NEW BIOMEDICAL TECHNOLOGIES

Immunocytochemical Localization of the α-Subunit of G-Proteins in Macrophages and *Escherichia coli* Interacting with Each Other

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The immunocytochemical localization of the α-subunit of G-proteins is established in murine macrophage-like P388D1 cells, in *E. coli,* and in L-forms of *E. coli.* It is shown that the cytoplasmic concentration of G-proteins is increased in macrophages interacting with the bacteria.

Key Words: *G-proteins; phagocytosis; E. coli; immunocytochemistry*

Heterotrimeric proteins (G-proteins) capable of binding guanine nucleotides mediate signal transduction from an activated cell receptor to the effector (adenylate cyclase, ion channels, etc.) [2]. Lipopolysaccharides, the major component of the cell wall of Gram-negative bacteria, serve as primary messengers along with other physical and chemical factors. Lipopolysaccharides stimulate the production of inflammation mediators by macrophages (MPH) via activation of the α-subunits of G-proteins and generation of second messengers [6,10]. The uptake of bacteria and endocytosis are associated with components of the cytoskeleton, primarily with the microtubules, which are controlled predominantly by G-proteins [8].

G-proteins have been found in a wide variety of mammalian, plant, and microbial cells; however, their localization in bacteria is unknown and their role in the interactions between bacterial and eukaryotic cells has not been investigated in sufficient detail. In the present study, we have demonstrated an increase in the cytoplasmic concentration of G-proteins in MPH interacting with bacterial cells. In addition, we have located for the first time G-proteins in *E. coli* and its L-forms.

MATERIALS AND METHODS

Murine P388D1 MPH were incubated for 96 h in modified Dulbecco's medium containing 10% fetal calf serum (Gibco). The suspended cells were washed three times with phosphate-buffered saline (PBS), the adherent cells were collected in PBS containing 0.02% EDTA, washed three times with PBS, and resuspended in PBS to a final concentration of 10^6 cells/ml. Pooled cells were incubated with *E. coli,* L-forms of *E. coli,* or latex particles (diameter 0.5-1.0 μ) for 1 h at 25°C.

After incubation with the bacteria or latex, a drop of the MPH suspension was transferred to a glass slide, air dried, fixed with absolute methanol or acetone for 5 min at room temperature, and washed in PBS-T

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(10 mM phosphate buffer, 0.5 M NaCl, and 0.1% Tween-20, pH 7.6). For localization of the α-subunit of G-proteins the samples were treated with rabbit antisera to the α_{common}-subunit of G-proteins (anti G-protein, GA/1, Du Pont) (primary antibodies). The bound primary antibodies were visualized with the use of a phycoerythrin-streptavidin kit (Amersham) for fluorescence microscopy, an avidin-biotin-peroxidase complex kit (Vectastain Elite, ABC-kit, Camon Labor-Service) for microscopy in the light field, and Nomarskii differential phase contrast microscopy in an Opton microscope with the appropriate filters [9].

Western blotting was performed to identify the type of α-subunit of bacterial G-proteins and to assess the specificity of primary antibodies (Fig. 1). Lysates of *E. coli* and their L-forms (30-35 μg protein per sample) were obtained by treatment with sodium dodecyl sulfate (SDS), and electrophoregrams were developed by polyclonal antibodies to the α_{common}- (GA/1), α_s- (stimulating, RM/1), and α_i- (inhibiting, AS/7) subunits of G-proteins (Du Pont) [9].

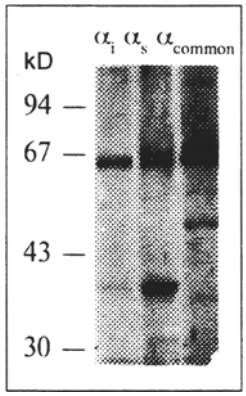

Fig. 1. Immunoblot of SDS lysate of *E. coli*. Incubation with antibodies against α_i-, α_s-, and α_{common}-subunits of G-proteins.

