

Manipulations of the allergic inflammation

S. Ahlstedt

Part A:

1. Theoretical background

An allergic reaction is initiated when the allergen hits the mucous membrane.

Allergic inflammatory reactions in the mucous membrane include mucous cells producing mucus transporting away the allergen, mast cells releasing mediators resulting in vasodilation, oedema and muscle constriction, lymphocyte mediating immunity and phagocytes. There are 2 types of mast cells; connective tissue mast cells (CTMC) adjacent to nerve fibres, vessels and mucosal mast cells (MMC) (Fig 1).

Lymph nodes with capacity for cell-mediated as well as humoral immunity are located adjacent to the inflammatory focus in the mucous membrane. To these regional lymph nodes and to the mucous membrane, inflammatory cells like lymphocytes, basophils and eosinophils originating from stem cells in the bone marrow are transported by the blood.

Allergen exposure of the mucous membrane stimulates neurons resulting in release of neuropeptides which act on the mast cells. The mast cell mediators released by this stimulation then open up the tight junctions between the epithelial cells in the membrane. The penetrating allergen triggers macrophages and T lymphocytes. This results in stimulation of T lymphocytes and antibody-forming cells in the regional lymph nodes, which migrate to the mucous membrane together with progenitor cells for mucosal mast cells and eosinophils. T cell immunity also induces progenitor cells to differentiate into mast cells and eosinophils and stimulates some epithelial cells to become mucous cells (Fig 2).

A signal is sent to the bone marrow to increase the production and release of progenitor cells into the blood for transport to lymph nodes and mucous mem-

branes. The cell traffic to the mucous membrane can become extensive (Fig 2).

A state of hyperreactivity to mediators seen in atopy is created by the exposure of the mucous membrane to the allergen, evoking neuropeptide release, cell accumulation and release of mediators (Fig 3). For the patient, the treatment of choice would be to turn off the hyperreactivity and cell traffic into the inflammatory focus and also to decrease the production of inflammatory cells and antibodies in the mucous membrane, regional lymph nodes and bone marrow (Fig 4).

Presently prescribed pharmacological treatment will only block the symptoms, or for some agents, the activity of mediators. There will, however, only be a minor effect on the production of mediators and inflammatory cells. Sometimes even an opposite effect may result after pharmacological treatment, which antagonizes but does not eliminate the inflammation-propagating elements. This may occur in even higher numbers of inflammatory cells which must be controlled by the pharmacological agent.

Regulation of the production of cells, antibodies and mediators can be achieved. The immune system can do this itself after developing a learned response, which could be the mechanism for immunotherapy.

2. What can be measured of the allergic inflammation?

Systems can be established according to the following:

a. Cell cultures

Peripheral blood leukocytes are prepared from the individuals to be tested by Ficoll-Paque separation. After washing, the cells are suspended in the medi-

um at a cell concentration of 2×10^6 cells/ml. The cells are cultured for indicated periods of time at 37°C in 95% humidity and 7% CO_2 atmosphere.

b. Proliferation

For tests of the proliferation of the cells, media such as RPMI 1640 or McCoy's medium 5A of pH 7.4 supplemented with human serum or preferably fetal calf serum and 2 mM glutamine (Flow Labs) and gentamycin are used. The cell density should be between 2×10^5 and 2×10^6 /ml, depending on the type of vial for the cell cultivation; such as u-formed or flat-bottomed wells. For T cell proliferation in culture, the optimal time in most systems is 3-4 days with radiolabelled thymidin added during the last 6-24 h. As stimulatory agents in these cultures, allergen or mitogen at concentrations of 1-30 $\mu\text{g/ml}$ can be used. For optimal B cell proliferation, 6-7 days in culture is sufficient with the radiolabelled thymidin present during the last 6-24 h. The amount of antigen used in these assays is the same as that used for T cell proliferation. The cultures can be harvested automatically in a Titertek cell harvester (Skatron, Libyen, Norway) and the incorporated radiolabelled thymidin measured after addition of (β)-scintillation solutions (1).

c. Regulation

Suppressive systems can be achieved after stimulation of peripheral blood cells for 24-48 h with mitogen, antigen or an inflammation mediator like histamine. When added to cell cultures as those mentioned above, cells or supernatants suppress proliferation and antibody formation (4). *In vitro* systems such as those described, monitor the suppressive mechanisms of the immune system which may be less efficient in atopics than normals; they have been used to follow the effect of immunotherapy (cf. poster IV:B:1 and Dreborg, Chap. III).

d. Antibody formation

When determining antibody formation, the cell culture medium can be analysed after 3-10 days of culture. Phadebas IgE PRIST® and Phadebas RAST® or ELISA can be employed. In these assays, antigen can be fixed on a solid phase, where-

after the antibody is added and the reaction is visualized by radio-labelled or enzyme-labelled antibodies to the antibody. It is important to use highly specific antibodies with specific labelling to get high sensitivity in the test (1,3, and cf. poster IV:B:1).

