

Short communication

Factors determining spontaneous histamine release from human basophils purified with Percoll gradients and Dynabeads

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Identification of factors influencing histamine release from purified and cultured basophil leukocytes is important for proper interpretation of results obtained on histamine release. This paper describes factors that influence spontaneous histamine secretion from human basophil leukocytes purified on Percoll gradients, followed by negative selection with Dynabeads. Anti-IgE and recombinant human interleukin-3 were used as model stimulants, and the purified basophil leukocytes were stimulated for 10 min and 6 h. The effect of the following conditions was examined: Percoll temperature, cell-suspension density, and serum in the media. The results showed that low Percoll temperature, high cell-suspension density, and the presence of serum in the media decreased spontaneous histamine release and increased maximal net histamine release upon stimulation.

**H. V. Nielsen, P. M. Shah,
P. O. Schiøtz**

Research Laboratory, Department of Pediatrics,
Aarhus University Hospital, Aarhus N, Denmark

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Henrik V. Nielsen, MD
Research Laboratory, Department of Pediatrics
Aarhus University Hospital
DK-8200 Aarhus N
Denmark

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Basophil leukocytes participate in the late phase of the allergic reaction, as they invade the inflamed tissue hours after the primary allergic reaction (1–3). Upon stimulation, basophil leukocytes secrete, among other substances, histamine, which plays a role in initiating and sustaining the allergic inflammation. Therefore, much interest has centered on characterizing histamine secretion from basophil leukocytes. However, experiments on basophil histamine release (HR) have yielded conflicting results. This could be due to differences in experimental conditions, as it is known that human serum albumin, ion content, temperature, pH (4), and buffer osmolarity (5) influence HR from basophil leukocytes.

In this study, Percoll gradient centrifugation and subsequent negative selection with Dynabeads for purification of basophils were employed, followed by culture in protein-free media. By stimulating basophil leukocytes with recombinant human interleukin-3 (rhIL-3) and anti-IgE as model secretagogues, it was found that small changes in the purification procedure and culture conditions induced substantial changes in spontaneous HR.

Thus, Percoll gradient temperature, serum in the media, and cell-suspension density could influence spontaneous as well as stimulated HR from basophil leukocytes purified on Percoll gradients, followed by negative selection with Dynabeads.

Material and methods

Materials

Dynabeads were obtained from Dynal, Oslo, Norway; monoclonal antihuman antibodies, CD8, CD19, CD8, CD4, CD14, CD16, and CD3, from Dakopatts Glostrup, Denmark; polyclonal rabbit antihuman anti-IgE from Behringwerke, Marburg, Germany; and rhIL-3 from Genzyme, USA.

Methods

Blood samples (50 ml) were obtained from healthy volunteers and anticoagulated with EDTA (final concentration: 5 mM). The blood samples were divided into two; one half was immediately chilled at 4°C, and the other half was left at room temper-

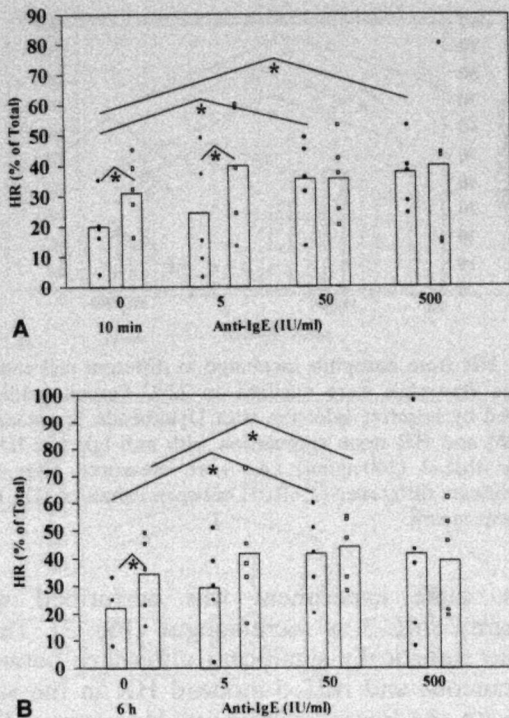


Fig. 1. HR from basophils stimulated with anti-IgE. Basophils were purified on 4°C (●) or 22°C (□) Percoll gradients, followed by negative selection with Dynabeads. Cells were incubated for 10 min (A) and 6 h (B). * = significant difference ($P < 0.05$) between values of HR. Bars represent means.

