Evaluation of Chicken Heterophil Adherence

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SUMMARY. Adherence of chicken heterophils was evaluated at 37 C using preconstructed columns containing various weights of nylon fiber (75 mg, 100 mg, or 125 mg) and whole blood anticoagulated with sodium heparin or 10% disodium ethylenediamine tetraacetic acid (EDTA). Additionally, 50-mg and 75-mg nylon fiber columns incubated at 41 C were used to evaluate heterophil adherence at an increased temperature. The mean percent adherence for heparin-anticoagulated blood applied to 75-mg, 100-mg, and 125-mg nylon fiber columns at 37 C was 76%, 92% and 97.4%, respectively. Samples applied to 50-mg and 75-mg columns at 41 C had adherence values of 27% and 85%, respectively. When paired samples of blood anticoagulated with EDTA or heparin were evaluated, the EDTA samples had significantly decreased heterophil adherence (paired t-test). Results indicate that increased or decreased adherence of chicken heterophils would best be detected using 75-mg nylon fiber columns incubated 37 C and whole blood collected in sodium heparin.

RESUMEN. Evaluación de la adherencia de los heterófilos de pollo.
Se evaluó la adherencia de los heterófilos de pollo a 37 C utilizando columnas previamente hechas que contenían fibras de nylon de diferentes pesos (75 mg, 100 mg, ó 125 mg) y sangre entera anticoagulada con heparina sódica o con el ácido etilendiamino tetraacético al 10% (EDTA). Adicionalmente, las columnas de fibra de nylon de 50 mg, 100 mg y 125 mg incubadas a 41 C fueron usadas para evaluar la adherencia de los heterófilos en una temperatura mayor. El porcentaje promedio de adherencia de la sangre no coagulada con heparina en las columnas de fibra de nylon de 75 mg, 100 mg y 125 mg a 37 C fue de 76%, 92% y 97.4%, respectivamente. Muestras mantenidas a 41 C en columnas de 50 mg y 75 mg tuvieron valores de adherencia de 27% y 85% respectivamente. Cuando el EDTA y la heparina se evaluaron en las mismas muestras de sangre no coaguladas, la adherencia de los heterófilos fue significativamente menor en las muestras con EDTA. Los resultados indican que el aumento o disminución en la adherencia de los heterófilos se puede detectar mejor usando columnas de fibra de nylon de 75 mg incubadas a 37 C y sangre entera tomada con heparina sódica.

Arrival of neutrophils at an inflammatory site requires margination, adherence to the vascular endothelium, diapedesis, and directional migration toward chemotactic gradients. Neutrophil adherence is dependent on glycolysis, surface charge, cell viability, divalent cations, microtubule integrity, and temperature (3). Scanning electron microscopy has demonstrated that individual neutrophils attach to nylon fibers and endothelial cells in vitro by fine, slender projections from a larger pseudopod involving a small portion of the cell membrane (6,7). Due to the similar morphologic appearance and individual cell attachment of neutrophils to nylon fiber and endothelial cells, nylon fiber columns often are used in the evaluation of neutrophil adherence.

Neutrophil adherence has been evaluated with nylon fiber columns during disease states or after the use of pharmacologic agents in people and in several animal species, including dogs, cattle, and swine (1,4,7). In swine with experimental salmonellosis, an association between increased neutrophil adherence and acute
inflammatory disease has been described (8). In people, adherence of neutrophils is augmented in septic shock (9). Heterophil adherence has been investigated in rabbits with induced peritonitis (2). During inflammation, increased adherence of neutrophils in humans and rabbits appears to be induced by a plasma augmenting factor (2,7,9). Neutrophil adherence in humans has been inhibited by pharmacologic agents such as aspirin, prednisone, and ethanol (4). The purpose of the present study was to determine whether chicken heterophils were similar to neutrophils in their adherence properties and to identify a single weight of nylon fiber column that could detect increases or decreases in adherence of chicken heterophils.

MATERIALS AND METHODS

Chickens. Twenty healthy adult broiler breeders were placed in a commercial chicken house on a 16-hour-light and 8-hour-dark cycle. The chickens were fed breeder ration and provided water ad libitum.