For determination of the amount of lymphokines in the cell supernatants, the cell culture supernatants are taken off at between 24 and 96 h of cultivation (4).

e. Inflammatory cells, basophils

The lymphokines, active on the maturation of basophils and mast cells, can be determined using other cell culture systems. The cells are then preferably cultivated at 1×10^6 cells/ml in McCoy's 5A medium in 12-well plastic plates for 14 days, each well containing 4 ml cell suspension. The cell maturation is stimulated with conditioned medium containing lymphokines as determined from the cultures mentioned above or from some established cell lines. Optimum amount of cell growth factors can be obtained if the cells used for lymphokine production are stimulated with 1-10 μg of allergen/ml or with some type of mitogen. The cell maturation can then be analysed by taking samples at day 0 day 4, 7, 10 and 14 during the cell cultivation period. An efficient method for staining the mast cells and basophils is liquid staining with Alcian blue, as described by Gilbert and Ornstein (5) (c.f. 4 and poster IV:B:2).

f. Cell growth factors

The lymphokine stimulating the basophils and mast cells can be separated using reversed phase FPLC technique (6). The culture medium with basophil-growth factor (BCGF) or mast cell growth factor (MCGF) is acidified with trifluoroacetic acid (TFA) to pH 2 and directly loaded onto a reversed phase column (PRO-RPC, Pharmacia Fine Chemicals). A water-acetonitrile gradient containing 0.1% TFA is run. The elution of protein is monitored by absorption at 280 nm or 206 nm. Each fraction is lyophilized and assayed for BCGF or MCGF activity by measuring % large basophils or mast cells after 14 days in a liquid peripheral blood leukocyte culture. The BCGF or

MCGF is eluted at a high per cent acetonitrile, whereas most other material is eluted earlier. The purification degree is high and all cell growth stimulating activity is recovered after fractionation (cf. poster IV:B:2).

g. Histamine determinations

The mediator, e.g. histamine, formed by the cultivated cells can be determined employing a fluorimetric assay (7) and a specific enzyme isotope method (8) with detection limits of 1 ng/ml and 100 pg/ml, respectively. The results of these methods show excellent correlation (4). The histamine determination methods can be employed for analysis of the composition and potency of allergic preparations, characterization of the reactivity of allergic patients and in experimental studies of the allergic inflammation (cf. poster IV:B:3).

h. Animal experimental systems

All these events can be studied in animal experimental systems which can be modulated by immunizations and cell and antibody transfer to obtain knowledge about the working mechanisms in an allergic inflammation (cf. poster IV:B:4).

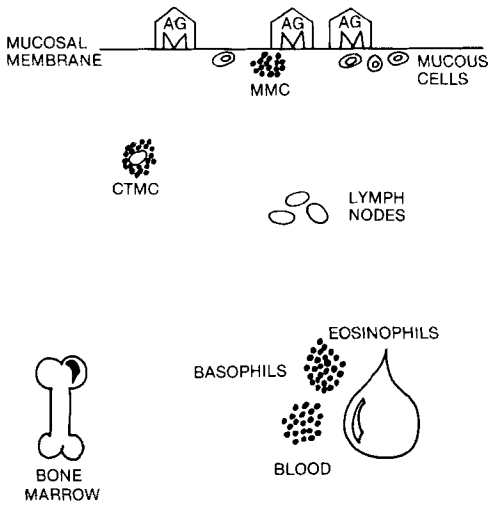
3. Conclusions

The important activities of antibodies, lymphocytes, basophils, mast cells, eosinophils and mucous cells both in mediating and regulating the allergic inflammation can be analysed *in vitro*. This may result in a more efficient diagnosis, therapy and therapy monitoring.

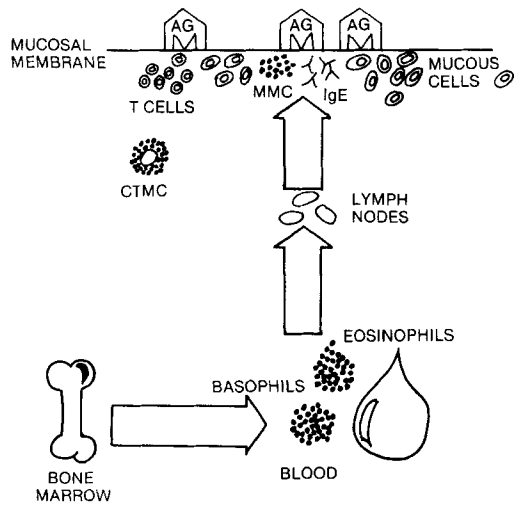
REFERENCES

1. Ahlstedt, S., Smedegård, G., Nygren, H. and Björkstén, B.: Immune responses in rats immunized with aerosolized antigen. Antibody formation, lymphoblastic responses and mast cell and mucous cell development related to bronchial reactivity. *Int. Archs Allergy Appl. Immun.* 72, 71, (1983).
2. Garovoy, M.R., Reddish, M.A. and Rocklin, R.E.: Histamine-induced suppressor factor (HSF): Inhibition of helper T cell generation and function. *J. Immunol.* 130, 357, (1983).
3. Ahlstedt, S., Carlsson, B., Hanson, L.Å., Kaijser, B., Mattsby-Baltzer, I. and Sohl-Åkerlund, A.: Application of the ELISA for determination of immunoglobulin class-specific antibodies. *Scand. J. Immunol.* 8, 119, (1978).
4. Ahlstedt, S., Hammarström, I., Into-Malmberg, M.B. and Denburg, J.: Appearance *in vitro* of large human histamine-containing basophilic cells. *Int. Archs Allergy appl. Immun.* In press.
5. Gilbert, H.S. and Ornstein, L.: Basophil counting with a new staining method using Alcian Blue. *Blood* 46, 279, (1975).
6. Ahlstedt, S., Hammarström, I., Björkstén, B. and Åkerblom, E.: Antigen-driven production of lymphokines stimulating regeneration of granula and maturation of basophilic cells in peripheral blood. *Scand. J. Immunol.* 20, 167, (1984).
7. Siraganian, R.P. and Hook, W.A.: Histamine release and assay methods for the study of human allergy. *Manual of Clinical Immunology*, p. 808, (1980).
8. Beaven, M.A. and Horáková, Z.: The enzymatic isotope assay of histamine. *Handbook of Experimental Pharmacology*. Vol XVII, Pt. 2, pp. 165-173, (1978).