ature (22°C). After 30 min, the blood was layered on Percoll gradients with the densities 1.079 and 1.071 mg/ml (2.5 ml of blood on each gradient). The gradients were prepared at either 4°C or room temperature (4°C or 22°C purification, respectively). The gradients were centrifuged (400 g, 20 min) at either 4°C or at 22°C. The rest of the negative selection procedure with Dynabeads was performed at 4°C, essentially as described by Bjerke et al. (6), except that the cells were finally washed once (400 g, 4 min, 4°C) in RPMI 1640 without serum. Upon staining with Alcian blue and counting, the cells were pelleted (400 g, 4 min, 4°C) and resuspended in serum-free RPMI 1640. Cell viability was assessed by the trypan blue exclusion test.

Purified basophils were incubated at a concentration of $1.25 \times 10^5/\text{ml}$ in RPMI 1640 for 10 min or 6 h at 37°C, 5% CO_2 , rhIL-3, anti-IgE, and heat-inactivated dialyzed neonatal calf serum (NCS, final concentration: 2%) were added, as indicated before incubation.

Upon incubation, the reaction was stopped by addition of cold Pipes buffer (150 mM Na-acetate, 10 mM Pipes, 5 mM K-acetate, 0.6 mM CaCl_2 , and 1.1 mM MgCl_2), and the pellet was sedimented by centrifugation (400 g, 10 min, 4°C).

Histamine was measured in pellet and supernatant by the microfiber-based method (7). HR was assessed in percentage of total histamine content. The experiments were performed in duplicate, and measurements of histamine were made in triplicate.

Data were analyzed with Wilcoxon's signed rank test for matched pairs. Results are presented as individual data points and mean.

Results

Effect of Percoll temperature on final purity

The purity of basophil leukocytes in the interface after Percoll gradient centrifugation was significantly higher in the 22°C gradients than in the 4°C gradients (4°C: $20.0 \pm 2.6\%$, 22°C: $32.4 \pm 3.9\%$, $P < 0.05$). However, the final purities after negative cell selection were not significantly different (4°C: $83.2 \pm 4.2\%$, 22°C: $89.6 \pm 1.5\%$, $P > 0.05$). Moreover, there was no significant difference in final basophil leukocyte number (4°C: $455\,000 \pm 64\,000$, 22°C: $424\,000 \pm 71\,000$). The viability of the basophil leukocytes at 4°C and 22°C was not significantly different (4°C purification: $99 \pm 0.5\%$, 22°C purification: $98 \pm 1.0\%$, $P > 0.05$). Thus, the different temperatures of Percoll did not change purity, yield, or viability of the basophil leukocytes.

Effect of Percoll temperature on HR

After obtaining homogeneous suspensions of purified and viable human basophils prepared at two different temperatures (4°C and 22°C), we examined their spontaneous as well as stimulated HR after 10 min and 6 h of incubation. After 10 min (Fig. 1A) of incubation, the 4°C preparation exhibited a significantly lower spontaneous HR than the 22°C preparations. Basophil leukocytes prepared at 4°C showed dose-dependent HR upon stimulation with anti-IgE. Basophil leukocytes prepared at 22°C were responsive to a much lesser degree.

After 6 h (Fig. 1B) of incubation, the basophil leukocytes prepared at 4°C also showed a significantly lower spontaneous HR than the basophil leukocytes prepared at 22°C. Basophils from both preparations showed comparable dose-dependent HR upon stimulation with anti-IgE. However, the maximal net HR (stimulated HR – spontaneous HR) was significantly higher in the 4°C preparation (4°C: $19.8 \pm 3.8\%$, 22°C: $9.8 \pm 2.4\%$, $P < 0.05$).