Sample collection. Blood samples (2.2 ml) were collected from the ulnar vein of fasted chickens into 3-ml disposable plastic syringes containing either 10% disodium ethylenediamine tetraacetic acid (EDTA) 100 μl/ml of blood or preservative-free sodium heparin (10,000 U/ml) 100 μl/ml of blood. A 1-ml whole-blood sample was used for each adherence column, and the remainder of the sample was used for total and differential leukocyte counts.

Heterophil adherence. Heterophil adherence was determined using heparin or EDTA-anticoagulated blood by a modification of the MacGregor method (1,4). The adherence column was constructed with disposable plastic tuberculin syringes, 21-gauge disposable needles, and nylon fiber (Leuko-Pak Filter, Fenwal Laboratories, Division of Travenol Laboratories Inc., Deerfield, Ill.). The nylon fiber was weighed on a balance and inserted into the syringe barrel with a wooden applicator stick. The fiber was compressed with the rubber-tipped syringe plunger, which was removed immediately before column use.

Adherence columns were placed in a plexiglass holder containing 10 × 75-mm siliconized glass test tubes. The unit was equilibrated at 37 C or 41 C in a humid 5% CO₂ atmosphere overnight and during the adherence tests. Differential and total leukocyte counts were obtained before and after adherence testing. Total leukocyte counts were performed using the technique of Natt and Herrick (5). Anticoagulated blood samples, 1 ml each, were placed in duplicate adherence columns and allowed to percolate through the nylon fiber by gravity flow for 10 minutes. Heterophil viability was evaluated by the trypan blue dye exclusion test. All samples were processed using this protocol.

Heterophil adherence was determined by the following equation:

$$\text{100} - \left( \frac{\text{heterophils/ml effluent blood}}{\text{heterophils/ml original blood}} \right) \times 100 = \% \text{ adherence.}$$

Adherence testing was performed at 37 C using 10 heparin-anticoagulated blood samples in adherence columns that contained 75 mg, 100 mg, or 125 mg of nylon fiber. For comparative purposes, eight paired blood samples (1 ml each) anticoagulated with EDTA or heparin were placed in 100-mg adherence columns at 37 C. Additionally, whole blood was anticoagulated with heparin and placed in ten 75-mg and five 50-mg nylon fiber columns at 41 C to determine the effects of increased temperature on heterophil adherence.

Scanning electron microscopy. After the whole-blood adherence procedure, columns were scored and broken, and the nylon fibers were removed. Nylon fibers were cut in two, trimmed, and quench-frozen in liquid nitrogen. The fiber mass was then transferred to the cold stage of an Edwards-Pearse Freeze Dryer, evacuated to 200 millitorr, and allowed to sublime 24 hours. The temperature was slowly increased to ambient, whereupon the fiber mass was removed from the chamber, mounted on a 2-cm aluminum stub, and sputter-coated with 30-nm gold-palladium using a Hummer V Sputter Coater. The specimen was examined with JSM-35 scanning electron microscope operating at 15 keV. Positive controls consisting of purified heterophils passed through nylon fiber columns were used for heterophil identification. All samples were processed by the method described.

Data analyses. Before analysis, percent adherence data for 75-mg, 100-mg, and 125-mg nylon fibers were transformed to arcsines. The data were analyzed by one-factor analysis of variance followed by a Tukey's W test. Percent adherence was regressed on the weight of nylon fibers using an analysis of variance for linear regression (multiple y factors per x). The paired samples from heparin- and EDTA-anticoagulated blood (100-mg nylon fiber) were analyzed using the paired t-test (alpha level = 0.05).

