The Relationship of Bartonella Bacilliformis to the Red Blood Cell as Revealed by Electron Microscopy

By Manuel Cuadra and Juan Takano

Barton (1905–1909) described the etiologic agent of Oroya Fever as a parasite located within the erythrocytes ("Barton's endoglobular bodies"). Strong et al. (1913) named the microorganism Bartonella bacilliformis and stated also that it was located within the red cells. Aldana (1929–1947) believed that B. bacilliformis was on the red cell, attached to its outer surface. Wigand et al. (1953) by examining replicas of parasitized blood smears with electron microscopy, concluded that the organisms were on the surface of the erythrocytes.

The epiglobular localization of B. bacilliformis has been utilized as a basis to explain the mechanism of the anemia. According to this theory the organisms would not injure the red cell. Therefore, the destruction of erythrocytes by phagocytosis is accidental since the red cells are considered vehicles of the B. bacilliformis.

In the present work, by means of ultrathin sections of erythrocytes parasitized by B. bacilliformis, we tried to determine whether the microorganisms were able to penetrate the red cells. Our results are discussed in relation to the fine structure of B. bacilliformis and the role of the organism in the production of anemia.

Materials and Methods

The present study utilized venous blood specimens taken from three patients who were affected by severe anemia, fever, malaise, headache, gastrointestinal disturbances and pain in joints, bones and muscles at the time of admission. On physical examination they appeared pale and weak. Lymph nodes were somewhat enlarged, the spleen was palpable in only one case. Clinical laboratory data are given in Table 1. Examination of red cells on Wright's stained smears of peripheral blood at admission time revealed a predominance of the coccoid type of B. bacilliformis parasitizing over 70 per cent of the red corpuscles (Fig. 1). Anisocytosis, poikilocytosis and polychromatophilia were observed. Reticulocytes were increased in number. Normoblasts, basophilic stippling, Cabot ring and Howell-Jolly bodies were occasionally seen. Abundant siderocytes were found using the technic of Sundberg and Broman.

On the basis of these data it was established that all 3 patients suffered from Oroya Fever. Patient number one was in the transition stage between the acute phase of the disease and...
Table 1.

| Subject | Age (yr.) and sex | Duration of illness, (days) | R.B.C. x 10⁶/mm² | Parasitized red cells % | B. Bacilliformis
<table>
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<tbody>
<tr>
<td>Case 1</td>
<td>27 M</td>
<td>10</td>
<td>2.4</td>
<td>98</td>
<td>90%</td>
</tr>
<tr>
<td>Case 2</td>
<td>56 M</td>
<td>15</td>
<td>0.84</td>
<td>80</td>
<td>100%</td>
</tr>
<tr>
<td>Case 3</td>
<td>13 M</td>
<td>15</td>
<td>1.4</td>
<td>70</td>
<td>100%</td>
</tr>
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(*) At the time of admission.
(**) Beaded appearance.

Fig. 1.—Smear of peripheral blood showing parasitized red cells by a coccoid type of B. bacilliformis. Wright's stain X 2,200.

Preparation of Materials for Electron Microscopy

Procedure A. Blood clot was fixed in 10 per cent formalin in isotonic saline and minced with a razor blade into very small pieces, approximately one mm³ in size. They were then postfixed for 30 minutes in one per cent osmium tetroxide solution buffered with phosphate buffer at pH 7.4, and dehydrated by passage through an ethyl alcohol series (70%, 80%, 95% and 100%); the specimens were then embedded in maraglass. Thin sections were cut with glass knives using a Porter-Blum ultramicrotome, placed on open 300 mesh copper grids and stained with lead hydroxide and uranyl acetate. The sections were examined in a Philips E.M. 200 microscope. This procedure was applied to cases 1 and 2.

Procedure B. Defibrinated blood sample was centrifuged and the serum removed; the remaining red cells were washed three times with isotonic saline, fixed in osmium tetroxide and transferred to neutral formaline for 30 minutes. The rest of the procedure as in "A."

Red cell stroma. A blood sample taken from case 2 was hemolyzed by repeated exposures to distilled water, until a residue, white in color, was obtained at the bottom of the centrifuge. This residue was then treated as described in "A."

convalescence; and cases 2 and 3 were in the beginning of convalescence. None of the patients was given antibiotics before the date of admission to the hospital.
Fig. 2.—Thin sections of a parasitized erythrocyte. Notice a rounded form near the cell surface. This form has a cell wall (W) and is composed of materials varying in density. A narrow space between this form and the red cell cytoplasm is seen (S). Lead hydroxide stain. $\times$ 102, 100.

The posthemolytic residue (packed red cell stroma) was processed according to procedure A.

Control blood samples. These were obtained from normal subjects and from a patient suffering hemolytic anemia not related to Bartonellosis (1'200,000 erythrocytes mm$^3$, 16 percent reticulocytes).

RESULTS

Rounded or bacillary forms resembling the electron microscopic appearance of the bacteria$^{10,11}$ were seen within the red cell sections, usually near the cell surface. We consider them to be Bartonella bacilliformis (Figs. 2, 3). The rounded forms measured around 1.5 by 0.25 microns. Two structural components were easily recognizable: a cell wall and cytoplasm. The cell wall consisted of an osmophilic layer measuring approximately 200 Angstrom units in thickness. The cytoplasm, sometimes limited by a thin membrane, was composed of materials varying in density filling almost the entire space enclosed by the cell wall; the lighter structures must be the bacterial nuclei (Fig. 3). A very narrow space between B. bacilliformis and the red cell cytoplasm was constantly seen. This space can be an artifact due to shrinkage of the microorganisms during fixation and dehydration procedure (Fig. 2). The close relationship between B. bacilliformis and red cell cytoplasm is morphologic evidence that these microorganisms are located within the red cells. Microorganisms were also seen within vesicle-like structures of red cells.
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Fig. 3.—Bacillary form which shows similar structure of rounded form described above. The lighter structures appear to be bacterial nuclei (N). Lead hydroxide stain. ×61,000.

