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Cell-to-Cell Interactions in Cultures of Swine Bone Marrow Cells and CPK-66b Cells Infected with African Swine Fever Virus

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In the presence of specific antiserum, cultured bone marrow cells from pigs infected with African swine fever virus form cell-to-cell junctions which are morphologically similar to those observed in antibody-dependent cellular cytotoxicity. This type of cytotoxicity does not determine the reaction of delayed hemadsorption, since this reaction is realized in a long-term culture of CPK-66b cells in the absence of the effectors of antibody-dependent cellular cytotoxicity. The sensitivity of delayed hemadsorption depends on the variant of infecting virus. A negative correlation is established between the area of the contact between erythrocytes and cells infected with different variants of the virus, on the one hand, and titers of antibodies in the delayed hemadsorption reaction, on the other.

Key Words: *antibody-dependent cellular cytotoxicity; delayed hemadsorption; African swine fever virus; cell culture*

Mononuclear phagocytes are the target for the virus causing African swine fever [3,8,10]. Replicating in monocytes/macrophages, this virus induces antigenic modulations consisting in the emergence of virus-induced proteins in the plasma membrane [2,5,6]. This phenomenon manifests itself as the ability of infected leukocytes or bone marrow cells to adsorb swine erythrocytes with serotype-specific abrogation of delayed hemadsorption (DHA) [1,9]. Hemadsorption is believed to be mediated by a virus-specific protein which is functionally and structurally similar to the CD2 surface antigen of T lymphocytes [7]. These membrane-associated viral proteins are the target for immune attack involving cytolytic effector mechanisms, specifically, antibody-dependent cellular cytotoxicity (ADCC) mediated by neutrophils and specific antibodies [4]. Thus, both ADCC and DHA are characterized by the same initial stage, namely, antigenic modulation of the plasma membrane of infected cell and reaction with antibodies. Antibody binding has opposite effects on cell-to-cell interactions in a heterogeneous subpopulation of cultured swine bone marrow cells which contains infected cells: stimulation of the attachment of ADCC effector cells or inhibition of the erythrocyte ad-

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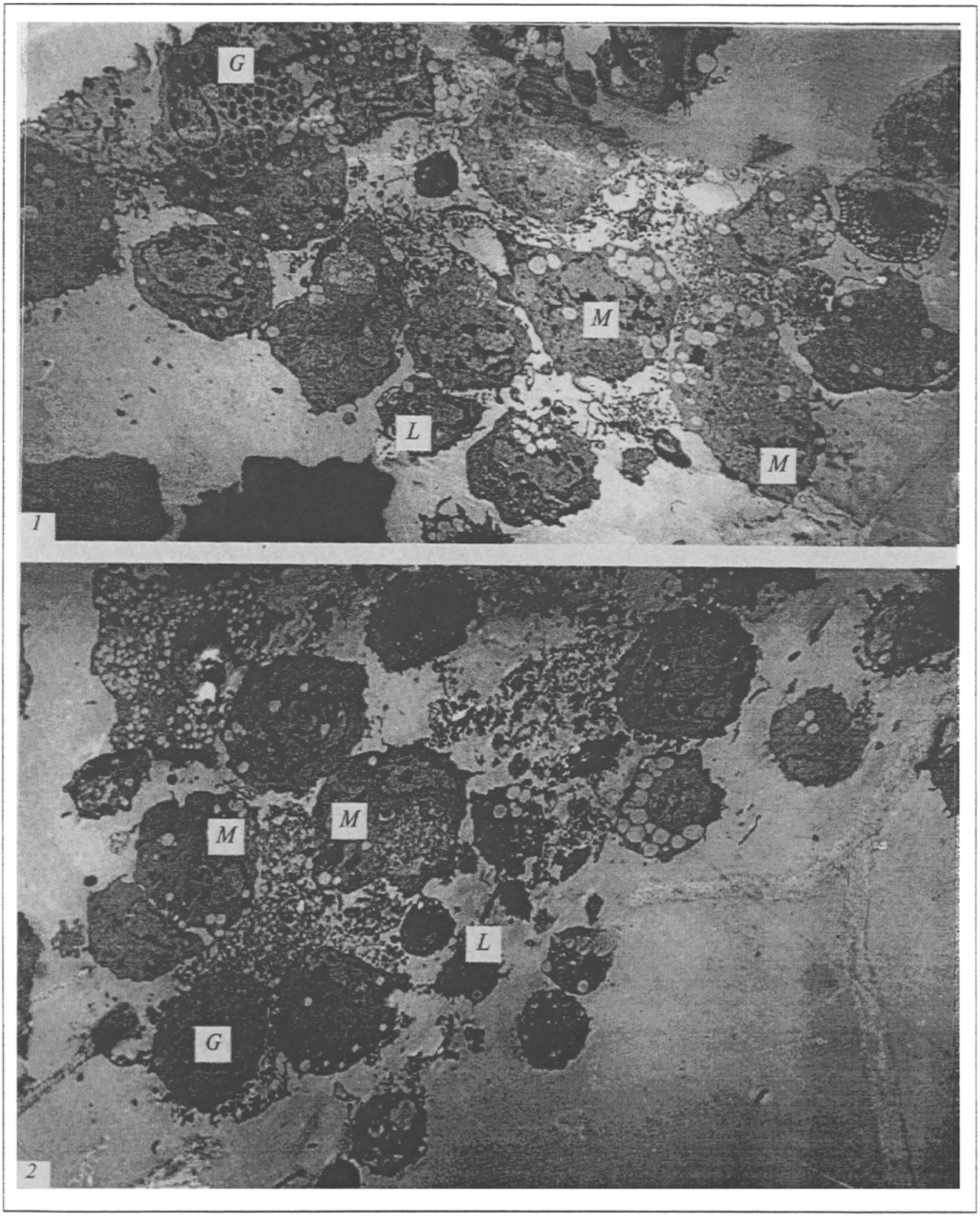