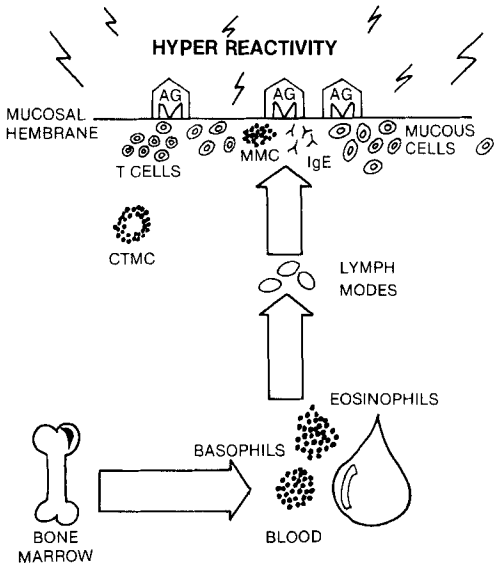
1.



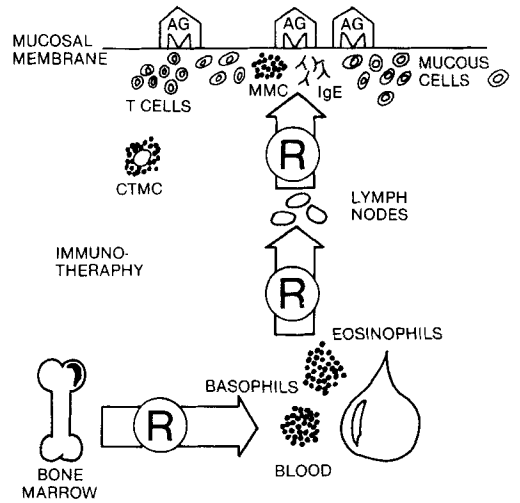
2.



3.



4.



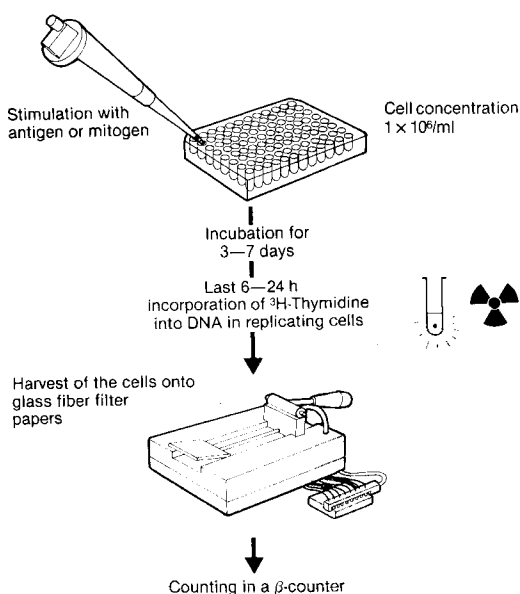
LYMPHOCYTE ACTIVATION IN VITRO

Jenny Borwell and Gunnar Nilsson

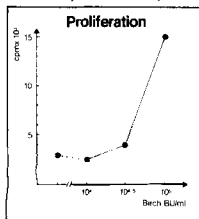
Separation
or Ficoll-

Lymphocytes

Cell Proliferation



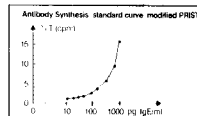
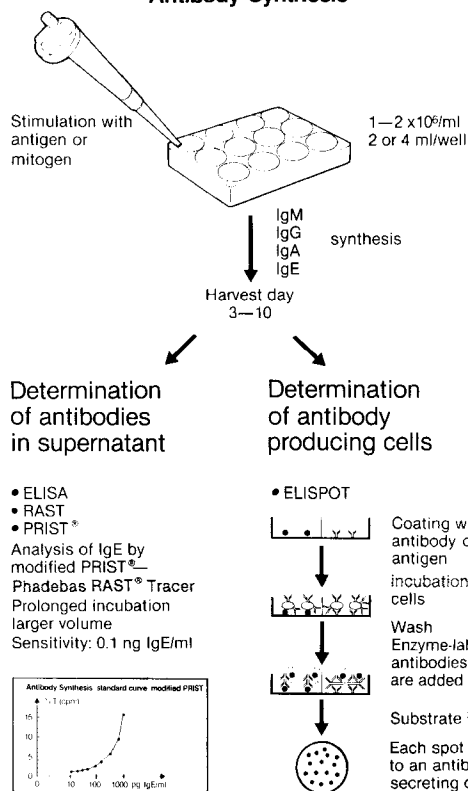
Dose-response Birch pollen



Reference:

Lymphocyte Proliferation
Barbara B. Mishell and Stanley M. Shiigi
Selected Methods In Cellular Immunology, 1980.
W.A. Freeman and Company, San Francisco

Antibody Synthesis



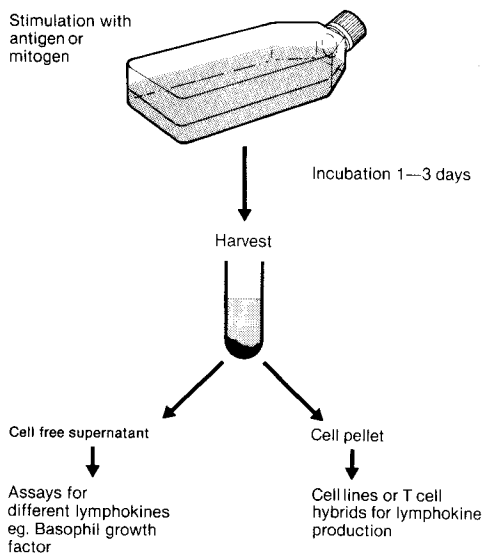
Reference:

