The bacterial lysate Lantigen B reduces the expression of ACE2 on primary oropharyngeal cells

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Abstract

Background: Vercelli and coworkers recently observed that a well-established bacterial lysate (OM-85, Vifor Pharma; CH) was able to downregulate the expression of Angiotensin-Converting Enzyme 2 (ACE2) on epithelial cells. This downregulation was also associated with a reduced infectivity of cells, resulting in a reduced viral titre. We evaluated whether another bacterial lysate (Lantigen B, Bruschettini Ltd; Italy) may have similar activities.

However, while OM-85 is given per os and has a systemic effect after absorption at the gut level, Lantigen B is given locoregionally. Thus, the concentration that the bacterial lysate can reach at the mucosal level seems to be promising.

Methods: Oropharyngeal cells were collected from healthy donors. After 24 hours of treatment in vitro with doses of Lantigen B comparable to those that are reached in vivo, the expression of ACE2 was evaluated by direct fluorescence and flow cytometry.

Results: A reduction in the number of ACE2-positive cells was observed in 80% of treated samples. Only a few donors had poor expression of ACE2, and in these donors, the downregulation was less evident or absent.

Conclusions: These results suggest that Lantigen B, at pharmacological doses, could be an interesting drug to reduce ACE2 expression on oropharyngeal cells, thus contributing to the prophylaxis of COVID-19 in humans.

Trial registration

Not applicable

Keywords

SARS-CoV-2; ACE2; COVID-19; Bacterial lysates; Lantigen B

Background

The cell surface molecule angiotensin-converting enzyme 2 (ACE2) is the main receptor for SARS-CoV-2 spike (S) protein attachment [1, 2]. The binding of the spike protein to the ACE2 receptor, together with other mechanisms, allows the fusion of the virus and the cell membrane, thus infecting the target cell. ACE2 has been shown in many different cell types,

including nasal, intestinal, kidney, testis, gallbladder, and heart cells, and, to a lesser extent, tongue, tonsil, and the olfactory region cells [3, 4]

The COVID-19 pandemic has pushed research to the limit, and in a few months, not only advanced protocols for virus identification but also active vaccines have been developed and introduced in humans [5, 6]. At present, few monoclonal antibodies are active against all viral variants [7], and novel drugs have been proposed or are under development [8].

In a recent interesting article [9], the authors showed that a bacterial lysate developed decades ago (OM-85) has the capability of reducing the expression of ACE2 both in cell lines and in primary cells from human airways. This finding suggests that the administration of OM-85, which would reduce the expression of ACE2, could to some extent inhibit respiratory tract target cell infection, acting as a prophylactic treatment to be used in association with vaccines and other future therapies.

Bacterial lysates have been shown to have the capacity to reduce the number of respiratory tract infections (or exacerbations) in treated patients [10, 11]. The detailed mechanisms are not completely described, although the capacity for activating dendritic cells, inducing a specific and functionally effective immune response, and recruiting innate immune-competent cells has been observed in the past [12, 13]. In addition, it has been shown that epithelial cells are able to recognize certain bacterial lysates (such as PMBL), resulting in epithelial cell activation, proliferation and tight junction sealing [14]. Lantigen B is a bacterial lysate that was originally developed in the 1960s and has been empirically used in recent decades to reduce the number of respiratory tract infections during the winter season. A recent clinical trial has clearly demonstrated the clinical activity of Lantigen B [15]. Lantigen B is administered under the tongue; thus, the drug enters into direct contact with the epithelial cells of the mouth and the oropharynx. If ACE2 expression modulation is a characteristic of bacterial lysates (and not only of OM-85), a possible effect of Lantigen B on

the same protein could be hypothesized. In this report, we show that epithelial cells of the oropharynx express ACE2 and that Lantigen B is able to reduce the expression of ACE2 after a 24-hour treatment at doses comparable to those used in vivo.

