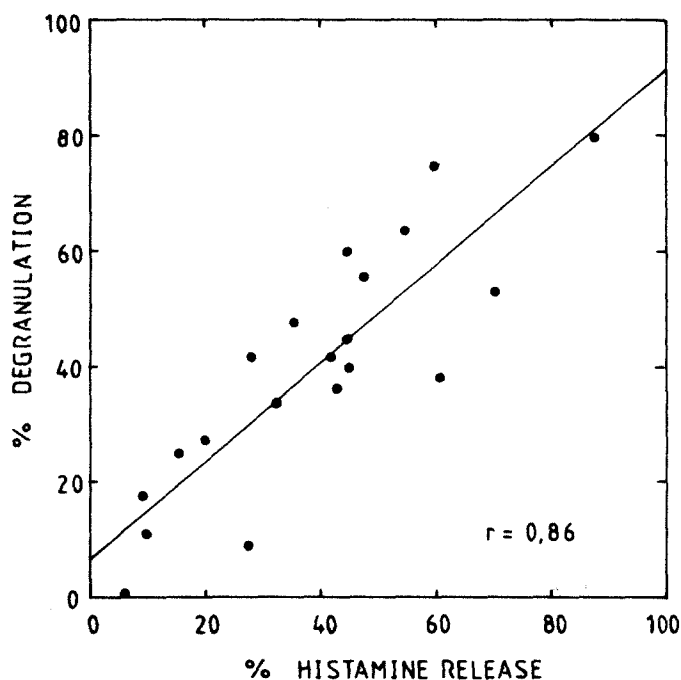


Fig. 7. Correlation between maximum percent basophil degranulation and maximum percent histamine release. Values were obtained from 10 experiments involving 20 determinations. The linear regression line was calculated by the least square method ($Y = mX + b$) where the slope of the line was $m = 0.840$ and the Y-axis intercept was $b = 7.000$. The linear correlation coefficient was $r = 0.86$.

neously. Experiments carried out to compare flow-cytometric and microscopic quantification of basophil counting showed that these two methods yielded a correlation coefficient of 0.94 (Fig. 2).

In order to assess basophil degranulation by flow-cytometry, dose-response curves of degranulation by anti-IgE and anti-IgG₄ were studied. Representative results of experiments on two donors out of 50 performed are shown in Fig. 3. It is clear from this figure that significant basophil degranulation can be induced by these antibodies against IgE and IgG₄. As described in detail elsewhere (18), there is a marked correlation in most donors in the degree of degranulation achieved by anti-IgE and anti-IgG₄. The effect of purified sheep and swine anti-IgG₄ preparations, devoid of aggregates and possible antigen-antibody complexes by passage through Sephadex G-200 column, was also examined on the same cell preparations; the efficacy of these purified materials was comparable

to that of the crude gammaglobulin fraction. Values for maximum percent degranulation by these purified anti-IgG₄ antibodies were 61.5 and 20.6, corresponding closely to the values obtained with the crude materials in these two experiments. The sample exposed to normal sheep serum, in order to obtain 'background values', showed less than 2.5% degranulation. In addition, dose-response curves also indicate that degranulation decreased when excess amounts of antibodies were added, and that the optimal concentration of antibodies to induce maximum degranulation differed from one donor to another.

Subsequently, experiments were performed on three donors each to study the influence of time and Ca⁺⁺ ions on degranulation. As the representative experiment in Fig. 4 shows, dealing with time course of basophil degranulation, this reaction is almost complete within 10 min, both after induction by anti-IgE (Fig. 4A) and by anti-IgG₄ (Fig. 4B).

The process of degranulation is Ca⁺⁺ dependent (Fig. 5). When suboptimal concentration of Ca⁺⁺ (0.06 mM) was employed in the degranulation process, only a low percent of basophils were degranulated by anti-IgE. The addition of 0.01M EDTA completely inhibited the reaction. Similar findings were also obtained upon exposure to anti-IgG₄.

Finally, experiments were undertaken to determine whether there exists a relationship between basophil degranulation and histamine release. A representative experiment among 10 is shown in Fig. 6. The dose-response curves of percent degranulation paralleled those of percent histamine release. Comparison made between maximum percent basophil degranulation and maximum percent histamine release in these 10 experiments involving 20 determinations showed a linear relationship with a correlation coefficient of 0.86 (Fig. 7).

DISCUSSION

The flow-cytometric technique described in this paper offers several advantages: simplicity in the procedure and the handling of large numbers of cells in a rapid, accurate and reproduc-

cible manner (1, 8, 22). The data presented here confirm that flow-cytometry can be applied to quantify human basophils, following alcian blue staining. Microscopically, it was observed that only basophils are stained a greenish blue color, which is suitable to differentiate stained cells from unstained cells on the cytograf (1), while other leukocytes are not stained at all. Comparison made between flow-cytometric and microscopic countings of basophils showed good agreement with each other (Fig. 2), implying that flow-cytometry can be substituted for microscopic evaluation. These results also indicate that the flow-cytometric procedure does not influence basophil counting. Gilbert et al. (5) also reported that the alcian blue staining method is suitable both for chamber basophil counting and automated sampling and electro-optical detection. They also reported that good agreement was found between these two methods.

