Persistence of *Staphylococcus aureus* L-form during experimental lung infection in rats

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Abstract

The course of pulmonary infection in rats infected by intranasal inoculation with a *Staphylococcus aureus* stable protoplast L-form was studied. Blood and bronchoalveolar samples were taken on days 3, 7, 14 and 30 after challenge and were investigated by microbiological, electron-microscopic, cytochemical and cyto-metric methods. The electron microscopic data and isolation of L-form cultures from bronchoalveolar samples at all experimental times demonstrated the ability of *S. aureus* L-form cells to internalize, replicate and persist in the lungs of infected rats to the end of the observation period, in contrast to the *S. aureus* parental form. It was found that persisting L-form evoked ineffectual phagocytose by alveolar macrophages and low but long-lasting inflammatory reaction in rats. The experimental model of pulmonary infection with *S. aureus* L-form suggests that the cell-wall-deficient bacterial forms may be involved in the pathogenesis of chronic and latent lung infections.

Introduction

*Staphylococcus aureus* is a major human and animal pathogen of increasing importance as a result of the spread of antibiotic resistance (Schito, 2006). It causes a wide range of diseases (arthritis, osteomyelitis, dermatitis, mastitis), and causes bacteremia, abscesses, toxemia and sepsis. The bacteria can survive outside the host by virtue of their adaptability and resistance to environmental conditions. *Staphylococcus aureus* is one of the most common infectious agents of pneumonia (Al-Ujayli *et al*., 1995). Staphylococcal persistence has been reported for several chronic pulmonary infections such as chronic bronchitis, emphysema and obstructive pulmonary disease (Bobkov, 1989; Crystal, 1991; Theegarten *et al*., 1999). The consequences of chronic lung inflammation as a response to the persistent pathogen may lead to considerably reduced lung function. Some resistant *S. aureus* strains are among the frequently identified pathogens in bronchopulmonary infections (Watanabe *et al*., 2000), and they might be also related to live bacteria, which actively replicate inside respiratory epithelial cells (Kahl *et al*., 2000). It has also been reported that ‘unusual’ bacterial forms occur inside host tissues and cells (Vesga *et al*., 1996), under a variety of conditions, especially at inducing concentrations of antibiotic agents (Theegarten *et al*., 1999; Bukharin, 2000). The development of antibiotic resistant and formation of L-forms during treatment with some β-lactam antibiotics are additional reasons for therapy failures. L-forms derived from *S. aureus* strains have been shown to be highly resistant to antibiotics of which the primary site of action is upon cell wall (Barbuti, 1976). Some studies indicated that persistent bacterial cell-wall-deficient forms (L-forms) could be the probable agents for the atypical pulmonary infections (Prozorovsky *et al*., 1981). They have been isolated from clinical specimens of patients suspected to have chronic infections (Jiang *et al*., 1994; Zhang *et al*., 1995; Guan *et al*., 1998; Mattman, 2001). These organisms could survive and persist in a latent state within the host and cause a pathologic response compatible with disease (Domingue & Woody, 1997).

Bacterial L-forms are difficult to identify by ordinary microbiological methods and light microscopy, and some antibiotic treatments do not act upon them. The mechanisms leading to the incidence of *S. aureus* L-form respiratory tract infections are largely unclear, and host-dependent factors are most probably involved. Little is known about the host-microbial interactions of cell-wall-deficient bacteria and their role as stealth pathogens. Our previous study indicated that induction and survival of *S. aureus* L-form could emerge spontaneously in vivo (Michailova *et al*., 2000).
Persistence of *S. aureus* L-form

The role and specific properties of stable protoplast-type *S. aureus* L-form in induction, development and persistence of pulmonary infection are still insufficiently investigated, the aim of this study was to examine the fate of *S. aureus* L-form in the lungs of intranasal infected rats.