Methods

Epithelial cells of the oropharynx were collected from a group of researchers and employees (14 males and 16 females, age ranging from 35 to 73) at Bruschettini Ltd, the Italian firm that produces Lantigen B. All donors gave verbal informed consent. Donors were first screened for the presence of SARS-CoV-2 antigens (using a rhino-pharyngeal swab and a standard antigenic lateral flow cassette). All donors were negative for SARS-CoV-2 infection and thus were recruited in the different sessions of the study. Epithelial cells were then collected by a sterile swab of the oropharynx of the donors. The cells were washed in saline and then resuspended in RPMI 1640 with 10% foetal bovine serum and gentamycin. The vitality of the cells was scored by trypan blue exclusion.

The expression of ACE2 on epithelial cells was measured by a specific anti-ACE2 monoclonal antibody (R&D Systems, labelled with Alexa Fluor 488) and flow cytometry (Cytoflex, Beckman Coulter). An isotypic control (FITC-labelled anti-CD83, Beckman Coulter) was used to evaluate the possible nonspecific binding to epithelial cells. To evaluate whether the modulation of ACE2 can be considered in the context of a more general mechanism, another surface molecule (CD54 – ICAM-1, receptors of rhinoviruses) [16] was used. These experiments were repeated three times using three different cohorts consisting of six donors. Polystyrene six micrometre microspheres (Polysciences, USA) were used to calibrate the flow cytometer and to evaluate the cell size.

Modulation of ACE2 by Lantigen B. Cells collected from donors were washed with saline and resuspended in RPMI (Euroclone, Pero, Italy) as described, and different

experimental conditions were provided. First, cells were incubated overnight in RPMI with FBS (HyClone, Sigma, USA) and gentamycin in the absence of any stimulus. Second, the cells were incubated with Lantigen B diluent containing polysorbate 80, chlorhexidine diacetate and sodium methyl parahydroxybenzoate. This condition was considered the experimental negative control. Third, the cells were diluted 1:1 with the drug used in humans. The drug contained the particulate and soluble fraction obtained by the chemical lysis of six different microbes of the respiratory tract (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis* and *Haemophilus influenzae*). After 24 hours of culture, the cells were collected, stained with the anti-ACE2 monoclonal antibody, and analysed on a cytofluorimeter. Vitality was evaluated for the same cells using trypan blue dye exclusion. The test was performed on 10 different donors in three different sessions.

Results

Expression of ACE2 on normal oropharyngeal cells. As described, the first series of experiments was focused on the identification of ACE2 on normal oropharyngeal cells immediately after cell collection with an oropharyngeal swab. Indeed, only in the presence of clear ACE2 expression could any attempt to downregulate this expression be started. Figure 1 shows the expression of ACE2 in the FITC channel and the same population treated with an isotypic control. As described, the assay was performed on cells from 18 different donors. ACE2 was expressed on the surface of oropharyngeal cells with a certain heterogeneity. The median fraction of ACE2-positive cells was 60% (ranging from 52 to 80%). Few samples were characterized by a reduced percentage of ACE2-positive cells (ranging from 37 to 47%). The intensity of fluorescence was more homogeneous. Of note, epithelial cells were large; the diameter measured using 6 micrometre plastic beads on the forward scatter channel

ranged between 18 and 56 micrometres. Under these conditions, the very large surface area caused a strong signal in the FITC channel. Additionally, autofluorescence was high but significantly lower than that of ACE2 mAb-stained cells.

Modulation of ACE2 on normal oropharyngeal cells after incubation with Lantigen B. Using the same conditions of the first experiments, donor oropharyngeal cells were tested for the presence of ACE2 after 24 hours of incubation in the presence and absence of Lantigen B. Treatment with Lantigen B resulted in a reduction in the number of ACE2-positive cells in 74% of samples (median reduction 44%, ranging from 18% to 69.5%). A reduction in the fluorescence intensity (representative of the number of surface molecules) was observed in 65% of samples (median reduction 12.4%, ranging from 4.2 to 43%). Figure 2 shows the results of the two different donors. Notably, not all samples where the percentage of ACE2-positive cells was reduced also showed a reduction in fluorescence intensity.

CD54 (ICAM-1) was used as a control, being a rhinovirus family receptor. Despite being well expressed on the surface of nearly all oropharyngeal cells, its expression intensity, as well as the number of positive cells, was unmodified by the administration of Lantigen B in this experimental setting.