(Fig. 4). These vesicle-like structures could be real or artifacts due to infolding of the red cell surfaces. Occasionally, the microorganisms appeared to be incompletely or completely segmented (Fig. 5).

In addition to Bartonella organisms there were also certain vesicles and rounded bodies containing electron dense material composed of minute particles (Fig. 6).

Frequently seen attached to the red cell surfaces were various sized small rounded bodies which were more dense than its contents of the red cell (Fig. 4).

The sections of hemolyzed red cell preparations showed the red cell stroma to consist of tortuous almost empty, membranes which have the appearance of “globules,” invaginated to each other. The bacterial cell bodies were easily identified within these “globules,” either very near the red cell membrane or directly beneath it (Figs. 7, 8).

Occasionally bacterial cells were seen outside these “globules.” The electron dense granular material contained in vesicles were also easily identified (Fig. 7).

DISCUSSION

Oroya Fever is an acute self-limited disease. Bartonella bacilliformis, the
B. bacilliformis organisms seen lying within a vesicle-like structure. One of these organisms shows an incomplete membrane (M). Upon the red cell membrane are seen granular rounded bodies (GB) varying in size, denser than the contents of the red cell. Unstained. × 18,750.

causative organism, varies in morphology and quantity during various stages of the disease. In severe cases of Oroya Fever, B. bacilliformis parasitizes virtually all the circulating red cells. In spite of being a pleomorphic organism, two essential types are distinguishable: bacilli or rod-shaped forms and coccoid forms. The first form, active or vegetative, predominates in the acute stage of the disease. The coccoid or inactive form predominates in the convalescent stage.

We studied three patients showing a predominance of coccoid forms in red cells. Our observations under the electron microscope of ultrathin sections of both red cells and their stroma, support the conclusion that B. bacilliformis were not only on the surface of the erythrocytes but that they lie within them. Therefore, they were able to penetrate into the red cells.

B. bacilliformis in the host red cell was identified ultrastructurally by its characteristic cell wall. Structures such as mitochondria, vacuoles and certain round bodies containing electron dense material were easily distinguished from B. bacilliformis.

The extracellular appearance of certain Bartonella organisms by light microscopy; the absence of evidence of intravascular destruction of the red cells and the incapacity of growing B. bacilliformis to parasitize red cells in vitro cultures, induced Aldana to conclude that the organisms are exclusively on the red cells, attached to their external surface. It is impossible to decide by
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Fig. 5.—A bacillary form segmented in three parts. Unstained, X 31,250.

Fig. 6.—Vesicles (V) and a rounded body (RB) contained electron dense material composed of minute particles which are seen in the red cell cytoplasm. Uranyl acetate stain. X 45,000.

light microscopy whether the organisms are in or on the red cells. The same problem has been encountered in the study of malaria.¹⁸,¹⁰

Due to the limitations of technic, Wigand et al.⁵ in their electron microscopical studies by the replica method, were confined to the demonstration
Fig. 7.—A “globule” resulting from hemolyzed red cell. At the inner surface are easily identified B. bacilliformis (B) and vesicles (V) containing electron dense granules. Unstained × 25,000.

Fig. 8.—B. bacilliformis attached to the inner surface of red cell membrane. Lead hydroxide stain. × 31,250.

of the organisms located on the red cells. Our studies, on the other hand, as performed by electron microscopy\textsuperscript{10,11} have demonstrated that B. bacilliformis is very similar in structure to bacteria. A cell wall was clearly visualized; furthermore, a residue of empty membranes was obtained by trypsin treatment.\textsuperscript{5,20} These facts, together with the inadequacy of the motility of the
organism and the unipolar flagella of the growing form in bacteriologic media\textsuperscript{20-22} place \textit{B. bacilliformis} closer to bacteria than rickettsia.\textsuperscript{20-23}

It is conceivable that \textit{B. bacilliformis} within red cells might damage the integrity of these cells making them more vulnerable to phagocytosis.

\textbf{SUMMARY}

Ultrathin sections of erythrocytosis parasitized by \textit{B. bacilliformis} have been examined by electron microscopy. The study concerns three Oroya Fever patients whose blood smears showed \textit{B. bacilliformis} predominantly in its coccoid form as parasitizing over 70 per cent of the red cells.

\textit{B. bacilliformis} is termed as a bacterium in its structure and appears to lie not only on the host red cells but predominantly within them. Therefore, this organism might have the capacity to penetrate into the red cell. This finding does not change the basic concept regarding the mechanism of the anemia of Oroya Fever.

\textbf{SUMMARIO IN INTERLINGUA}

Sectiones ultratenue de erythrocytosis parasitisa per \textit{Bartonella bacilliformis} esseva examine per microscopia electronic. Le studio concerne tres patientes con febre de Oroya in qui le frottis de sanguine monstrava \textit{B. bacilliformis} predominantemente in su forma coccoide in le processo de parasitar plus que 70 pro cento del erythrocytos.

\textit{B. bacilliformis} es designate como bacterio a base de su structura. Illo pare jacer non solo al superficie del erythrocytos hospite sed etiam—e predominantemente—intra illos. Per consequente, on debe supponer que iste organismos possede le capacitate de penetrar ad in erythrocytos. Iste conception non modifica le conception fundamental relative al mecanismo del anemia de febre de Oroya.

\textbf{ACKNOWLEDGMENT}

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