Fig. 1. Cell rosettes in a culture of bone marrow cells infected with African swine fever virus in the presence of specific antiserum.
M: macrophage;
L: lymphocyte;
G: granulocyte.

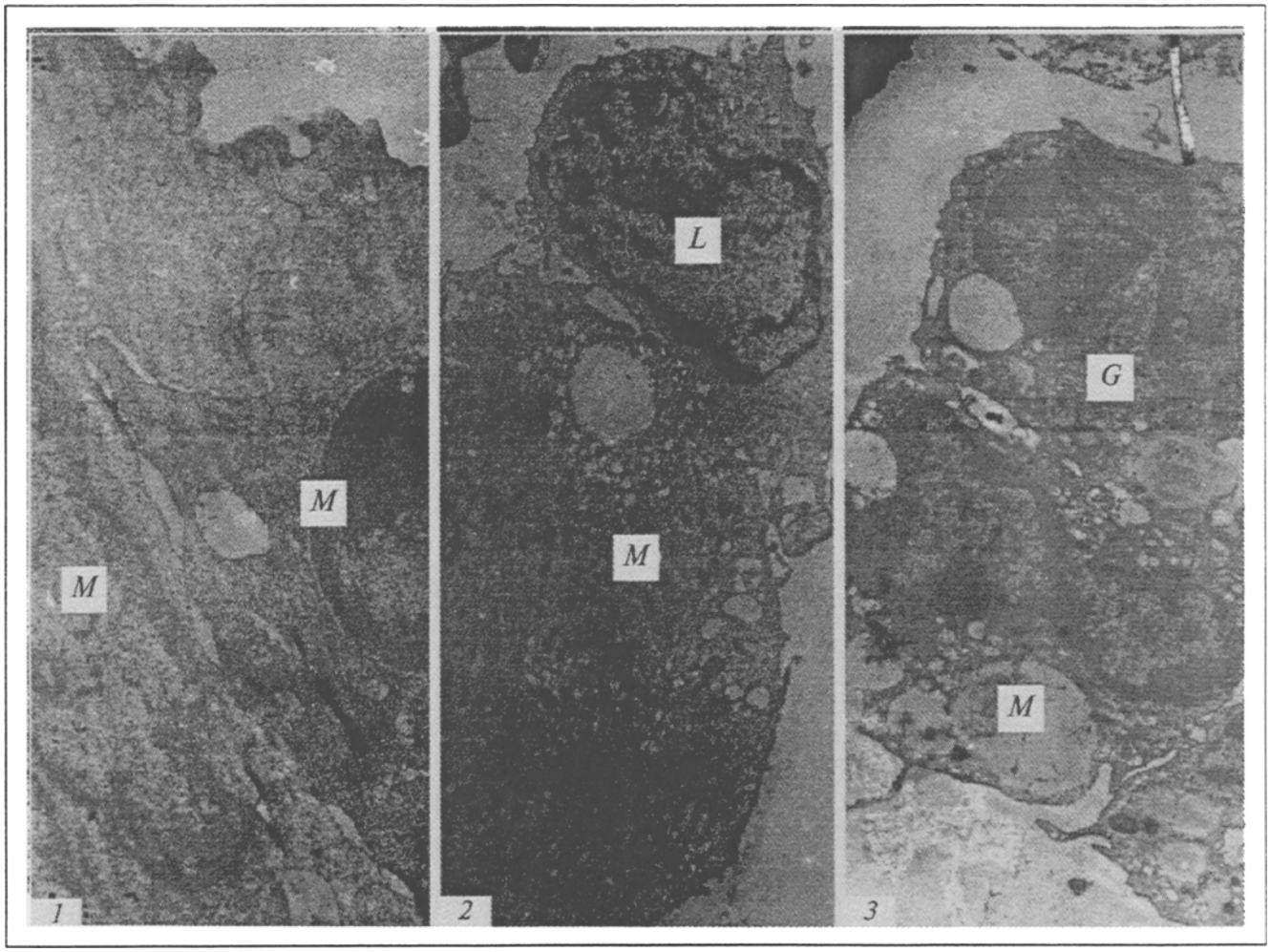

Fig. 2. Cell-to-cell interactions in a culture of bone marrow cells infected with African swine fever virus in the presence of specific antiserum. *1*) macrophage (*M*)+macrophage; *2*) lymphocyte (*L*)+macrophage; *3*) granulocyte (*G*)+macrophage.

sorption. Therefore, it is likely that ADCC-related lysis of target cells in infected cultures may completely or partially compete with hemadsorption and simulate its delay.

In this study we attempted to characterize the "relationship" between ADCC and DHA by investigating cell-to-cell contacts in infected cultures at the light and electron microscopy levels and by ultrastructural examination of hemadsorption caused by various variants of African swine fever virus (ASFV).

MATERIALS AND METHODS

The major characteristics of ASFV, primary cell cultures of swine bone marrow cells, and the methods of culturing and infection were described previously [2-4]. The DHA reaction was conducted in a culture of bone marrow cells by conventional techniques [1]. When a long-term culture of CPK-66b cells was used, swine erythrocytes were added to a final concentration of 0.1% 24 h after infection and addition of specific antiserum. The reaction was read within a 3-4-day period after the addition of erythrocytes. For preparation of ultrathin sections cells were fixed with 4% glutaraldehyde in cacodylate buffer (pH 7.3) and 1% osmium tetroxide in phosphate buffer (pH 7.3), dehydrated in rising ethanols, and embedded in Epon-Araldite. The sections were contrasted with saturated aqueous solution of uranyl acetate and lead citrate and studied in a JEM-100 electron microscope at the magnification range 10,000-40,000.

RESULTS

The fact that DHA may be promoted by the involvement of infected bone marrow cells in ADCC is confirmed by the following observations. On day 2 after infection in the absence of specific antiserum, a pronounced hemadsorption was observed in a culture of bone marrow cells infected with ASFV strain FK-135. There was no visible hemadsorption in cultures infected in the presence of specific antiserum. However,

light microscopy of these cultures revealed cell-to-cell contacts morphologically similar to those formed in the ADCC reaction: several cells adsorbed on the target cell, forming tight contacts and rosettes which were often multilayered. Some rosettes contained erythrocytes, which were then internalized by the effector cells. This was not observed in intact bone marrow cells cultured with and without specific antiserum and in cultured cells infected with ASFV in the absence of antiserum.