Discussion

A recent article by Vercelli and coworkers showed that a bacterial lysate (OM-85) has the capability of reducing the expression of ACE2 on epithelial cells after in vitro culture. In the same experiments, it was demonstrated that OM-85 inhibited SARS-CoV-2 infection of epithelial cells. This result is interesting because it can be hypothesized that drugs with this capability can be used in the context of the prophylaxis of COVID-19. Even more interestingly, it has been demonstrated that vaccination induces a strong systemic immunological response lasting months. However, in the same patients, the locoregional

response at the mucosal level is shorter [17, 18]. This finding could be one of the explanations for the inability of vaccination to prevent reinfections, while severe disease is better controlled by systemic immunity. Thus, already available drugs that can reduce the infection of cells, representing the first barrier, are extremely useful. However, these results were obtained using high concentrations of the bacterial lysate (from 0.24 to 1.92 mg/mL), doses that seem to be difficult to reach in vivo, considering that a single pill of OM-85 contains 7 mg of the active principle.

Lantigen B is administered at the mucosal level, and this could be one added value of this treatment. Indeed, the assays performed in vitro on freshly isolated oropharyngeal epithelial cells indicate that a significant reduction in expression is achieved using concentrations of the drug that are easily reached in vivo. In addition, the observed reduction is observed after 24 hours, while OM-85 has effects observed after a longer period in the experimental conditions described.

This effect seems to be strictly related to the presence of the bacterial antigens because the excipients and preservatives have no effect when used alone. Furthermore, the surface expression of CD54, a rhinovirus receptor, was unmodified by in vitro treatment with Lantigen B, thus suggesting that, at least in these experimental conditions, the downregulation of ACE2 seems to be bacterial lysate specific.

The major limitation of this study is represented by the absence of any assay of SARS-CoV-2 infection of oropharyngeal cells and its inhibition mediated by Lantigen B. However, even if not formally demonstrated, this seems to be highly possible at least comparing the results of the reduction in the expression of ACE2 in these experiments with those described by Vercelli et al.

In conclusion, Lantigen B, a bacterial lysate used for decades in the prophylaxis of recurrent respiratory infections, has well-established activity in reducing the number of

infections in clinical trials. Our novel findings add that Lantigen B has activity that could be extremely useful in the prophylaxis of COVID-19 (or at least in the prevention of SARS-CoV-2 asymptomatic infections), particularly in the period that seems to be less protected by vaccines. Thus, in addition to the well-known effects of bacterial lysates on the adaptive and innate immune response, the finding of this capability of Lantigen B adds to the mechanism of action of bacterial lysates.

List of abbreviations

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

ACE2: Angiotensin-Converting Enzyme 2

COVID-19: COronaVIrus Disease-2019

ICAM-1: Intercellular Adhesion Molecule 1

FITC: Fluorescein isothiocyanate

mAb: monoclonal Antibody

Declarations

Ethics approval and consent to participate

The donors involved in this study were all employees of Bruschettini ltd, were all volunteers and had gave an verbal informed consent to collect oropharyngeal cells by a swab.

Consent for publication

Not applicable

Availability of data and materials

The experimental design and the raw data are all included in the laboratory files of Bruschettini ltd. Data are available upon request.

Competing interests

CP, ADA, PP and GR are employees of Bruschettini ltd. GM is a consultant.

Funding

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Authors' contributions

CP, ADA and PP carried out the in vitro experimental study. CR and GM designed the study. GM wrote the manuscript.

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CP, ADA and PP are biologists or biotechnologists and carried with expertise in the field of cellular biology, immunology and quality controls of Bacterial Lysates. CR is the Medical Director of Bruschettini, expert in clinical trials on Bacterial lysates. GM is a scientist active in the field of immunology, virology, allergy, and clinical pathology since late seventies. In the last two decades, GM has focused on the mechanism of action of bacterial lysates and their clinical efficacy.

