Neutrophil-erythrocyte rosette formation: case report and proposed mechanism

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Learning points for clinicians

Erythrocyte rosetting refers to the aggregation of red blood cells around a central leukocyte resembling a flower. Antibodies are often thought to account for erythrocyte-leukocyte bridging. Neutrophil-erythrocyte resetting, however, may also be due to neutrophil activation by IL-8, resulting in the expression of surface molecules that interact with erythrocytes.

Case report

A 21-year-old male presented with fever, abdominal pain, and jaundice. He denied the use of illicit drugs, excessive alcohol consumption, unsafe sexual intercourse, and exposure to animals or insect bites.

Upon admission, his vital signs were normal. Physical examination was notable for jaundice and mild abdominal tenderness in the right upper quadrant. Blood analysis showed elevated liver markers: Alanine transaminase 396 U/L (normal range 10-49), aspartate aminotransferase 382 U/L (0-34), alkaline phosphatase 182 U/L (46-116), gamma-glutamyl transferase 226 U/L (0-73), lactate dehydrogenase 1060 U/L (120-246), total bilirubin 195 μmole/L (5-21), direct bilirubin 139 μmole/L (0-5). Complete blood count was unremarkable and C-reactive protein showed an increase with a value of 2 mg/dL (0-0.5). Extensive serological investigation indicated acute Epstein-Barr virus (EBV) infection and a polymerase chain reaction detected a viral load of 81 x 10^3/ml.

Following admission, hemoglobin level decreased from 14.2 to 11.9 gr/dL and additional investigation revealed a ferritin value of 4556 ng/ml (22-322), undetectable haptoglobin, and a considerably increased soluble IL-2 receptor value of 3835 U/ml (0-1000). Direct Coombs test was positive for nonspecific cold agglutinin.
Serum cytokine analysis revealed IL-8 levels of 146 pg/ml (0-70) and IL-1β levels of 5.5 pg/ml (0-0.6). IgG subclasses were within normal limits. Blood smear demonstrated neutrophil erythrocyte rosettes (NER; Figure 1a). The patient recovered within several days under supportive treatment.

**Discussion**

Cold type autoimmune hemolytic anemia (AIHA) is a known complication affecting approximately 60 percent of EBV patients, although clinically significant hemolysis is rare. Typically, spherocytes and red blood cell agglutination can be observed.\(^1\) There are few reports on neutrophil-erythrocyte rosettes in association with AIHA and the underlying mechanism is unclear. Previously suggested mechanisms involved an interaction between surface Fc receptors on neutrophils and the IgG1 or IgG3 coated erythrocytes (Figure 1b).\(^2\) However, our findings could not lend support to this hypothesis. Furthermore, a recent report of a COVID-19 patient with NER and no evidence of AIHA suggested the possibility of an alternative, inflammation-based, explanation. Strikingly, inflammation was predominant in all previously reported cases of NER.\(^3\) Accordingly, elevated markers of inflammation were observed in the patient presented herein, manifested as increased values of C-reactive protein, ferritin and soluble IL-2 receptor.

Therefore, we propose a cytokine-mediated mechanism for NER formation (Figure 1B). We found significantly elevated serum levels of IL-1β and IL-8. *In vitro* studies demonstrated that IL-1β stimulates IL-8 expression \(^4\) and that exposure of neutrophils to IL-8 induces the expression of the adhesion molecule CD11b/CD18.\(^5\) In turn, the I domain of CD11b/CD18 integrins were shown to be bound by erythrocyte-expressed ICAM-4 \(^6\), which is a member of the immunoglobulin superfamily. This
explanation is based on the cytokine profile of the patient as well as previously reported in vitro data which warrant in vivo validation.

In summary, we propose that the cytokine-milieu plays a predominant role in the formation of NER, with IL-8 being a pivotal stimulant of neutrophil-erythrocyte adhesion.
Consent

Written consent was obtained from the patient prior to the submission of this report.

Conflict of interest: None declared.
References


Figure legend

Figure 1: (a) A neutrophil-erythrocyte rosette structure as observed in the patient's blood film. (b) proposed mechanisms underlying neutrophil-erythrocyte adherence manifested as rosettes (created with BioRender.com)