Z.S. Hemady; F. Bjornberg, R.E. Rocklin
Journal of Allergy and Clinical Immunology 1982, 69, 156

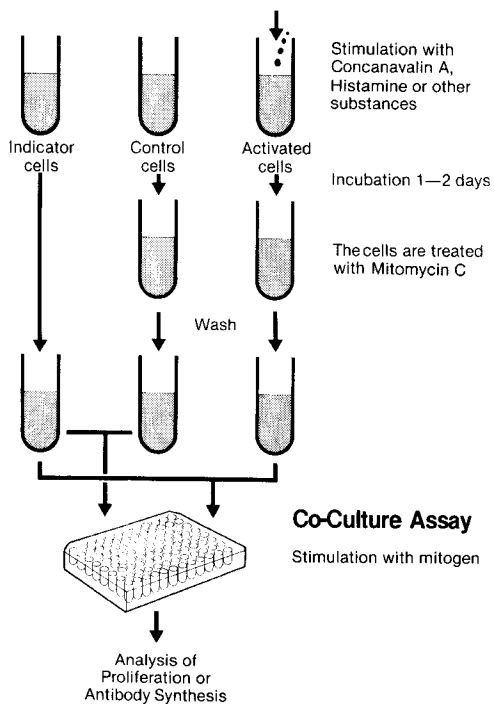
Reference:

C. Gzerniksky, L. Å Nilsson, H. Nygr, O. Ouchterlong and A. Tarkowski
J. Immunol Methods, 1983, 65, 109

Lymphokine Production



Induction of Helper or Suppressor cells



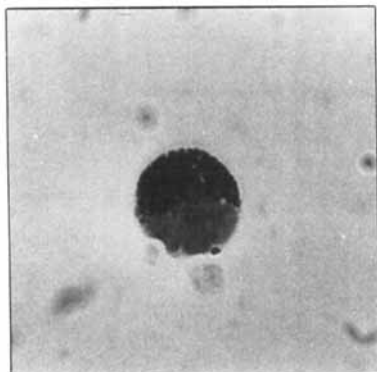
Reference:

Ross E. Rocklin and Angela Haberek-Davidson
Journal of Clinical Immunology 1, 1981:73-79