Stimulating basophil leukocytes prepared at 4°C with rhIL-3 (Fig. 2A and B) resulted in a significantly higher HR at an optimal IL-3 concentration of 100 ng/ml compared to the basal HR after both 10 min and 6 h. The basophil leukocytes from the 22°C preparation showed no significant differences

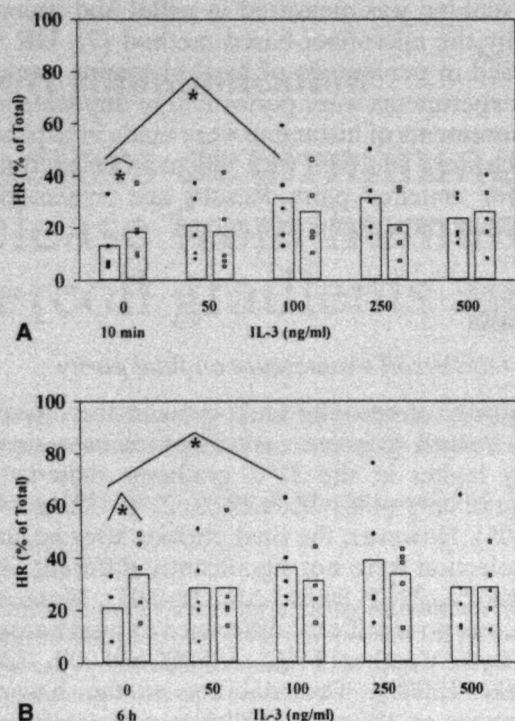


Fig. 2. HR from basophils after incubation with rhIL-3. Basophils were purified on 4°C (●) or 22°C (□) Percoll gradients, followed by negative selection with Dynabeads. Cells were incubated for 10 min (A) and 6 h (B). * = significant difference ($P < 0.05$) between values of HR. Bars represent means.

between basal HR and maximum HR after stimulation with rhIL-3.

In conclusion, spontaneous HR was significantly lower when the basophil leukocytes were purified on the 4°C Percoll gradients than on the 22°C Percoll gradients. Upon stimulation with polyclonal anti-IgE, the basophil leukocytes prepared at 4°C were more responsive. When purified on cold Percoll gradients, basophil leukocytes secreted histamine dose-dependently upon stimulation with rhIL-3.

Effect of basophil leukocyte cell concentrations on HR

Basophil leukocytes were purified from five donors at 22°C and resuspended to final concentrations of either 1.25×10^5 or 5×10^6 basophil leukocytes/ml. Subsequently, the cells were incubated for 6 h (Fig. 3). Spontaneous HR and HR upon stimulation with anti-IgE (50 IU/ml) were significantly lower in the samples with the greater density. Furthermore, the net HR (stimulated HR – spontaneous HR) was significantly higher in the samples with a high concentration of cells ($14.3 \pm 3.7\%$ vs $9.8 \pm 2.4\%$, $P < 0.05$).

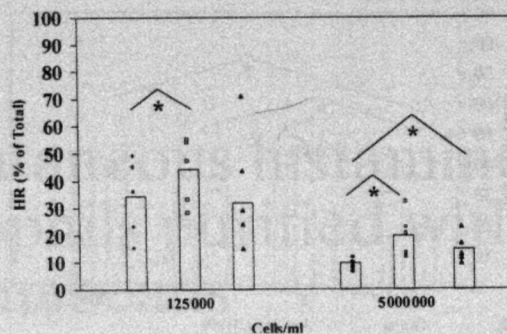


Fig. 3. HR from basophils incubated at different cell concentrations. Basophils were purified on 22°C Percoll gradients, followed by negative selection with Dynabeads. Spontaneous HR (●) and HR upon stimulation with anti-IgE (50 IU/ml) (□) or rhIL-3 (100 ng/ml) (▲) were measured after 6 h. * = significant difference ($P < 0.05$) between values of HR. Bars represent means.

The same experiment was performed with 100 ng/ml rhIL-3 as secretagogue (Fig. 3). There was no statistically significant difference between spontaneous and rhIL-3-induced HR in the samples with the lower cell density. However, rhIL-3 induced a statistically significant higher HR in the samples with the higher density.

Effect of serum on HR from basophil leukocytes

The effect of addition of serum to the culture medium was investigated. The suspensions of basophil leukocytes were incubated with 2% NCS for 10 min and 6 h, and spontaneous and anti-IgE-induced HR was measured. After 10 min (Fig. 4A) and 6 h (Fig. 4B) of incubation, there was no significant difference in spontaneous HR from the basophil leukocytes prepared at either 4°C or 22°C. Both preparations showed comparable dose-dependent HR. Thus, NCS did level the histamine release in the two preparations.