Electron microscopy of ultrathin sections cut though the rosettes showed that some of them consisted of 20 cells (Fig. 1, *1*). Morphologically, 70% of these cells were macrophages, and the others were granulocytes, lymphocytes, and erythrocytes. Generally, the section was cut thought 2-4 cells contacting with each other (Fig. 1, *2*). The type of the contact depended on the cell type. Macrophages contacted with each other by large areas of the plasma mem-

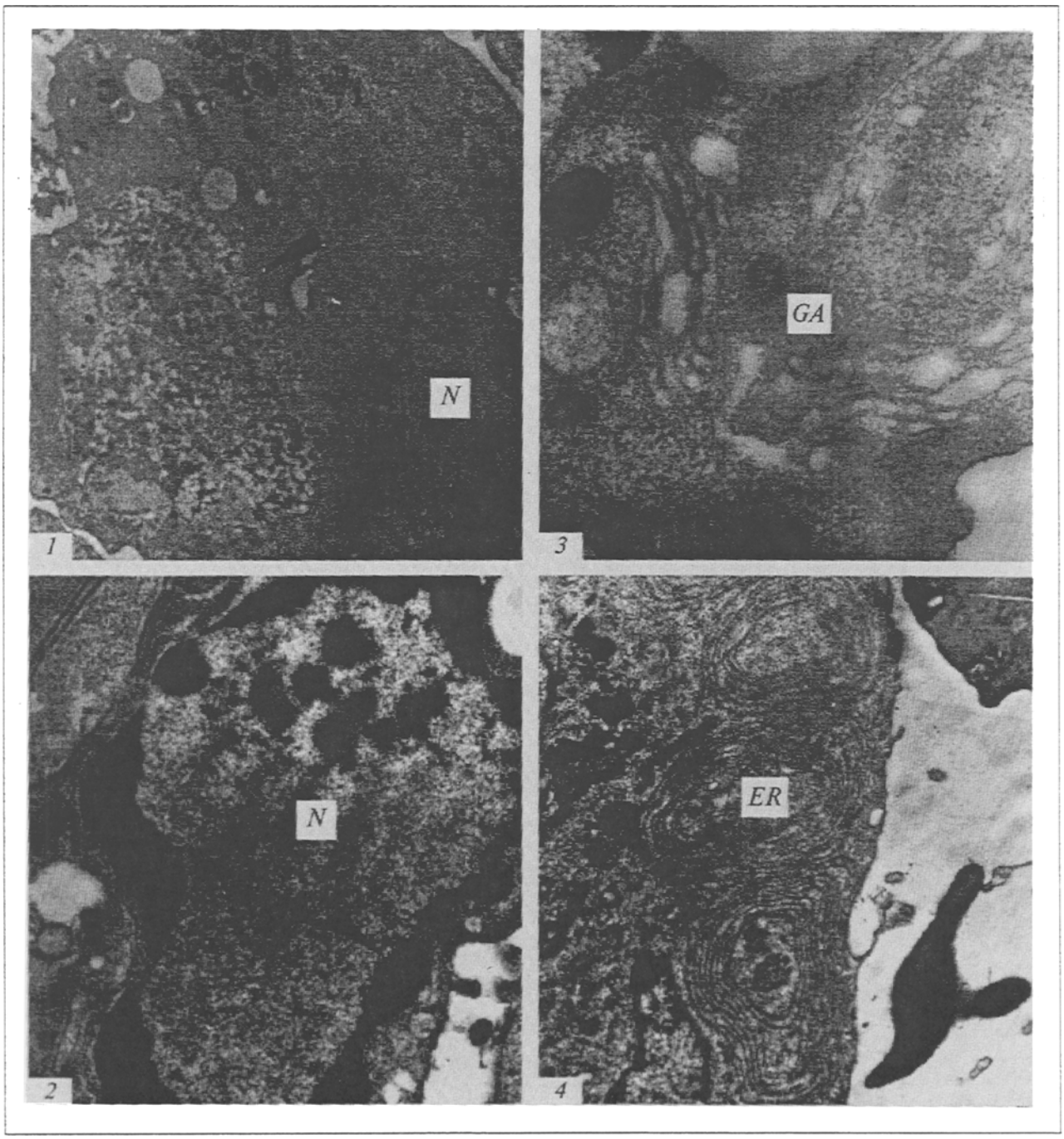

Fig. 3. Changes in ASFV-infected macrophages in rosettes. *1*) necrotized cytoplasm (arrow); *2*) condensation