As shown here for the first time, flow-cytometry can also be applied to quantify basophil degranulation. Microscopically, 'degranulation' of basophils is observed as a decrease in the total number of basophils. As exposure of basophils to degranulating agents results in prompt and explosive discharge of granules (4, 23), it was reasonable to expect that degranulated basophils would lose their stainability by alcian blue. However, numerous experiments not reported here indicated that the methods previously reported for counting basophils in whole blood by flow-cytometry (5, 22) are not applicable, if degranulation is carried out in whole blood. Paradoxically, apparent increase in electronic counts following the application of degranulating agents is probably due to secondary phenomena such as cell or platelet aggregation or ingestion of granules by accessory cells.

Our data clearly reconfirmed the previous findings by Ishizaka et al. (14) that anti-IgE can degranulate basophils. As is the case in histamine release from leukocytes upon exposure to anti-IgE (12), the dose-response curves of degranulation by anti-IgE demonstrated that excess amounts of antibodies rather suppress the reaction, the optimal concentration for maximum degranulation differs from one case to another (Fig. 3), and that the degranulation pro-

cess is Ca^{++} dependent (Fig. 5). Basophil degranulation is almost completed within 10 min (Fig. 4), which has been also found by microscopy (4).

Moreover, our data supported the evidence that anti-IgG₄ can degranulate basophils in the same manner as anti-IgE. Many investigators have recently identified this IgG₄ subclass as a new kind of reaginic antibody (3, 19, 26, 27). For example, inhibition of reagin-mediated PCA reactions by IgG₄ in monkeys (27) and in baboons (26) and significant histamine release from human leukocytes by anti-IgG₄ (27) were observed. In our experiments, anti-IgG₄ can induce basophil degranulation (Figs. 3–6) and histamine release (Fig. 6) in a dose-dependent manner. Degranulation by anti-IgG₄ is also Ca^{++} dependent, and the supraoptimal concentration of antibodies decreases the reaction. These results also indicate that IgG₄ are bound to basophils, as well as IgE. Furthermore, experiments dealing with passive sensitization of basophils with IgG₄ myeloma protein, followed by exposure to anti-IgG₄, exhibited that IgG₄ can passively sensitize basophils. The fact that preincubation of basophils with IgG₄ myeloma considerably increases degranulation by anti-IgG₄ but not by anti-IgE, aside from the absence of cross-reactivity detectable by radioimmunoassay between the anti-IgG₄ and anti-IgE used, confirms that degranulation observed with anti-IgG₄ is not due to cross reaction with Fab from IgE. These findings, which are reported in detail elsewhere (18), suggest that the human IgG₄ subclass also plays some role in immediate hypersensitivity.

Finally, our experiments indicated that the degree of basophil degranulation correlates well with that of histamine release (Figs. 6, 7). This correlation between human basophil degranulation and histamine release might suggest that there is an 'all-or-none' response in regard to histamine release from the degranulated human basophils. A similar linear correlation between basophil degranulation and histamine release by antigen and ionophore was also reported recently by Pruzansky et al. (21). It should be noted, however, that comparative studies with histamine release showed that the degree of de-

granulation by antibodies determined in our experiments includes the 'spontaneous degranulation', which could be assessed by measuring the spontaneous histamine release. The degree of spontaneous degranulation was approx. 10%. In any event, values obtained by flow-cytometry and microscopic evaluation, and comparison made between percent degranulation and percent histamine release, both yielded good agreement with each other in our system; flow-cytometric analysis of human basophil degranulation provides a new reliable method to assess *in vitro* the morphological basis of immediate hypersensitivity. Investigations applying this method to quantify allergen-induced basophil degranulation in hay fever and bee venom allergic patients are currently under way.

ACKNOWLEDGMENTS

The authors wish to acknowledge Drs. F. Skvaril and D. C. Heiner for their gift of anti-IgG₄ preparations and their helpful discussions; Dr. M. C. Conroy for performance of histamine measurement and her helpful discussions; and Mrs. J. Tilch and Miss M. Kings for their expert technical assistance.