**Materials and methods**

**Bacterial strains**

*Staphylococcus aureus* BM 3041 strain (parental, S-form) and its stable protoplast L-form, a kind gift from INSERM U. 65, France, were used in this study. The strain’s characteristics were described by El Solh & Schmitt-Slomska (1985). The L-forms were cultivated in tryptic soy broth (TSB, Difco) supplemented with 1% yeast extract, 2% pleuropneumonia-like organisms (PPLO) serum fraction (Difco) and 3% sodium chloride at 37 °C for 48 h. The normal S-form of *S. aureus* BM3041 was cultivated in TSB at 37 °C for 18 h. Before use, the bacterial culture was centrifuged, resuspended in sterile pyrogen-free saline so that it contained c. 10⁷ CFU mL⁻¹. CFU units in the L-form suspension used for infection were controlled by plating on semisolid tryptic soy agar (TSA) supplemented with 1% (w/v) yeast extract, 2% (v/v) PPLO serum fraction (Difco) and 3% (w/v) sodium chloride. The plates were incubated at 37 °C for 48 h, and the colonies with typical for L-forms ‘fried egg’-like shape were enumerated.

**Experimental procedure**

Male and female Wistar rats weighing 200 g, at 4 weeks of age, were used. The animals were divided into three groups: (1) those infected with the L-form of *S. aureus*; (2) those infected with the normal S-form of *S. aureus*; and (3) control – those infected with saline. All rats were anesthetized with ether, and 0.2 mL of bacterial suspension was instilled unilaterally into the nostril cavity with a 22-gauge feeding needle. Control rats received the same quantity of sterile saline. On days 3, 7, 14 and 30 after challenge, five animals per interval were sacrificed and samples of bronchoalveolar lavage fluid were removed for evaluation. The broncho-alveolar fluid samples were taken and subjected to the following procedure: the lungs were taken off aseptically. A sterile catheter-tipped syringe was inserted into the trachea and a 5 mL volume of sterile saline was injected into the lungs. A 4.5 mL volume of broncho-alveolar lavage fluid was aspirated by a syringe and poured into a sterile bottle. The procedure was repeated three times for each rat and the summarized lavage fluid (13.5 mL per rat) was pooled, centrifuged, washed and used for assessment of inflammatory cell response by automated cell counting (Hoffman La Roche), electron microscopic investigations and inoculation of broth for isolation of L-form cultures. Because of their osmotic fragility, direct isolation of L-forms from animals and the enumeration of colonies were impossible. This is why the samples were inoculated and cultivated initially in liquid TSB supplemented for L-forms (1% yeast extract, 2% PPLO serum fraction and 3% sodium chloride). The identity of isolated L-form cultures was confirmed by electron microscopy. The formation of typical L-form ‘fried egg’-like colonies through subsequent plating of liquid subcultures on TSA also confirmed that the isolated cultures belonged to L-forms.

Blood samples were collected and evaluated at the same intervals after challenge.

**Electron microscopy**

The transmission electron microscopy (TEM) examination included samples of bronchoalveolar fluid, taken at the intervals indicated above. The cells were isolated in ice-cold 0.85% sodium chloride, centrifuged for 20 min at 2000 g, washed in 0.1 M cacodylate buffer, supplemented with 0.1% (w/v) MgSO₄ and 4.5% (w/v) sucrose, pH 7.4, and fixed in 2.5% (v/v) glutaraldehyde in the same buffer for 2 h at 4 °C. After washing with the same buffer, the macrophages were incubated for 1 h, at 37 °C, in medium containing 3% (w/v) p-nitrophenylphosphate (Merck), 0.12% (w/v) lead nitrate and 7.5% (w/v) sucrose, dissolved in 0.1 M acetate buffer, pH 5.5. Cytochemical controls were incubated with the omission of substrate p-nitrophenylphosphate or incubated in full medium with addition of 0.01 M NaF as an inhibitor. The samples were postfixed in 1% (w/v) OsO₄ in symm-kollidin buffer (Fluka), pH 7.2, for 2 h at 4 °C. After a short-duration dehydration in graded ethanol series and propylene oxide, the cells were embedded in Epon-Araldite (Fluka) for polymerization at 56 °C for 48 h. Ultrathin sections were cut on a Reichert–Jung Ultracut microtome, stained with uranylacetate and lead citrate and observed with a Zeiss 10C electron microscope at 60 kV.

For scanning electron microscopy (SEM), the samples were fixed and dehydrated as described above and standard screening was performed with gold in an argon medium in an Edwards-S 150 A vacuum apparatus with a 30 Å (1 Å = 0.1 nm) thickness of the coating. The observations were made on a scanning device on the same electron microscope at 20 kV.