Discussion

The original findings by Lichtenstein & Osler (4) showed a clear dependence of HR from basophil leukocytes on temperature. In the present study, spontaneous HR from basophils suspended in protein-free media was dependent on the temperature of the Percoll used during purification. In fact, this was so even though the basophils were processed for yet another 1.5 h at 4°C before incubation. Perhaps unspecific activation of basophil leukocytes could take place during gradient centrifugation. Therefore, the lower temperature (4°C) could protect the basophil leukocytes against this activation because a decrease in temperature lowers the metabolism. Percoll in itself is chemically inert, and

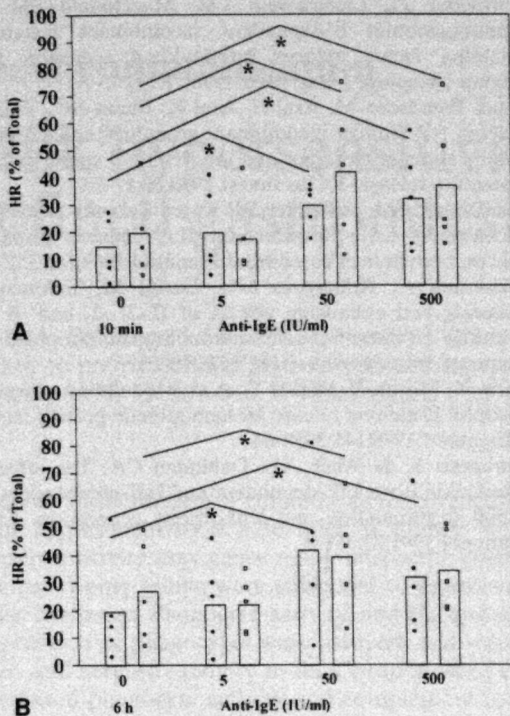


Fig. 4. Histamine release from basophils incubated in media with 2% neonatal calf serum and stimulated with anti-IgE. Basophils were purified on 4°C (●) or 22°C (□) Percoll gradients, followed by negative selection by Dynabeads. Cells were incubated for 10 min (A) and 6 h (B). * = significant difference ($P < 0.05$) between values of HR. Bars represent means.

injection with 60% Percoll in the ovary and uterine horn of rabbits did not cause inflammatory reaction (8). It is a common experience that hard handling (pipetting) of basophil leukocytes can induce degranulation. Therefore, it cannot be excluded that the actual mechanical distress caused to the basophil leukocytes by the Percoll solution activated the cells.

The present study demonstrated that high concentrations of cells efficiently decreased the spontaneous HR as well as increased the net HR upon stimulation with anti-IgE or rhIL-3. This effect of cell density on spontaneous HR was recently also reported by others (9). Many other authors have reported lower spontaneous HR in preparations of basophil leukocytes from non-food-allergic or healthy control subjects than we found in our basophil preparations at low cell density (10–12). However, most experiments, including those cited, were performed with only partially purified basophil leukocytes, with foreign proteins added, and at much higher total cell density than used in our experiments. Probably the difference in our reported HR and that of others are due to differences in the experimental conditions, especially the cell densities employed.

May & Remigio (10) were the first to report a donor-specific inhibitory effect of serum on basophil HR. The very high spontaneous HR from basophils of some food-allergic donors were inhibited dramatically by autologous serum, but it was increased by the addition of serum from normal subjects. We dialyzed and heat-inactivated our NCS before use in order to avoid potential activating or inhibiting substances, finding that 2% NCS induced a modest decrease in spontaneous HR from our basophil suspensions.

IL-3 has been shown to stimulate histamine secretion from basophils from a subset of atopic donors (13–15). Other researchers did not find this stimulatory effect of IL-3 (16, 17). We found that basophil leukocytes purified on Percoll gradients, followed by negative selection with Dynabeads and serum-free incubation, could release histamine upon rhIL-3 stimulation, if purified on chilled Percoll gradients or incubated at high cell concentrations.

Identification of factors influencing HR from purified and cultured basophil leukocytes is important for proper interpretation of obtained results on HR. In this study, we describe factors that influence HR from basophil leukocytes purified on Percoll gradients, followed by negative selection with Dynabeads. The results show that HR is dependent on Percoll temperature, serum in the incubation medium, and the cell concentration in the basophil leukocyte suspension during incubation.

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