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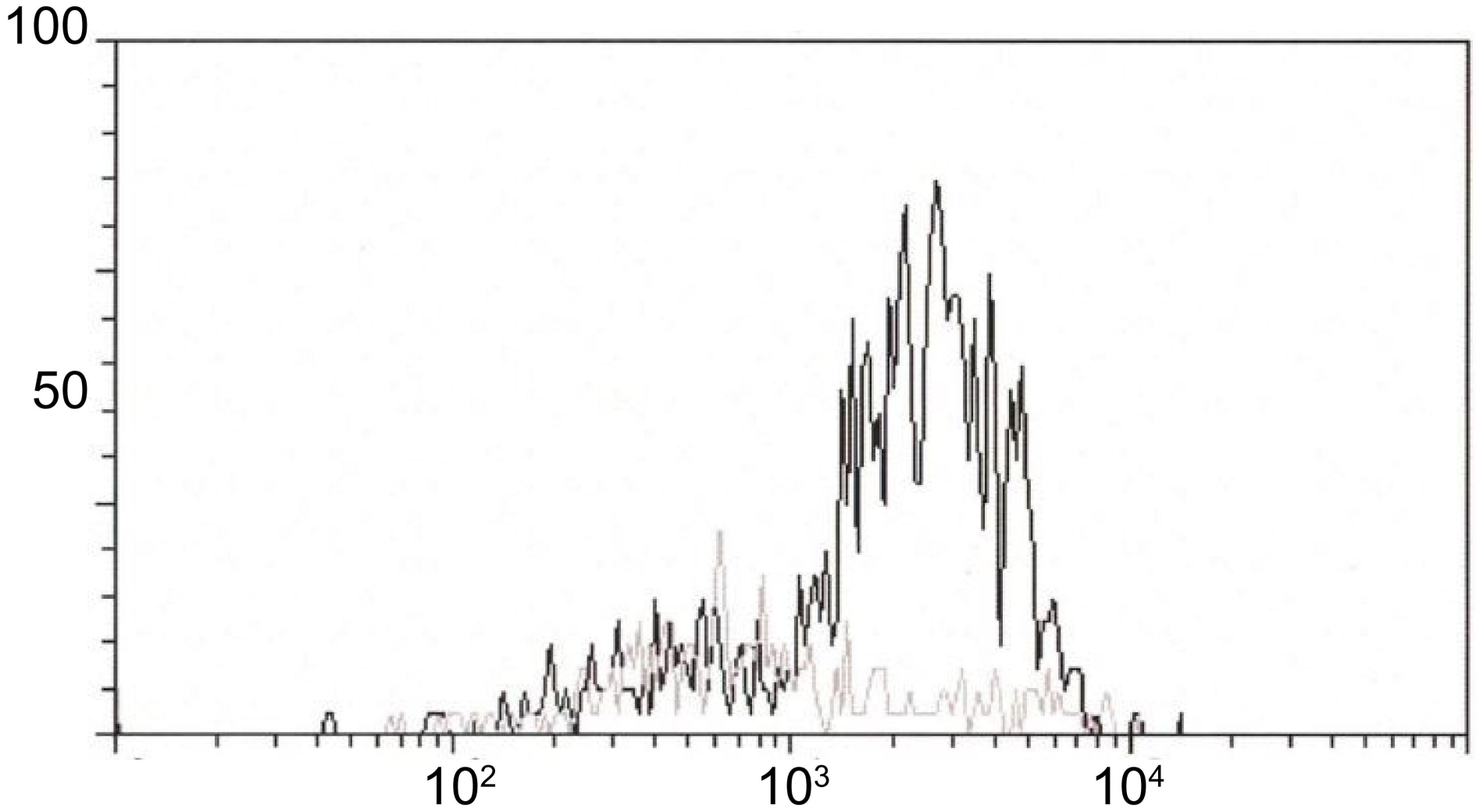
Figures legend

Figure 1. Demonstration of the presence of the ACE2 protein on the surface of oropharyngeal cells. Black profile represents the distribution of ACE2 on cell surface. In grey, the isotypic negative control shows weak autofluorescence.