**Statistical analysis**

The results are expressed as the mean value ± SD. To assess the significance of differences within experiments, Student’s t-test was used. Statistical significance was defined as *P* < 0.01.
Results

Monitoring of infection

Viable L-forms were isolated from bronchoalveolar lavage of the infected rats in liquid media (TSB) at all sample times till the end of examination, day 30 after challenge. Direct isolation of L-forms on solid media from animals was impossible. The identity of the isolated L-form cultures was confirmed by electron microscopy. In contrast, viable S-forms were isolated from broncho-alveolar fluid until day 14 after challenge.

Table 1 presents the dynamics of the total and differential counts of broncho-alveolar cells in rats infected with parental S-forms and L-forms of Staphylococcus aureus. As shown in L-form-infected rats, a statistically significant increase in broncho-alveolar leukocyte number was registered at days 14 and 30 after challenge. Changes in the distribution of different leukocyte types within the inflammatory broncho-alveolar cell population were characterized by a predominant increase in the percentage of monocytes-macrophages on days 7, 14 and 30 after challenge. In contrast to L-form-infected animals, the peak value of broncho-alveolar leukocyte count was found in the S-form-infected animals on day 7 after challenge. The total number of leukocytes was significantly higher than in the control and in the L-form group. The differential leukocyte count was characterized in this group by an increase predominantly in the number of granulocytes. The data presented in Table 2 give information about the total and differential count of leukocytes in blood. A low grade of leukocytosis in the blood of L-form-infected rat was registered at days 7, 14, and was retained at this level till the end of experiments. The percentage of granulocytes increased significantly on days 7 and 14, and that of monocytes-macrophages at 14 and 30 days after challenge. In contrast, a high level of leukocytosis with predominance of granulocytes was found in the S-form-infected rats on day 3 after challenge. On days 7 and 14, the number of leucocytes diminished, while on day 30 it decreased approximately to control values.

Morphological examinations

Figure 1 shows the micrographs of control bacterial cells from broth culture of the protoplastic S. aureus L-form performed by SEM (a, b) and TEM (c, d, e). The L-form cell population demonstrated a typical L-form culture morphology – large bodies multiplying by division (a, c) or by extracellular budding, and formation of elementary bodies of different size (b, d, e).

The interactions between L-form cells of S. aureus and lung phagocytes were also visualized by SEM and TEM. As shown on Fig. 2, the SEM observations revealed alveolar macrophage morphology and interactions of macrophages with S. aureus L-form. Formation of undulating ruffled membrane, microvilli, pseudopodia and lamellipodia in the surface of the macrophages was observed, attesting to macrophage activation. A high grade of macrophage membrane undulation in comparison with the morphology of macrophages from control noninfected animals (Fig. 2a) was observed at day 7 after challenge with the L-form (Fig. 2b and g) and also at day 30 (Fig. 2c and i). The occurrences of attraction, adhesion and engulfment of the L-form cells by macrophages at day 3 after challenge are shown in Fig. 2d–f. The occurrences of cell interactions at day 30 after challenge demonstrated lysis of some macrophages, the release of L-forms in the intercellular space (Fig. 2h) and

Table 1. Dynamics of broncho-alveolar leukocytes during infection with S- (parental) and L-forms of Staphylococcus aureus in intranasal infected rats