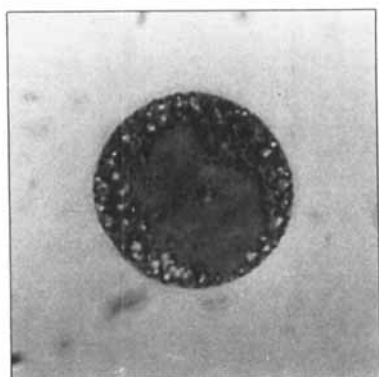
BASOPHIL GROWTH IN HUMAN CELL

I. Hammarström, P. Lehtonen, O. Hall, E. Åkerblom

Aim: Cells from allergic individuals produce factors that induce basophil growth

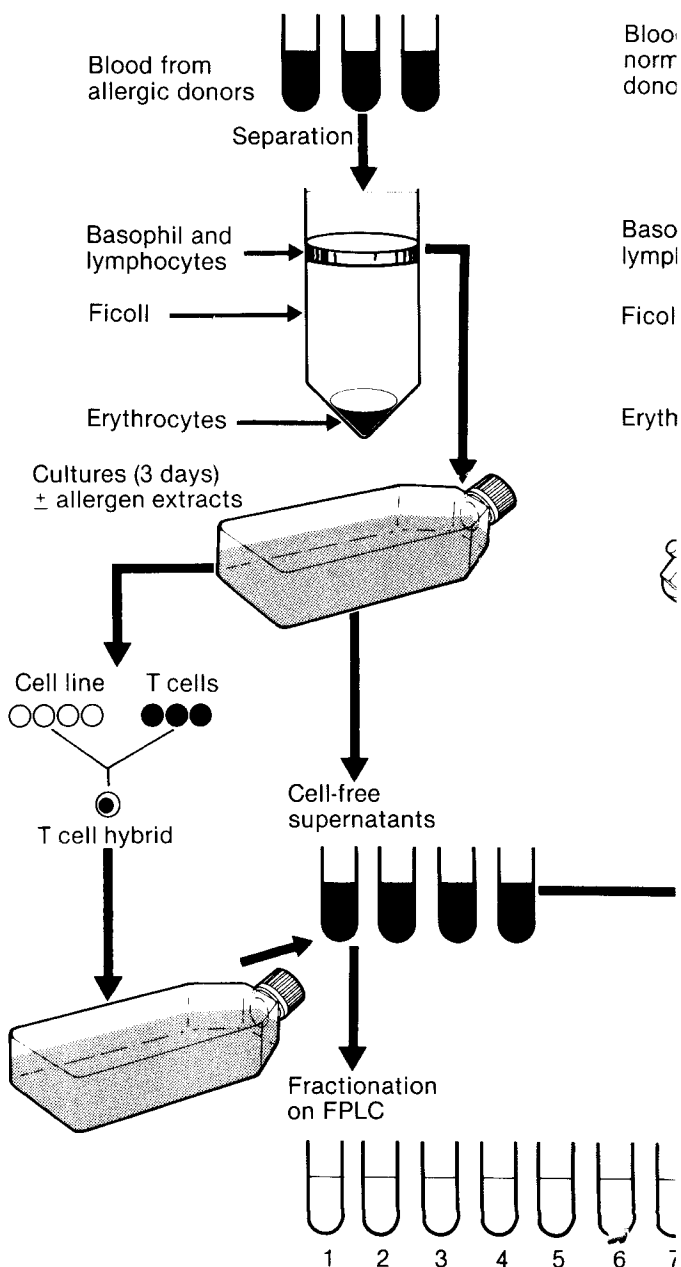


Small basophil



Large basophil

Preparation of basophil growth medium



Test act

Blood from normal donors

Basophil lymphocytes

Ficoll

Erythrocytes

Cell line

T cell hybrid

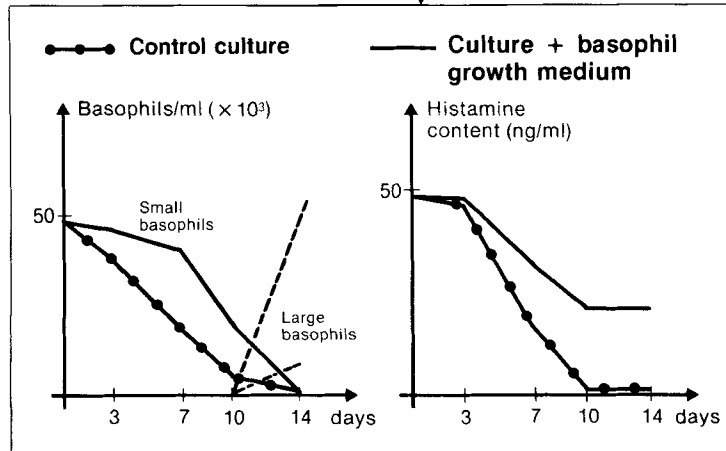
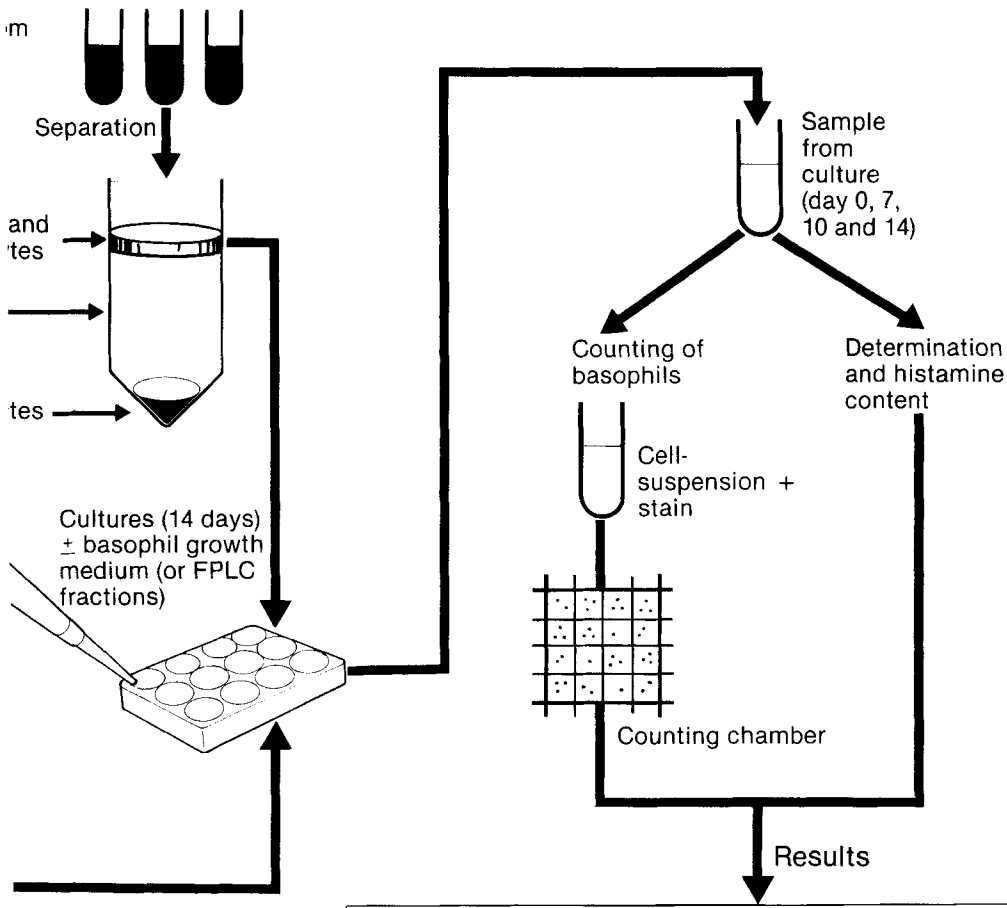
Cell-free supernatants

Fractionation on FPLC

1 2 3 4 5 6 7

CULTURES

or basophil growth
y



HISTAMINE ASSAYS IN ALLERGY RESE

INTRODUCTION

Determination of histamine from mast cells and basophilic leucocytes can be utilized in allergy research, e.g. to analyze the composition and potency of allergenic preparations, to characterize the reactivity of allergic patients and for experimental studies of allergic inflammation.

There are a number of methods today to assay histamine. We will present two of them and relate them to the clinical and experimental research.