REFERENCES

1. Adams, L. R.: Staining for the cytograf and cytofluorograf. *J. Histochem. Cytochem.* 25, 965–975, 1977.
2. Benveniste, J., Egido, J., Millet, V. G. & Camussi, G.: Detection of immediate hypersensitivity in rabbits by direct basophil degranulation. *J. Allergy Clin. Immunol.* 59, 271–279, 1977.
3. Bruynzeel, P. L. B. & Berrens, L.: IgE and IgG₄ antibodies in specific human allergies. *Int. Arch. Allergy Appl. Immunol.* 58, 344–350, 1979.
4. Dvorak, A. M.: Biology and morphology of basophilic leukocytes. In Bach, M. K. (ed.): *Immediate hypersensitivity. Modern concepts and developments*, pp. 369–405. Marcel Dekker Inc., New York, 1978.
5. Gilbert, H. S. & Ornstein, L.: Basophil counting with a new staining method using alcian blue. *Blood* 46, 279–286, 1975.
6. Heiner, D. C., de Weck, A. L., Skvaril, F., Müller, U. & Underdown, B.: IgG₄ antibody responses to 5 bee venom antigens. In abstracts for 36th annual meeting of the American Academy of Allergy, Abstract No. 124. Atlanta, 1980.
7. Hirsch, S. R. & Zastrow, J. E.: Basophil degranulation: A new method of observation and its correlation with skin testing. *J. Allergy Clin. Immunol.* 50, 338–347, 1972.
8. Horan, P. & Wheelless, L. L., Jr.: Quantitative single cell analysis and sorting. *Science* 198, 149–157, 1977.

9. Ishizaka, K. & Ishizaka, T.: Human reaginic antibodies and immunoglobulin E. *J. Allergy* 42, 330-363, 1968.
10. Ishizaka, K. & Ishizaka, T.: Mechanisms of reaginic hypersensitivity. *Clin. Allergy* 1, 9-24, 1971.
11. Ishizaka, T., DeBernardo, R., Tomioka, H., Lichtenstein, L. M. & Ishizaka, K.: Identification of basophil granulocytes as a site of allergic histamine release. *J. Immunol.* 108, 1000-1008, 1972.
12. Ishizaka, T., Ishizaka, K., Johansson, S. G. O. & Bennich, H.: Histamine release from human leukocytes by anti- α E antibodies. *J. Immunol.* 102, 884-892, 1969.
13. Ishizaka, T., Sterk, A. R. & Ishizaka, K.: Demonstration of Fc γ receptors on human basophil granulocytes. *J. Immunol.* 123, 578-583, 1979.
14. Ishizaka, T., Tomioka, H. & Ishizaka, K.: Degranulation of human basophil leukocytes by anti- γ E antibody. *J. Immunol.* 106, 705-710, 1971.
15. Lichtenstein, L. M., Levy, D. A. & Ishizaka, K.: *In vitro* reversed anaphylaxis: Characteristics of anti-IgE mediated histamine release. *Immunology* 19, 831-842, 1970.
16. Lichtenstein, L. M. & Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena IX. Histamine release from human leukocytes by ragweed pollen antigens. *J. Exp. Med.* 120, 507-530, 1964.
17. Nakagawa, T., Stadler, B. M. & de Weck, A. L.: Determination of rat mast cells by flow-cytometry. *J. Immunol. Methods* 33, 87-92, 1980.
18. Nakagawa, T., Stadler, B. M., Heiner, D. C., Skvaril, F. & de Weck, A. L.: Flow-cytometric analysis of human basophil degranulation II. Degranulation induced by anti-IgE, anti-IgG $_4$, and the calcium ionophore A23187. *Clin. Allergy* (In press).
19. Parish, W. E.: Evidence for human IgG antibodies anaphylactically sensitizing man. In Bach, M. K. (ed.): Immediate hypersensitivity. Modern concepts and developments, pp. 277-299. Marcel Dekker Inc., New York, 1978.
20. Petersson, B. Å. & Stålenheim, G.: Induction of histamine release and desensitization in human leukocytes. Effect of anti-IgE or protein A from *Staphylococcus aureus*. *Scand. J. Immunol.* 4, 103-112, 1975.
21. Pruzansky, J. J., Zeiss, C. R. & Patterson, R.: A linear correlation between histamine release and basophil degranulation using either antigen or ionophore A23187. In abstracts for 36th annual meeting of Americal Academy of Allergy, Abstract No. 25. Atlanta, 1980.
22. Saunders, A. M.: The automation of cytochemical methods for automated cytophotometers. *J. Histochem. Cytochem.* 25, 1001-1012, 1977.
23. Shelly, W. B. & Juhlin, L.: New test for detecting anaphylactic sensitivity: Basophil reaction. *Nature (Lond.)* 191, 1056-1058, 1961.
24. Siraganian, R. P.: Refinements in the automated fluorometric histamine analysis system. *J. Immunol. Methods* 7, 283-290, 1975.
25. Skvaril, F., Roth-Wicky, B. & Barandun, S.: IgG subclasses in human gammaglobulin preparations for intravenous use and their reactivity with *Staphylococcus* protein A. *Vox Sang.* (In press), 1980.
26. Stanworth, D. R. & Smith, A. K.: Inhibition of reagin-mediated PCA reactions in baboons by the human IgG $_4$ sub-class. *Clin. Allergy* 3, 37-41, 1973.
27. Vijay, H. M. & Perelmutter, L.: Inhibition of reagin-mediated PCA reactions in monkeys and histamine release from human leukocytes by human IgG $_4$ subclass. *Int. Arch. Allergy Appl. Immunol.* 53, 78-87, 1977.

Address:

Dr. Alain L. de Weck

Institute of Clinical Immunology Inselspital
CH-3010, Bern
Switzerland

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.