<table>
<thead>
<tr>
<th>Days after challenge</th>
<th>Control</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes × 10⁹/mm³</td>
<td>1.7 ± 0.3</td>
<td></td>
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<tr>
<td>(L) →</td>
<td>1.2 ± 0.4*</td>
<td>2.3 ± 0.2*</td>
<td>3.8 ± 0.5*</td>
<td>3.6 ± 0.3*</td>
<td></td>
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<tr>
<td>(S) →</td>
<td>3.9 ± 0.2*</td>
<td>4.8 ± 1.2*</td>
<td>3.3 ± 0.6*</td>
<td>3.9 ± 0.7*</td>
<td></td>
</tr>
<tr>
<td>Differential count (%)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Lymphocytes</td>
<td>49.7 ± 7.8</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(L) →</td>
<td>52.3 ± 6.7</td>
<td>43.5 ± 8.5</td>
<td>48.4 ± 4.8</td>
<td>53.2 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>(S) →</td>
<td>39.3 ± 8.4</td>
<td>38.0 ± 6.8</td>
<td>53.0 ± 6.2</td>
<td>49.6 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Monocytes /Macrophages</td>
<td>27.2 ± 3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L) →</td>
<td>31.4 ± 3.2*</td>
<td>37.3 ± 6.5*</td>
<td>39.8 ± 2.5*</td>
<td>35.9 ± 1.8*</td>
<td></td>
</tr>
<tr>
<td>(S) →</td>
<td>22.5 ± 3.4</td>
<td>24.3 ± 1.2</td>
<td>28.2 ± 2.3</td>
<td>30.0 ± 4.5</td>
<td></td>
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<tr>
<td>Granulocytes</td>
<td>23.1 ± 5.4</td>
<td></td>
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<tr>
<td>(L) →</td>
<td>16.3 ± 3.2*</td>
<td>19.2 ± 4.1*</td>
<td>11.8 ± 5.8</td>
<td>10.9 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>(S) →</td>
<td>37.8 ± 8.4*</td>
<td>37.6 ± 4.8*</td>
<td>18.8 ± 3.4</td>
<td>20.4 ± 5.4</td>
<td></td>
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</tbody>
</table>

*Significantly different from the control (uninfected animals) group (P < 0.01).  
*Significantly different from the S-form group (P < 0.01).

Control, uninfected animals. Values are means ± SD (five samples per interval).
attraction again of these L-form cells by new migrating macrophages (Fig. 2i).

The TEM observations of ultrathin sections from broncho-alveolar samples revealed certain peculiarities in the pattern of phagocyte–L-form interactions at the examined intervals (Figs 3 and 4). The initial phases of phagocytosis in vivo at days 3 and 7 after challenge, including adhesion and attachment of L-forms to alveolar macrophages, are shown in Fig. 3. At day 3, the activation of macrophages was manifested by cytochemical labeling of acid-phosphatase location on the undulating membrane surface and numerous macrophage microvilli (Fig. 3a and b) or on the adhered L-form cell (Fig. 3c). At day 7 after challenge, the process of adhesion, enclosing, engulfment and ingestion of L-forms in the macrophages continued. The multiplication by budding or division in the phagocytes

Table 2. Dynamics of blood leukocytes during infection with S- (parental) and L-forms of Staphylococcus aureus in intranasal infected rats

<table>
<thead>
<tr>
<th>Days after challenge</th>
<th>Control</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes × 10⁹/mm³</td>
<td>11.9 ± 2.2</td>
<td>8.1 ± 3.1*</td>
<td>19.4 ± 1.8*</td>
<td>23.2 ± 4.5*</td>
<td>17.4 ± 1.5*</td>
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<tr>
<td>(L)</td>
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<tr>
<td>(S)</td>
<td></td>
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<tr>
<td>Differential count (%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>88.5 ± 6.3</td>
<td>86 ± 8.2</td>
<td>77.9 ± 12.2</td>
<td>79.9 ± 9.2</td>
<td>84.2 ± 4.5</td>
</tr>
<tr>
<td>Monocytes/Macrophages</td>
<td>7.2 ± 1.2</td>
<td>63 ± 12.4*</td>
<td>59.2 ± 16.3</td>
<td>77.9 ± 9.4</td>
<td>81 ± 11.2</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>4.3 ± 0.9</td>
<td>4.7 ± 1.1*</td>
<td>15.2 ± 3.2*</td>
<td>7.6 ± 0.7*</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td>(L)</td>
<td></td>
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<td></td>
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<tr>
<td>(S)</td>
<td></td>
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*Significantly different from the control group (P < 0.01).
+Significantly different from the S-form group (P < 0.01).

Control, uninfected animals. Values are means ± SE (five samples per interval).
was also examined at this time point (Fig. 3d–f). Signs of phagolysosomal fusion and digestion of L-forms were not observed, regardless of good expressed acid-phosphatase labeling in the cytoplasmic membrane of L-forms and in the lysosomes around them (Fig. 3e and f). Similar processes of adhesion and ingestion were registered on day 14 after challenge. L-forms of variable size and shape were observed outside or in the activated phagocyte cells (Fig. 4a–c). On day 30 postinfection, intracellular multiplication and persistence of L-form cells in phagocytes continued. Fusion of small phagosomes containing L-forms and formation of larger ones were observed, but phagolysosomal fusion was not found (Fig. 4d and e), suggesting ineffectual phagocytosis of L-forms. Cell lysis and liberation of replicating L-form bodies in the intercellular space were observed (Fig. 4f and g). It should be noted that alveolar macrophages showed the characteristic signs of activation till the end of examination. This was manifested by formation of numerous protuberances and acid-phosphatase cytochemical labeling, suggesting increased macrophage activity.