of heterochromatin (arrows) at the periphery of the nucleus (*N*); *3*) hypertrophy of the Golgi apparatus (*GA*); *4*) hypertrophy of endoplasmic reticulum (*ER*).

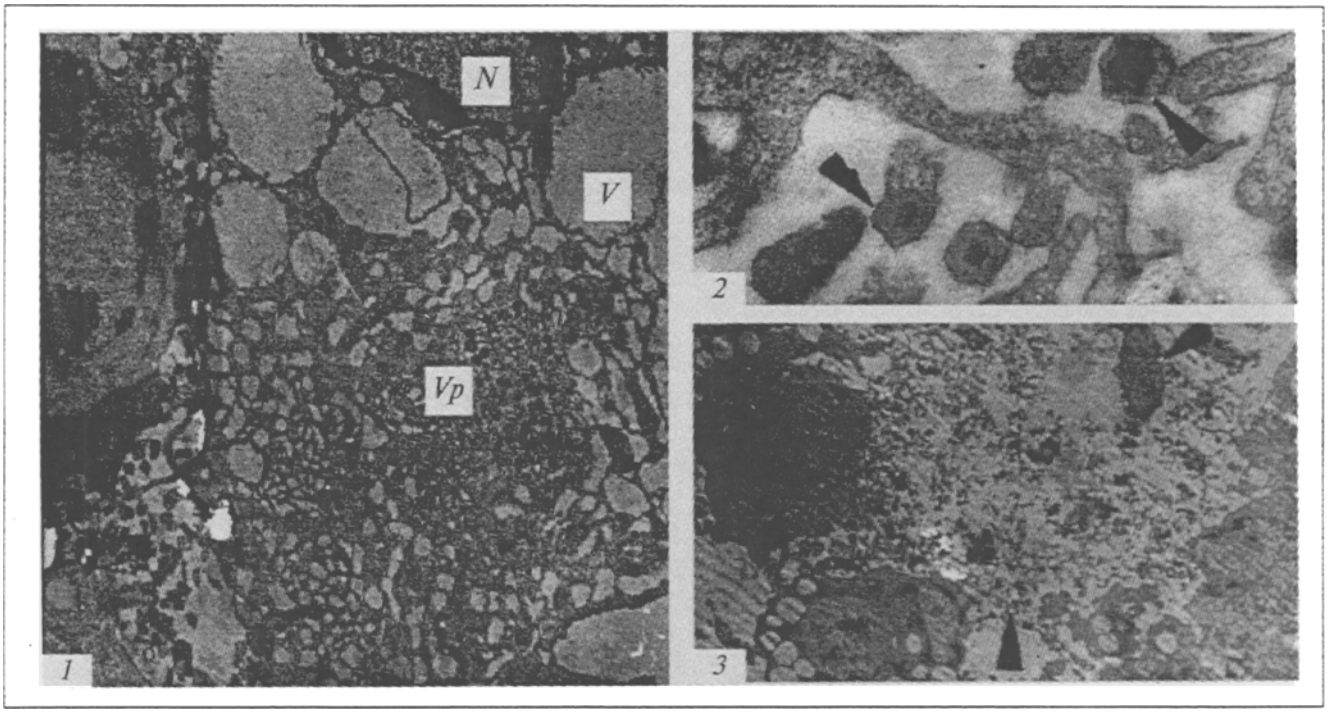

Fig. 4. Destruction of ASFV-infected macrophage (*1*). *N*: nucleus; *V*: vacuole; *Vp*: viroplast; *2*) virions (arrows) among cellular detritus; *3*) fragments of destroyed cells (arrows).

brane without processes (Fig. 2, *1*). Generally, granulocytes and lymphocytes interacted with macrophages by processes sometimes interweaved, which is typical of so-called interdigitation (Fig. 2, *2*, *3*).