Figure 2. Downregulation of ACE2 on oropharyngeal cells. The analyses were performed on oropharyngeal cells after 24-hour incubation in the presence of pharmacological doses of Lantigen B. In black, cells from donors treated with drug excipients alone. In grey, cells from donors after treatment with the drug. Panel A shows a representative example of the downregulation observed in 74% of donor samples. In Panel B, a different behaviour (absent or poor reduction in the expression of ACE2) was observed in the remaining samples.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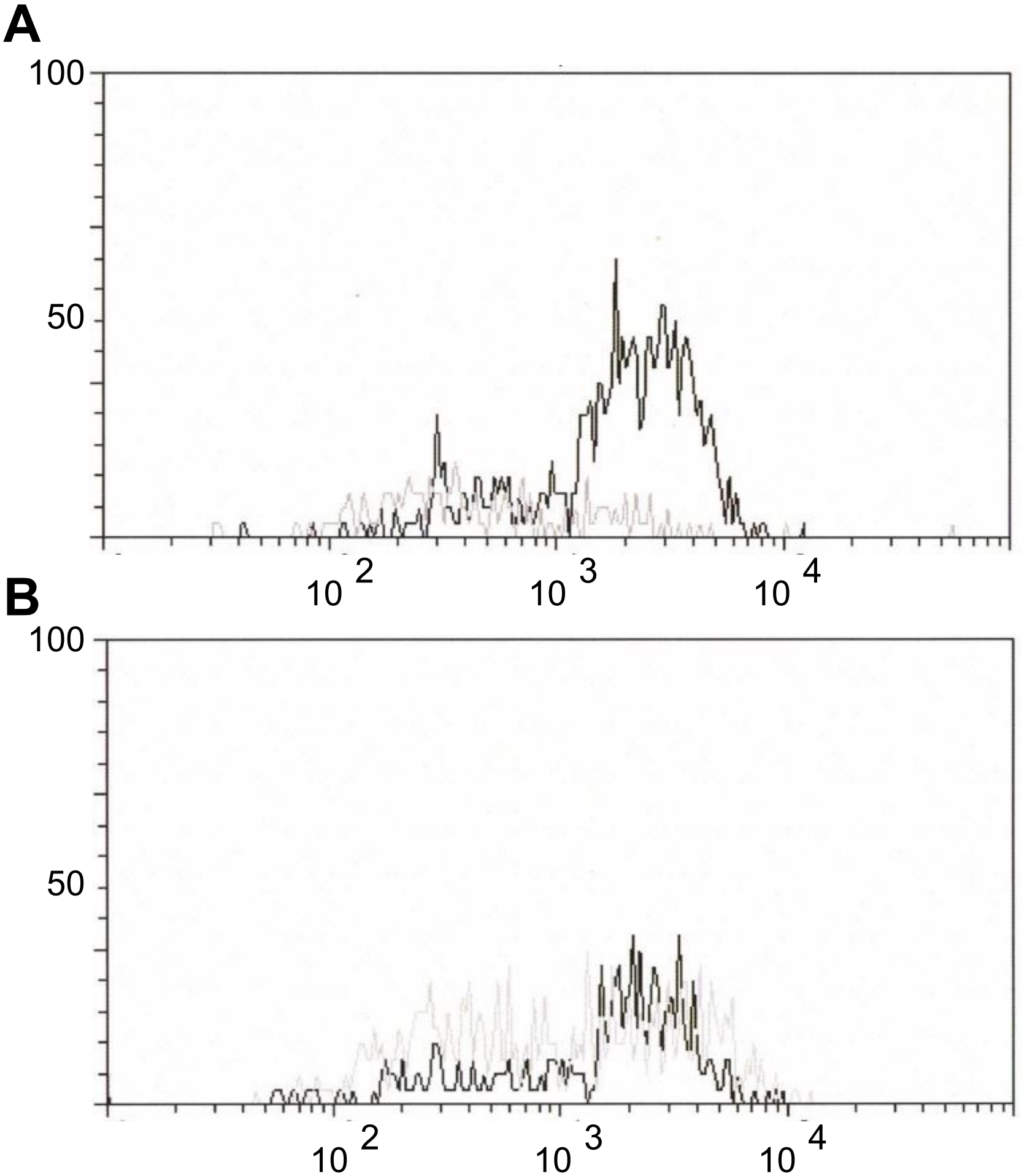
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