The interaction between the alveolar macrophages and the S. aureus parental coccal form (as a control) at days 3 and 7 of infection is shown in Fig. 5. Activation of macrophages (Fig. 5a), internalization of cocci in the macrophage phagosome (Fig. 5b) and processes of phagolysosomal fusion (Fig. 5c–g) and digestion of staphylococci (Fig. 5c–i) were observed. Cytochemical labeling of the acid-phosphatase location preferably in the lysosomes and its release in the phagosomes and phagolysosomes (Fig. 5d–g) indicate the processes of phagolysosomal fusion and digestion of parental bacterial form of S. aureus.

**Discussion**

Clinical and experimental data indicate that microbial cell wall deficiency of some pathogens frequently contributes to *in vivo* longevity and persistence following antibiotic administration (Acar & Sabath, 1978; Watanakunakorn, 1979). Of particular interest was the appearance of the L-form stage under certain conditions unfavorable to the organisms. These forms, which some authors consider silent but potentially important pathogens, were also reported to be associated with atypical, chronic or latent infection and expression of disease (Prozorovsky et al., 1981; Domingue, 1982; Domingue & Woody, 1997; Mattman, 2001). Of considerable interest are the atypical respiratory tract infections (atypical pneumonia), which are usually not clinically severe, and the presence of the causative agents in these cases is difficult to demonstrate by isolation.

In the present study, we investigated the capacity of S. aureus L-form to induce lung infection in rats following intranasal administration. It is known that rats eliminate bacteria faster and show the mildest and most rapidly organized inflammatory response, and they are more resistant to localized wound infection with S. aureus than are hamsters and mice (Donnelly & Stark, 1985). The
established dynamics of paraclinical parameters in L-form-infected rats demonstrate development of a modified infection process, less intense but longer than the one provoked by the respective parental bacterial form (Michailova et al., 2000). The hematological parameters in L-form-infected rats showed a low-grade leukocytosis with a relatively increased percentage of granulocytes at day 7, and later at day 14 of monocytes and macrophages, which was retained till the end of experiments. Lung inflammation was noted 14 days after inoculation of the *S. aureus* L-form and was characterized by an increase in the total number of broncho-alveolar cells. Cytometric evaluation revealed a broncho-alveolar infiltrate rich in monocytes and macrophages. In contrast, extreme and early blood leukocytosis with rapidly progressive acute course of infection induced by the parental, isogenic S-form strain of *S. aureus* has been found in our preliminary study (Michailova et al., 2000), and in the study of other authors (Fritsch et al., 1983).

Regarding development of lung inflammatory parameters, the electron-microscopic investigations provide activation of the macrophage surface and some details on phagocyte–bacterial interactions in a rat model of pulmonary *S. aureus* L-form infection. Similar cell interaction processes of peritoneal macrophages with the L-form of *S. aureus* were observed by TEM in another study (Shmitt-Slomska et al., 1986).

**Fig. 3.** TEM of the interaction between alveolar macrophages and L-form of *Staphylococcus aureus* on day 3 (a–c); and on day 7 (d–f) after challenge: acid-phosphatase location (arrow); L-form cells (L), lysosomes (Ly). Bar = 0.2 μm.
It is well known that macrophage activity and neutrophil influx represent the major natural defenses against respiratory pathogens, and the effects of phagocytosis are a decisive factor for the outcome of microbial–host interaction (Silverstein & Loike, 1980; Wright & Detmers, 1991). Some data suggest that the ability of *S. aureus* to exploit the inflammatory response of the host by surviving inside neutrophils is a virulence mechanism for this pathogen and that modulation of the inflammatory response is sufficient to alter significantly the morbidity and mortality induced by this infection (Gresham *et al.*, 2000).

