An autoradiographic study of human blood basophils

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Synopsis. The aim of this paper is the selective visualization of human blood basophils in autoradiographs. [3H]Thymidine-labelled basophils in buffy coat smears were fixed in methanol-formaldehyde followed by 5-aminoacridine hydrochloride and stained with basic Aldehyde Fuchsin prior to autoradiographic processing. The technique described represents a simple method for quantitative autoradiographic studies on basophil kinetics and the interaction of these cells with IgE-mediated atopias.

Introduction

The solubility of specific cytoplasmic granules in blood basophils is a major obstacle in autoradiographic processing. Fixatives and stains otherwise suitable for preservation of human blood basophils (Parwaresch & Lennert, 1967) produce considerable chemo-graphic artifacts, which make a quantitative analysis of the results impossible. On the other hand, autoradiography is still the most accurate method for recording radioactivity in individual cells. It is the best technique for cytokinetic studies of life-span, circulation time, and renewal rate.

Gaps in our knowledge of the kinetics of human blood basophils and recent results on the participation of these cells in atopic (IgE-mediated) reactions (Ishizaka & Ishizaka, 1970) stress the importance of a suitable technique for autoradiographic studies.

The present paper describes a procedure which enables first, labelled basophils in autoradiographs to be distinguished clearly, and second, a quantitative analysis of the labelling intensities when required.

Materials and methods

Isotopic labelling

2–3 days after an intravenous injection of 0.1 μCi [3H]thymidine/g body weight (specific activity 1.9 Ci/mM; Radiochemical Centre, Amersham, England), blood samples were collected from the cubital vein. Smears were prepared from leukocyte concentrates (Desaga & Parwaresch, 1970).
Fixation and staining
Air-dried smears were placed in a mixture of absolute methanol and formalin (9:1 by vol) as described by Kaplow (1955) for 5 min at 4°C. For a final fixation, the slides were transferred to 0.4% 5-aminoacridine hydrochloride solution in 50% ethanol for 30 min. They were then rinsed in the methanol-formalin mixture and stained for 30 min in 0.5% basic Aldehyde Fuchsin solution in 65% ethanol, to which 1 ml paraformaldehyde and 1 ml conc. hydrochloric acid had been added (Gomori, 1950) to adjust the pH to 1. The slides were rinsed in 0.5% borax solution (Na₂B₄O₇·10 H₂O) in 80% ethanol, differentiated in 70% ethanol, and air-dried.

Autoradiography
Smears from leukocyte concentrates were fixed and stained as described above, dipped in the same batch of Kodak nuclear trace liquid emulsion NTB-2 at 43°C and cooled to 4°C in humidified chambers. They were then exposed in lightproof boxes at 4°C for 60 days, developed in Kodak-D19 for 3 min, rinsed in distilled water, fixed in an aqueous solution of 20% sodium thiosulphate and 2.5% potassium metabisulphite for 5 min at 18°C and, finally, rinsed in tap water for 15 min. Haemalum was used for nuclear counterstaining when required.

To detect any positive or negative chemographic effect, caused by the various agents used, grains of about 100 segmented nuclei per slide were counted and the values were compared with those of control slides. Prior to the autoradiographic processing, the controls had been subjected to either (a) methanol fixation; or (b) methanol-formalin fixation; or (c) methanol-formalin fixation followed by 5-aminoacridine hydrochloride fixation; or (d) the same as (c) plus Aldehyde Fuchsin staining and subsequent borax treatment. The probability that 5-aminoacridine hydrochloride accumulates in the specific granules and thereby leads to a greater grain production over the cytoplasm of the basophils, was determined by comparing the mean cytoplasmic grain counts of 100 blood basophils with that of 100 other leukocytes.

Statistical treatment
Assuming a random distribution, all item rules were subjected to variance analysis with the F-test as an overall test for independent populations. Means were computed for a significant difference on the basis of a 95% p-level using the t-test.

Results and discussion
The staining technique applied allows blood basophils to be distinguished clearly (Fig. 1). The cytoplasm of the basophils was well preserved and was light violet in colour. Other leukocytes did not stain at this pH and were, therefore, easily distinguishable from basophils even at low magnifications. Evidence of artificial cell destruction, common to most staining procedures owing to leaching of the water-soluble basophil granules, was not found. There was no significant difference between the background grain dispersion of the control slides and the smears subjected to the full treatment (Table 1). This was also true for grain counts over the cytoplasm.

No significant difference could be found between the mean labelling intensities (mean grain count per nucleus) of the segmented leukocyte nuclei in slides not subjected to treatment (except for nuclear staining after developing of the autoradiographs) and those