Some ASFV particles and matrices were identified only in macrophages (Fig. 3, *1*). These cells were characterized by changes typical of apoptosis: peripheral condensation of nuclear chromatin (Fig. 3, *2*), hypertrophy of Golgi apparatus and endoplasmic reticulum (Fig. 3, *3*, *4*), and formation of giant apoptotic bodies: vacuoles filled with fragments of granulated cytoplasm (Fig. 4, *1*). Generally, rosette-forming cells preserved their structure and surrounded destroyed target cells; ASFV particles were seen among cellular detritus (Fig. 4, *2*, *3*). The morphology of cell-to-cell contacts in a culture of bone marrow cells from a pig infected with ASFV in the presence of specific antiserum indicates the development of ADCC under the given conditions, when all major components of this reactions are present, namely, antigen-modulated target cell, antibodies to its plasma membrane antigens, and effector cells.

The effect of ADCC on DHA was examined in a culture of CPK-66b cells infected with adapted ASFV (strain FK-135). This allowed us to exclude the effectors of ADCC, which are present in a heterogeneous culture initiated from the bone marrow. A distinct DHA was observed in cultured CPK-66b cells infected in the presence of antiserum: erythro-

TABLE 1. Titers of Hemadsorption-Delaying Antibodies in Various Cultures and Variants of Strain FK-135 ASFV

Number of antiserum	Cell culture and ASFV variant		
	Swine bone marrow		CPK-66b
	virus unadapted to CPK-66b	virus adapted to CPK-66b	
1	1:40	1:1280	1:1280
2	1:320	1:2560	1:2560
3	1:160-1:320	1:2560	1:5120
4	1:160	n.d.	1:2560
5	1:1000-1:2000	n.d.	1:8000-1:16,000

Note. n.d. — not determined.