The present electron microscopic observations visualized the process of interactions of *S. aureus* L-form with alveolar phagocytes *in vivo*. Long persistence of the L-forms outside or inside the phagocyte cells was demonstrated without an evident process of phagolysosomal fusion, suggesting that the phagocytosis of L-forms was ineffectual. The TEM investigation of other authors (Kahl *et al.*, 2000) revealed that internalized live *S. aureus* bacteria resided within endocytic vacuoles of pulmonary epithelial cell line without lysosomal fusion in a 24-h period. The possibility of intracellular staphylococcal replication of *in vitro* infected human pulmonary macrophages or human endothelial cell culture with reduced or demonstrated phagolysosomal fusion were observed by some authors (Galbenu *et al.*, 1979; Lowy *et al.*, 1988). Of special interest was our finding

![TEM of the interaction between alveolar macrophages and L-form of *Staphylococcus aureus* on day 14 (a–c) after challenge: adhesion, engulfment and multiplication of L-forms in the activated phagocytes. Intracellular multiplication and persistence of L-forms in phagocyte cells on day 30 of infection: fusion of small phagosomes containing L-forms and formation of larger ones (d, e); cell lysis and liberation of L-form bodies in the intercellular space (f, g). L-form cells (L). Bar = 0.2 μm.](https://academic.oup.com/femsle/article-abstract/268/1/88/594959)
of lysosomal activation demonstrated by cytochemical acid-phosphatase labeling and an absence of the phagosome-lysosomal fusion in contrast to these well expressed processes found in the parental coccal-form phagocytosis. This finding might suggest a correlation between the pathogenesis of *S. aureus* and its intracellular localization in phagocytes. The bacterial L-forms are generally considered to be good persisters inside the host (Domingue & Woody, 1997). It was observed that after intra-phagosomes formation of protoplast L-form cells, the phagocytes were incapable of the digestive process. It is possible that this is due to the cell wall antigen insufficiency in L-forms, and they may remain unrecognized inside the alveolar macrophages. In a recent study, we showed the presence and location of membrane-bound protein A in the *S. aureus* L-form, which reduced in quantity perhaps due to its unstable binding to the L-form cytoplasm-membrane cell surface (Ivanova et al., 1989). As far as staphylococcal protein A has been found to be involved in the bacterial–host cell interactions (Jung et al., 2001), its reduction may contribute to atypical properties of these forms in vivo and modification of infection induced by them. Differences in the production of protein A by normal *S. aureus* and L-forms, as a significant reduction of muramic acid, indicating the lack of mureopeptide, and also lack of cell-wall ribitol teichoic acid in L-forms of *S. aureus* were also reported (Pratt, 1966; Forsgren, 1969). It is known that cell wall (or cytoplasmic membrane of L-forms) as a surface organelle function allows bacterial pathogens to interact with their environment, in particular the tissues of the infected host. Surface proteins of Gram-positive bacteria possess cell-wall-sorting signals, especially protein A of *S. aureus*, lipoteichoic acid, peptidoglycan, and some mechanisms of their targeting the cell wall envelope are recognized (William & Olaf, 1999; Thiemermann, 2002).

Although a wide variety of important bacterial ligands and possible eukaryote receptors have been described, the precise mechanisms leading to persistent normal *S. aureus* and L-form colonization and even more importantly associated infections have not been elucidated in detail. This may be a consequence of the fact that most of the adherence

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**Fig. 5.** TEM of interaction between alveolar macrophages and normal *Staphylococcus aureus* strain (parental S-form): phagolysosomal fusion (c–g), and digestion (e, f, h, i). *Staphylococcus aureus* (Sa); lysosomes (Ly); acid-phosphatase location and phagolysosomal fusion (arrow). Bar = 0.2 μm.
factors have been studied individually in simplified in vitro systems, not considering the complexity of multi-factorial in vivo cell–cell interactions. The present experimental parameters demonstrated that the inflammatory response in L-form S. aureus-infected rats was modified and less intensive, appeared later and remained longer than infection with the respective parental normal S. aureus form. The observed results are due to the missing cell wall envelope and antigen signals in the protoplast L-form cells, causing ineffectual phagocytosis and the ability of L-forms to survive and persist for a long time in the host. On the other hand, the morphological findings of this in vivo phagocytosis indicate that the significant intracellular survival of the S. aureus L-form might be a factor contributing to the failure of antibiotic therapy.

References


