Fc Receptor Expression and Immune Phagocytosis in Chicken Monocytic Cell Lines

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Fc-receptor (FcR) on monocytes/macrophages plays important roles in phagocytosis and antibody-dependent cellular cytotoxicity. FcR is also involved in the removal of immune complexes and triggering of enzyme secretion from phagocytic cells. Monocytic cell lines have expressed FcR at various degrees in vitro. Several cytokines and phorbol esters have enhanced FcR expression of monocytic cell lines, but rarely induced FcR-mediated phagocytosis [2, 7, 8, 11]. In addition, lipopolysaccharide has been described to be inhibitory to FcR expression and FcR-mediated phagocytosis [1, 8]. Complement receptor is also reported to be involved in FcR-mediated phagocytosis [4-6, 13]. The mechanisms for the transduction of FcR-mediated phagocytosis are yet unclear. Monocytes/macrophages also play an important role in the chicken immune response. Distribution of mononuclear cells bearing FcR in various lymphoid organs of the chicken has been reported [3, 16]. The present note describes FcR expression and FcR-mediated phagocytosis in chicken monocytic cell lines, IN24 and LSCC-NP1.

IN24 cells [8], established from a spontaneous myelocytic leukemia in a chicken were cultured in RPMI1640 medium, supplemented with 10% fetal bovine serum at 37°C. LSCC-NP1 cells [9], derived from an enlarged bursa of a chicken with lymphoid leukemia were cultured in Ham's F10 medium, supplemented with 10% fetal bovine serum and 10% tryptose phosphate broth at 37°C. IN24 cells were treated with 40 U/ml human interferon-γ (IFN-γ, Chemicon International Inc., U.S.A.) or 5 nM phorbol-12-myristate-13-acetate (PMA, Sigma Chemical Co., U.S.A.) for 72 hr. For FcR and phagocytosis experiments, chicken antiserum to sheep erythrocytes (SEs) was prepared as described [8]. Antiserum was inactivated at 56°C for 45 min. SEs were incubated with antiserum at 20°C for 45 min. For FcR assay, the cells were incubated with IgG-coated SEs (IgG-SEs) at 4°C for 30 min and then were viewed microscopically for the examination of rosettes. Normal chicken serum was used as a source of complement. IgG-SEs were incubated with fresh chicken serum at 37°C for 30 min. For phagocytosis assay, the cultured cells mixed with IgG-SEs or complement-coated SEs (C-SEs) were incubated at 37°C for 60 min. For electron microscopy, the cells incubated with IgG-SEs or C-SEs were fixed in 2.5% glutaraldehyde and centrifuged to form a pellet. Pellets were post-fixed in 2% osmium tetroxide for 60 min at 4°C. The pellets were dehydrated in ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

Both LSCC-NP1 and IN24 were adherent cell lines and expressed FcR as indicated by the rosette-formation with IgG-SEs. Almost all the LSCC-NP1 cells and about 65% of IN24 cells showed FcR-positive in the routine cultures. LSCC-NP1 cells also showed active phagocytosis of IgG-SEs. Most of the LSCC-NP1 cells ingested many rosette-forming IgG-SEs after incubation for 60 min (Fig. 1). By comparison, IN24 cells showed weak phagocytosis of IgG-SEs. Although about 65% of the IN24 cells exhibited rosette-formation with IgG-SEs (Fig. 2), only 20% of the cells ingested a few IgG-SEs after incubation for 60 min. A large number of FcR-positive IN24 cells failed to ingest binding IgG-SEs. However, IN24 cells showed active phagocytosis of C-SEs. About 60% of the cells ingested many C-SEs after incubation for 60 min. The ratio of IN24 cells showing phagocytosis of C-SEs was level to that of FcR-positive cells.

Electron microscopically, LSCC-NP1 cells relatively showed mature characteristics compared to IN24 cells. Lysosomes and phagocytic vacuoles were frequently seen in the cytoplasm of LSCC-NP1 cells (Fig. 3). However,
Fig. 2. IN24 cells showing rosette-formation with IgG-SEs. Most of binding IgG-SEs are not ingested. × 100.