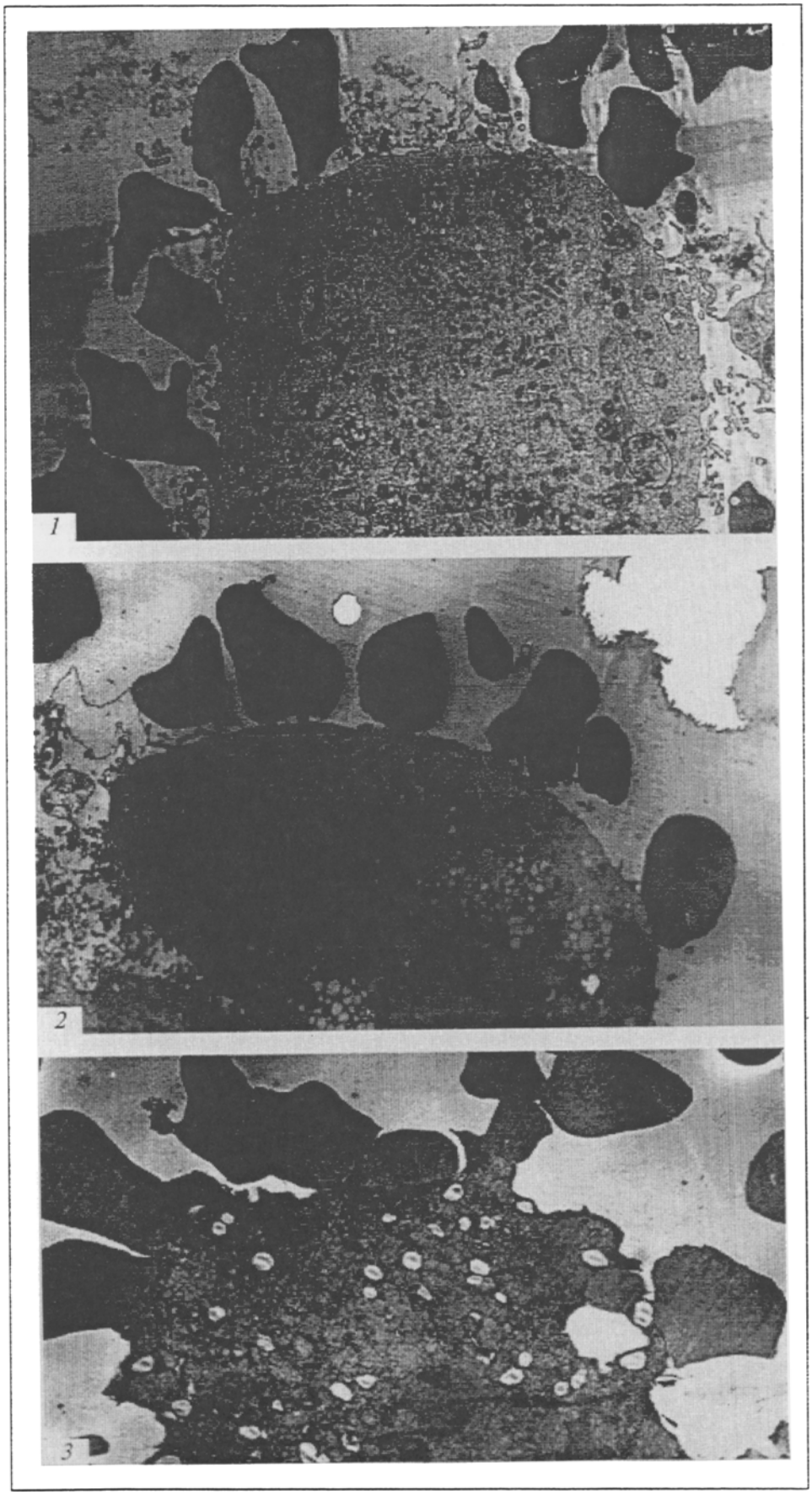

Fig. 5. Hemadsorption: *1*) in a culture of bone marrow cells infected with ASFV variant adapted to CPK-66b cells, 2) in a culture of CPK-66b cells infected with adapted variant of ASFV; 3) in a culture of bone marrow cells infected with unadapted variant of ASFV.

cytes did not attach to infected cells during a 4-day period, after which destruction of the cell monolayer started. The reaction was serotype-specific; hemadsorption induced by serotype IV FK-135 ASFV was not delayed by serotype I or II specific antisera.

The sensitivity of DHA was markedly increased in CPK-66b culture, which manifested itself as an increase in the titer of hemadsorption-delaying antibodies. This increase depended on the variant of infecting ASFV and might be associated with a lower expression of the plasma membrane antigen responsible for hemadsorption onto CPK-66b cells infected with adapted variant of ASFV compared to cells infected with unadapted virus (Table 1).

In fact, comparative electron microscopic cytometry of hemadsorption in the two cell cultures showed that the contacts between erythrocytes and the plasma membrane of bone marrow cells infected with CPK-66b-adapted ASFV were formed by 5.25± 0.62% of the circumference of infected cell (n=20) (Fig. 5, *1*). A similar length of cell-to-cell contacts (5.6±0.54%, n=20) was observed in a culture of CPK-66b cells infected with adapted virus (Fig. 5, *2*). However, in bone marrow cells infected with unadapted (wild type) virus this parameters was 34.2± 7.5% (n=20) (Fig. 5, *3*). These data imply a lower expression of the hemadsorption antigen by CPK-66b-adapted ASFV in both cultures.

Our findings indicate that ADCC and DHA are independent processes occurring in a culture of infected bone marrow cells in the presence of specific antiserum. Bearing in mind that ASFV is not neutralized during the disease, ADCC may account for the protective effect of specific antiserum which manifests itself as alleviation of the disease symptoms [11].

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