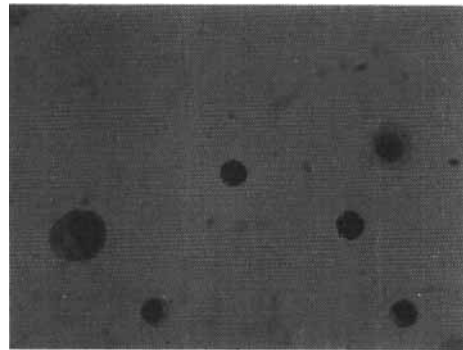
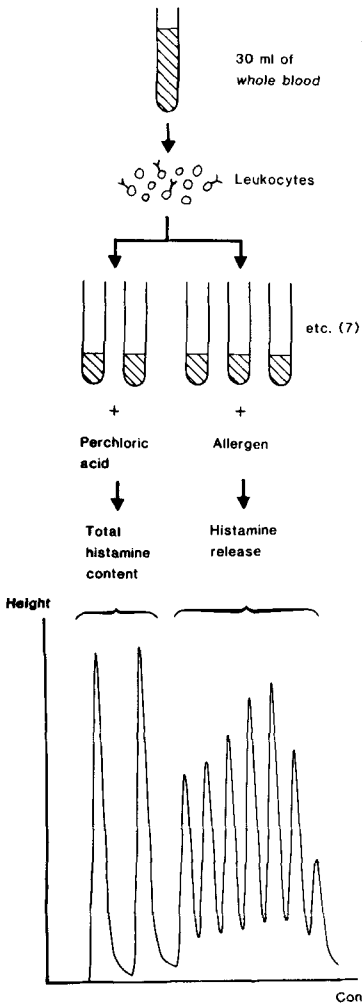
PRINCIPLE

Fluorometric assay: Histamine release from basophilic leucocytes/mast cells.

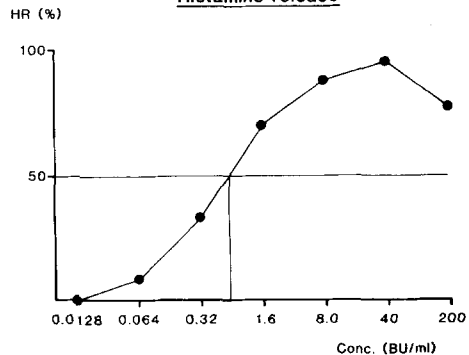
Enzymatic isotopic assay: Identification of histamine containing cells.

METHODS

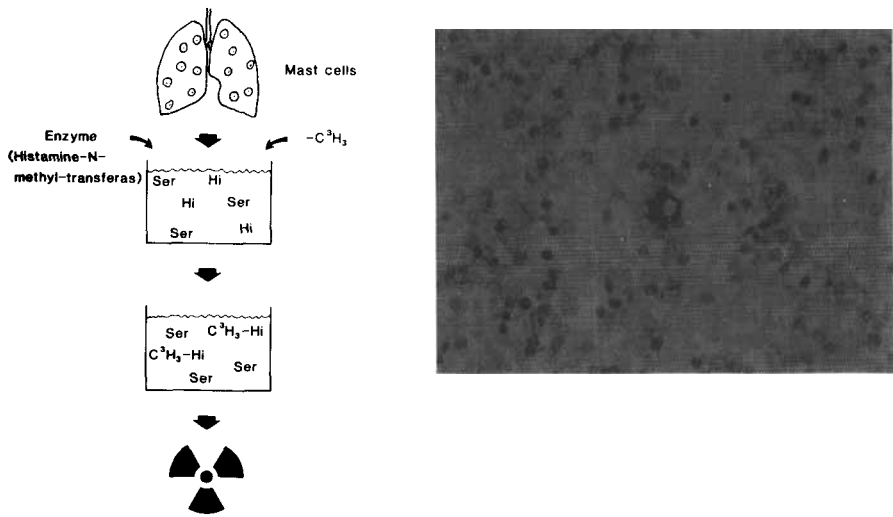
Fluorometric assay



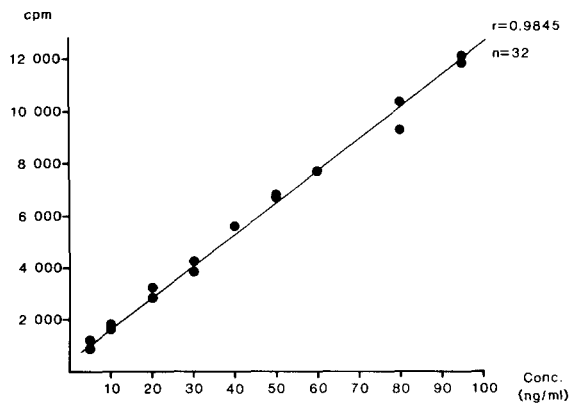
Histamine release



METHODS
Enzymatic isotopic assay

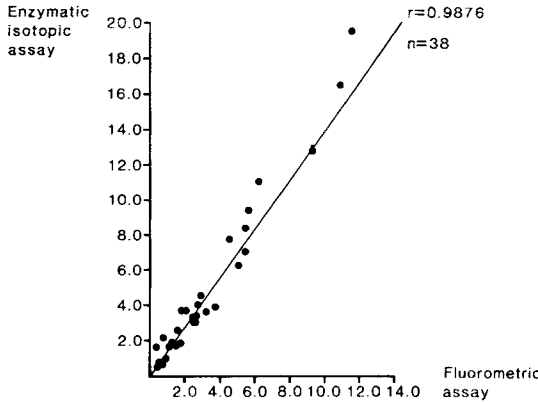


Calibration



	<u>Fluorometric assay</u>	<u>Enzymatic isotopic assay</u>
Sample	Basophil/mast cell in liquid	Cells in tissue extract
Specificity	No	Yes
Detection limit	1 ng/ml	0.1 ng/ml
Coeff. of variation	10–15%	20%