RESULTS

Heterophil viability was greater than 98%, as determined by trypan blue dye exclusion. Heterophil adherence increased as the weight of nylon fiber increased. The mean percent adherence for heparin-anticoagulated blood at 37 C was 76% for a column with 75 mg of nylon fiber, 92% for 100 mg, and 97.4% for 125 mg. Significant differences (F = 35.5, df = 2/27, P < 0.001) were present between each group's
Chicken heterophil adherence

mean percent adherence. The percent adherence had a significant linear increase ($F = 31.6$, $df = 1/27$, $P < 0.001$) as the weight of nylon fiber increased (regression line $y = 29.3119 + 0.4343x$). A significant decrease ($t = 9.62$, $df = 7$, $P < 0.001$) was present for the mean percent heterophil adherence when EDTA-anticoagulated blood (14.6%) was compared with heparin-anticoagulated blood (90.1%) (Table 1). The mean percentages for 50-mg and 75-mg nylon fiber column at 41 C were 27% and 85%, respectively.

Scanning electron microscopic examination revealed that heterophils attached to nylon fibers singly via a small portion of the cell membrane (Fig. 1). Indirect cell-to-cell attachment was not observed for leukocytes. Very small aggregates of flat elliptical cells, presumed to be erythrocytes, were attached to nylon fibers. Leukocytes were neither entrapped between fibers nor attached to erythrocyte aggregates.

**DISCUSSION**

Increased weight of nylon fibers resulted in increased heterophil adherence. Similar findings have been reported for human neutrophil adherence (4). Additionally, the percent adherence of chicken heterophils (76%, 92%, and 97.4%) approximated values reported for human neutrophils (70%, 99%, and 100%) at 75-mg, 100-mg, and 125-mg nylon fiber weights at 37 C (4). The statistical analysis of fiber weight vs. percent heterophil adherence demonstrated a linear relationship between increasing weights and increasing adherence values. Increased adherence is probably due to the presence of a

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*100-mg nylon fibers. There were significant differences between group percentage means ($t = 9.62$, $df = 7$, $P < 0.001$, paired t-test).
greater surface area for leukocyte attachment to nylon fibers.

In people, neutrophil adherence has been shown to be temperature-dependent, with maximum adherence occurring between 25 and 42 C and decreased neutrophil adherence at temperatures above 42 C (3). In people and domestic mammals, adherence testing is usually performed at 37 C (1,4,8). This has proven to be satisfactory even though body temperatures of domestic mammals are higher than human body temperature. In this project, adherence testing also was performed at 41 C to approximate chicken body temperature. Heterophil adherence in 75-mg nylon fiber columns increased from 76% to 85% at temperature increased from 37 C to 41 C. Because an adherence of 85% probably would be too high to detect an increase in adherence during disease conditions, we attempted to use 50-mg weight nylon fibers at 41 C. The 50-mg columns resulted in very low adherence values (27%). These low values may have resulted from the decreased surface area provided by 50 mg of nylon fiber or from the difficulty in uniformly distributing the small amount of fiber in the adherence column. An incubation temperature of 37 C provided a reproducible mid-range (76%) adherence value. This temperature also is convenient for most laboratories, because it is a standard temperature setting for incubators.

Heterophil adherence decreased significantly when a divergent cation chelator, EDTA, was used as the anticoagulant. Divalent cations, especially magnesium and calcium, are required for neutrophil adherence (4). In vivo, cations are thought to provide a positive charge to bridge the negatively charged endothelium with resultant cell adherence (6). This also may occur in vitro, because neutrophil attachment to endothelium and nylon fiber appears to be similar (6,7). In people, chelation of magnesium and calcium with EDTA reduced neutrophil adherence by 87% when compared with heparin anticoagulation of the same patient sample (4).

Single heterophil-to-fiber attachment was demonstrated by scanning electron microscopy. The absence of passively entrapped leukocytes in cellular aggregates indicates that nylon wool columns reflect heterophil-to-fiber adherence, as has been demonstrated for neutrophils (6).

Nylon columns provided a rapid, simple method to evaluate chicken heterophil adherence. Under conditions of accurate nylon fiber weight, the adherence assay can be adapted so that any percentage of heterophils can be retained by varying the fiber weight within the column. Blood that was anticoagulated with sodium heparin, then placed in 75-mg nylon fiber columns and incubated at 37 C, resulted in 76% heterophil adherence. This column weight should be sensitive enough to allow detection of increases or decreases in heterophil adherence during disease states.

REFERENCES


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