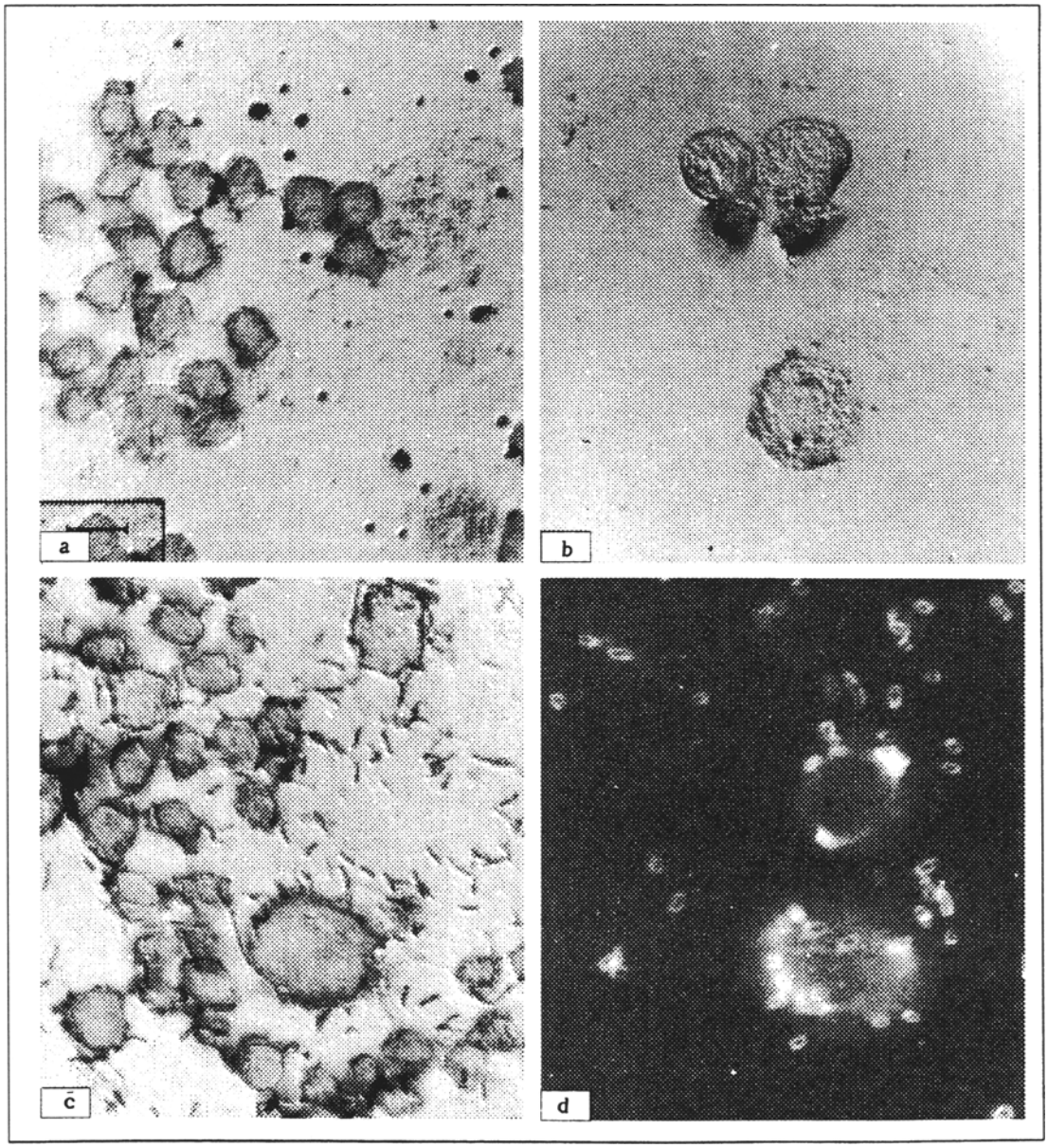

Fig. 2. Immunohistochemical localization of the α_{common}-subunit of G-protein in macrophages incubated for 60 min with L-forms of *E. coli* (*a*), *E. coli* (*b, d*), and latex particles (*c*). Primary antibodies were visualized with secondary antibodies conjugated with biotin-avidin-peroxidase (a-c, Nomarskii's interference phase contrast) or phycoerythrin (*d*).

RESULTS

Two cell types were found in the culture of P388D1 MPH: small MPH with a narrow cytoplasmic halo and condensed nuclear chromatin and large MPH with a wide cytoplasmic halo and diffuse nuclear chromatin. In small MPH G-proteins were localized in the plasmalemma and predominantly in the perinuclear space, while in large MPH they were confined to cytoplasmic vacuoles. Small MPH interacted with *E. coli*, their L-forms, and latex particles more actively than large MPH. The specific G-protein reaction was more intense in the plasmalemma of MPH with adherent bacteria, L-forms, and latex particles and in the forming phagosomes (Fig. 2, *a-c*). This was particularly evident in the samples where the fluorescent probe was applied (Fig. 2, *d*).

When the peroxidase and phycoerythrin techniques were employed, a positive staining for the α_{common}-subunit was observed in the cell wall of *E. coli* and in the midbody of dividing bacteria; in L-forms the staining was confined to the plasma membrane and the cytoplasm. In some bacteria the fluorescent marker was localized at the poles. The specificity of the immunochemical reaction was confirmed by the absence of positive staining in preparations incubated without primary antibodies.

The presence of G-proteins not only on the plasma membrane but also in the cytoplasm of MPH is consistent with recent evidence that these proteins are involved in membrane transport and endo-, and exocytosis [3]. Moreover, it has been hypothesized that in the evolutionary sense the participation of the heterotrimeric G-proteins in the control over the intracellular membrane transport is primary, while their involvement in the transduction of the extracellular signal is secondary [3]. The discovery of antigenic sites binding anti-G proteins in the cytoplasm of the bacterial cell merits closer study. There is evidence that bacteria contain compounds similar to mammalian hormones and receptors for such hormones as insulin, neurotensin, and catecholamine that modulate the growth of Gram-negative bacteria [7]. In addition, adenylyl cyclase, the main effector protein of hormones, has been found in bacterial plasma membranes [5]. This suggests the presence of an intermediate between hormone receptor and effector protein, for example, heterotrimeric G-proteins or other GTP-binding proteins (*ras*-proteins). Immunoblotting of bacterial lysates provides additional evidence favoring the existence of G-proteins in *E. coli*. It reveals a band in the 40 kD region reacting primarily with antibodies to the α_s-subunit (stimulatory, RM/1) of G-proteins. It is noteworthy that the GTP/GDP-binding FtsZ-protein detected in *E. coli* and termed an essential protein of bacterial cell division [1] is localized in this same area. In addition to the typical bands in the 40 kD region, where the α-subunits of mammalian G-proteins are located, bands above 60 kD and below 30 kD are revealed in *E. coli* lysates. This indicates that there are proteins cross-reacting with the antibodies to the α-subunits of G-proteins. Bearing in mind the conservative structure of GTP-binding proteins and their possible origin from a common primordial protein [4], we can postulate that these proteins have similar epitopes binding anti-G-protein antibodies and belong to the GTPase superfamily which, in addition to G-proteins, includes the low-molecular-weight *ras*- and *rab*-proteins.

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