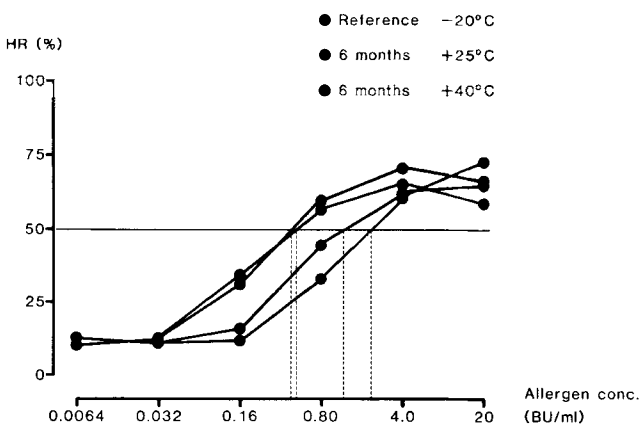
CORRELATION



APPLICATIONS

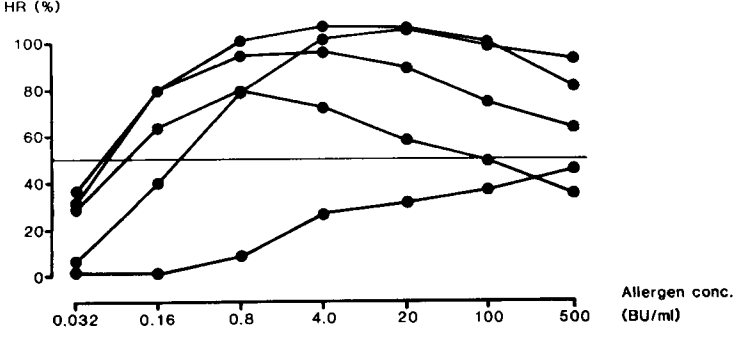
Allergenic preparation

Stability

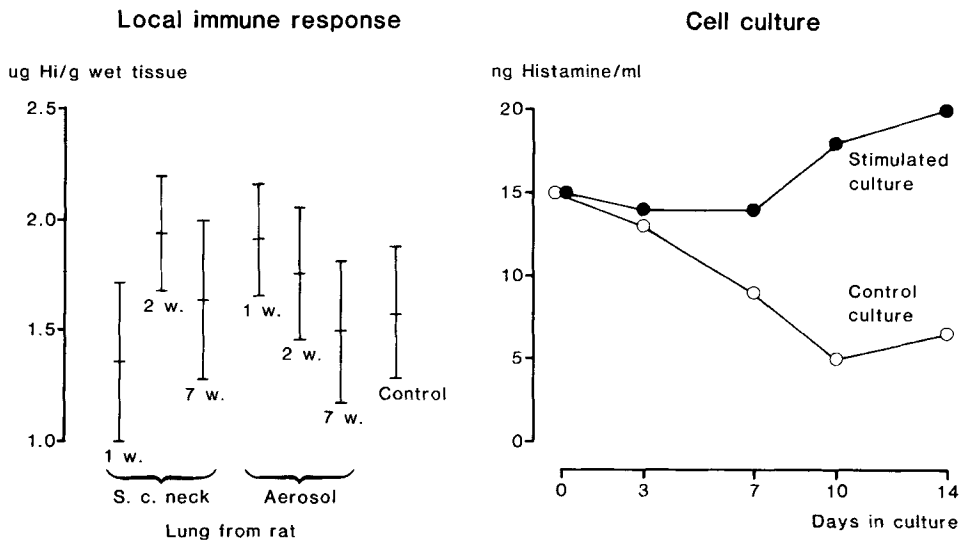


Allergic patients

Diagnosis



Experimental research



CONCLUSION

The histamine assays provide sensitive and reliable tools for

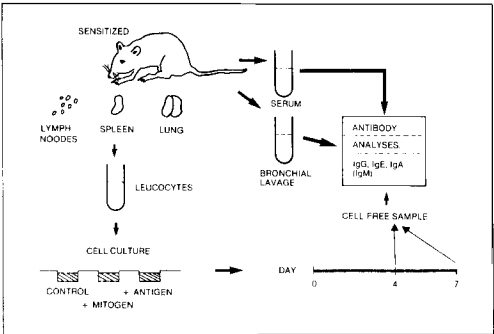
- the potency of diluted allergen preparations.
- the sensitivity of basophils/mast cells of allergic patients.
- the amount of histamine containing cells in tissue and cell cultures.

INFLAMMATORY RESPONSES AND CLIN

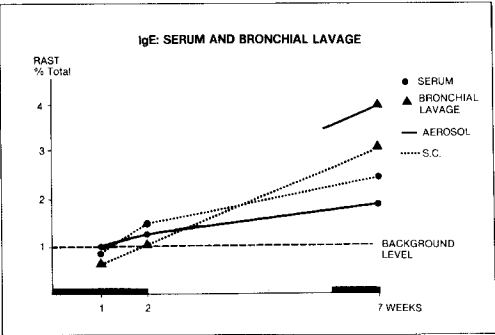
Kjell Alving, Ingrid Enander, Jan Hård, Erik Olaisson, and Ann-Kristin Ulfgren.