Fig. 3. LS3C-NP1 cell after incubation for 60 min with IgG-SEs. Ingested IgG-SEs are surrounded with lysosomes. × 6,600.

Fig. 4. LS3C-NP1 cell showing ingestion of IgG-SE (SE). Aggregated microfilaments (arrow head) are seen near IgG-SE. × 21,000.

Fig. 5. IN24 cells after incubation for 60 min with IgG-SEs. IgG-SEs are entrapped by microvillous projections, but not ingested. × 6,600.
there were fewer lysosomes in IN24 cells, which had abundant free ribosomes (Figs. 5 and 6). Many IgG-SEs were ingested by LSCC-NP1 cells after incubation for 60 min (Fig. 3). Ingested IgG-SEs were surrounded by lysosomes. Bundles of microfilaments were also frequently seen between the lysosomes and near ingested IgG-SEs (Fig. 4). IgG-SEs were entrapped by microvillous projections of IN24 cells, but not ingested into the cytoplasm (Fig. 5). Microfilaments were dispersed in the cytoplasm. This finding indicated that microfilament organization was not induced in IN24 cells by binding of IgG-SEs. On the other hand, many C-SEs were ingested by IN24 cells after incubation for 60 min (Fig. 6). Bundles of microfilaments were also frequently seen in the cytoplasm.

IN24 cells treated with PMA or IFN-γ for 72 hr showed cellular differentiation. Morphologically, development of filopodium-like projections and cell organelles was discernible. Phagocytic vacuoles were frequently seen in the extended cytoplasm. Although all of the cells strongly expressed FcR, only 30% of the cells showed phagocytosis of rosette-forming IgG-SEs. Most of FcR-positive IN24 cells induced by IFN-γ or PMA did not show significant phagocytosis of IgG-SEs.

LSCC-NP1 cells showed FcR expression and active immune phagocytosis. By comparison, IN24 cells expressed FcR, but showed weak immune phagocytosis. FcR-mediated phagocytosis by monocytes/macrophages may be divided into binding, ingesting and digesting processes. Cytoskeletal organization is thought to play an important role in ingesting process of opsonized particles [13, 14, 17]. Electron microscopically, bundles of microfilaments were frequently seen in LSCC-NP1 cells ingesting IgG-SEs, but not in IN24 cells. Binding of IgG-SEs to FcR did not act as the triggering of cytoskeletal organization in IN24 cells. The reason for functional differences of FcR expressed on LSCC-NP1 and IN24 cells is unclear.

Morphologically and functionally, IN24 cells have possessed an immature cellular character compared to LSCC-NP1 cells and shown cellular differentiation in response to cytokine derived from mitogen-treated chicken T-lymphocytes and IFN-γ [8, 9]. PMA and IFN-γ enhanced FcR expression of IN24 cells. However, FcR-positive IN24 cells induced by IFN-γ and PMA did not show significant immune phagocytosis. It has been reported that PMA and IFN induce an augmentation of FcR expression in monocyte cell lines and monocytes/macrophages [2, 7, 11]. However, PMA has not predominantly augmented FcR-mediated phagocytosis in these cells. The finding concerning the effect of IFN on FcR-mediated phagocytosis has been controversial [15, 18]. We have previously shown that mitogen-stimulated chicken spleen cell culture-medium augments FcR expression of IN24 cells, but are not sufficient for the induction of immune phagocytosis [8]. Recently, phagocytosis inducing factors derived from T-lymphocytes [12] and neutrophils [10] were reported. These factors enhanced FcR-mediated phagocytosis, but did not induce the other macrophage functions. FcR expression and FcR-mediated phagocytosis are thought to be distinct steps during cellular differentiation.

Phagocytosis of immune complexes by macrophages is thought to be mediated by FcR. Although macrophages can not ingest particles via their complement receptor, complement receptor-mediated binding enhances FcR expression and FcR-mediated phagocytosis [4–6, 13]. IN24 cells showed active phagocytosis of C-SEs. Bundles of microfilaments were frequently seen in IN24 cells ingesting C-SEs. The binding of C-SEs could act as the triggering of cytoskeletal organization in IN24 cells. Complement receptor-mediated binding may be required for the development of functional FcR during the differentiation of IN24 cells.

REFERENCES