► DAILY MONDAY THROUGH FRIDAY
► 2 WEEKS WITH 4 WEEKS INTERVAL

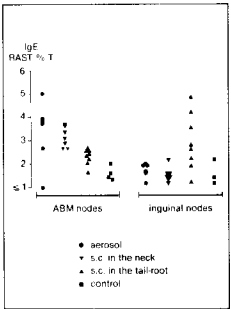
ANTIBODIES



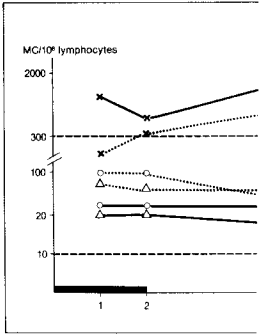
IgE: SERUM AND BRONCHIAL LAVAGE



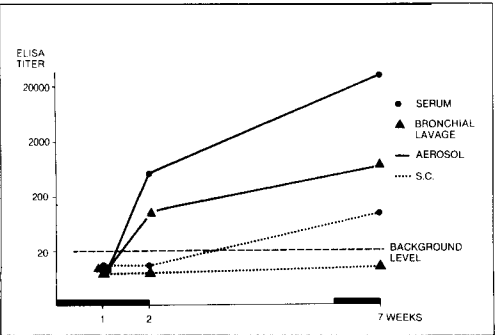
IgE in vitro:



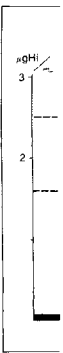
MAST CELLS



IgG: SERUM AND BRONCHIAL LAVAGE



HISTAMINE



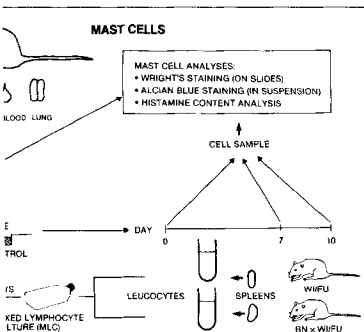
REFERENCES: • Ahlstedt, S., Smedegård, G., Nygren, H., Björkstén, B. "Immune responses in rats sensitized with aerosolized antigen". Int. Archs Allergy appl. Immun. 72:71—78, 1983.
• Smedegård, G., Ahlstedt, S., Björkstén, B., Nygren, H. "Bronchial reactivity in relation to immune responses in rats after subcutaneous sensitization without adjuvant". Allergy. 39:249—258, 1984.
• Alving, K., Enander, I., Hård, J., Olaisson, E., Ulfgren, A.

CLINICAL REACTIVITY IN RATS

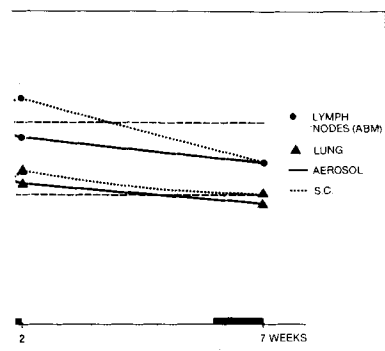
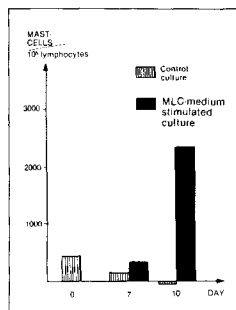
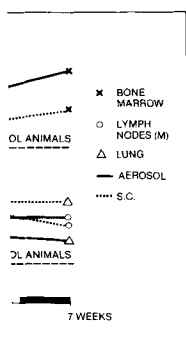
WEEKS

- RATS (BN x WU) 6-8 WEEKS OLD
- PURE PROTEIN ANTIGEN (OVALBUMIN)
- NO ADJUVANTS
- SUBCUTANEOUSLY: 100-300 ng
- AEROSOL INHALATION: 1 % W/V, 30 MINUTES

MAST CELLS

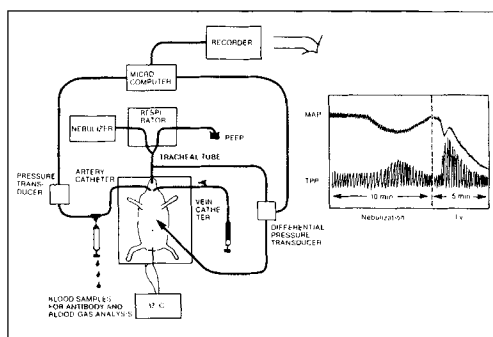


MLC — MEDIUM STIMULATED MAST CELL DIFFERENTIATION

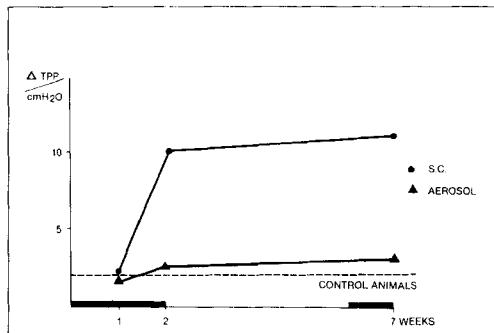


CLINICAL REACTIVITY

- ANESTHETIZED ANIMALS
- CONSTANT RESPIRATORY FREQUENCY
- THE TIDAL VOLUME IS SET TO GIVE CORRECT BLOOD GAS LEVELS, WHICH ARE DETERMINED IN AN AUTOMATIC BLOOD GAS ANALYZER.
- BLOOD IS DRAWN FOR ANTIBODY ANALYSES.
- THE ANIMALS ARE CHALLENGED IN TWO WAYS: FIRST BY AEROSOL INHALATION AND THEN BY INTRAVENOUS (i.v.) INJECTION.
- THE REACTION IS FOLLOWED BY CHANGES IN TRANSPULMONARY PRESSURE (TPP), MEAN ARTERIAL BLOOD PRESSURE (MAP) AND BLOOD GAS LEVELS.
- TPP AND MAP ARE RECORDED ON A UV OSCILLOGRAPH.



CLINICAL LUNG REACTIVITY



Björkstén, B., Hesselmar, B., Nygren, H., Alving, K., Olsson, E.
 "Immunized regional inflammatory responses mediating lung reactivity in rats."
 82—288, 1985.