Amino Acid Transport into Cultured McCoy Cells Infected with Chlamydia trachomatis

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Amino acid transport into McCoy cells infected with strains representative of the two major biovars of Chlamydia trachomatis has been studied to determine if uptake is increased during infection. Preliminary work suggested that the transport systems L, A/ASC (for neutral amino acid transport), N (for transport of Asn, Gln, and His) and y+ (for cationic amino acids) were present in McCoy cells. With lymphogranuloma venereum biovar strain 434, little difference in the influx of representative amino acids Trp, His, and Lys or the analogue 2-aminoisobutyric acid (AIB) was observed during infection. With trachoma biovar strain DK20, a small increase in the initial entry rate and equilibrium concentration of each amino acid was found. McCoy cells appear to have great capacity for concentrating amino acids, which might obviate the need for transport induction by chlamydiae under conditions favoring the growth of infectious organisms.

Chlamydia trachomatis is the major infectious cause of blindness in the developing world and the single largest cause of bacterial genital tract infection. However, because chlamydiae are obligate intracellular bacteria which grow and develop inside an inclusion, they are difficult to study. Basic biochemical and genetic information is limited, since there is currently no system available for gene transfer into these organisms. Chlamydiae rely on the soluble pool of metabolites in the host cell for their survival, and competition between host and parasite is likely (18, 31). In vitro evidence suggests that restriction of available metabolites induces the production of noninfectious organisms (6, 18, 29, 30), which can appear morphologically abnormal (6). Indeed, in recent studies of C. trachomatis infection of McCoy (mouse L fibroblast) cultures, we have demonstrated that a small reduction in the amino acid concentration of medium to 75% of control levels is enough to induce the growth of aberrant chlamydial forms with reduced infectivity (17). More importantly, such forms were also induced when infected cultures were supplied with medium containing amino acids in the concentrations found in plasma. Atypical chlamydiae have also been observed in vivo (20, 32, 36, 38), probably as a result of natural or immunologically induced nutritional deficiency (17). In vitro, a reversal to infectious forms can be brought about by the reintroduction of amino acids into starved cultures or by the addition of cycloheximide (CIH) (1, 18, 29). An important question arising from these studies is whether chlamydiae influence their own productive infection by inducing amino acid transport into the host cell. Among other intracellular organisms, certain viruses and protozoa are known to increase the permeability of the host cell plasma membrane, making it more permeable to a wide range of solutes, including amino acids (3, 4, 8, 12, 13, 26, 37). Infection of human red blood cells with the malarial parasite Plasmodium falciparum leads to an induction in transport of Gln of up to 100-fold (7), which may provide a more favorable environment for parasite development. Parasite-induced transport pathways may involve modification of the lipid or protein constituents of the membrane by the organism. The malaria parasite inserts new polypeptides into the infected erythrocyte membrane, giving it altered antigenic properties (21). There is increasing evidence for chlamydial modification of the host cell membrane (45), either by glycosylation (39) or by insertion of parasite-derived molecules such as the genus-specific lipopolysaccharide antigen (23, 33), the 155-kDa species-specific C. trachomatis antigen (42), or a glycolipid antigen (40). Potential new transport pathways could constitute targets for protective immune responses or could be an important means of targeting inhibitory (to chlamydiae) or cytotoxic drugs into infected cells (4, 11, 12).

This study examined the effect of chlamydial infection on the transport of amino acids into the host cell. Following on previous work (17), the C. trachomatis strains investigated were L2/434/Bu (strain 434; lymphogranuloma venereum biovar) and E/DK-20/ON (strain DK20; trachoma biovar). The host cells were McCoy cells (strain L murine fibroblasts), which are widely used for propagation of chlamydiae and studies of host-chlamydia interaction (31). McCoy cultures (ATCC CRL-1696; ICN Biomedicals Ltd., Thame, United Kingdom) were judged free of mycoplasma contamination by mycoplasma detection (Boehringer Mannheim Biochemica, Lewes, United Kingdom). Cells were cultured at 37°C in Eagle minimal essential medium with Earle’s salts, to which 2 mM L-glutamine (Gibco, Paisley, United Kingdom), 5% (vol/vol) fetal bovine serum (ICN), and 100 µg of streptomycin sulfate (Evans Medical Ltd., Horsham, United Kingdom) per ml were added. Organism stocks were routinely propagated in CH (Sigma Chemical Co., Poole, United Kingdom)-treated McCoy cell monolayers (6) and infected by centrifugation (1,580 × g for 30 min at 37°C). CH was not used in subsequent experiments.

Preliminary work investigated the amino acid transport systems present in McCoy cells, since to our knowledge they had not been identified. Many mammalian cells have amino acid transport systems A, ASC, and L in their membranes, which serve for neutral amino acids (9). System y+ commonly transports cationic amino acids in fibroblasts (44), and the activity of system N, selective for Asn, Gln, and His, has been observed in hepatocytes, muscle tissue, and human red blood cells (28). It
was considered adequate for these studies to limit the transport system characterization to the determination of an uptake time course for representative amino acids of a particular transport system recognized in other cells and to use known competitive substrates to reduce the amino acid influx under initial entry rate conditions. Amino acid uptake into monolayer McCoy cells was measured using the 24-well tray technique for rapid measurement of solute transport (10, 43). Time course determinations of uptake of the natural amino acids L-Trp, L-His, and L-Lys, representing amino acid transport systems L, N, and y+, respectively, were performed. The effect of putative competitors on the transport of these amino acids and on the amino acid analogue 2-aminoisobutyric acid (AIB), a substrate of systems A/ASC, was examined.

McCoy cell monolayers, established overnight in 24-well trays (Nunc, Paisley, United Kingdom), were washed with 1 ml of Earle’s salt solution (Gibco) at 37°C and preincubated for 1 h at 37°C in 250 μl of Earle’s salt solution supplemented with 25 mM HEPES (Sigma) and 5% (vol/vol) dialyzed fetal bovine serum. Medium was added, and incubations with 14C-labeled amino acids in Earle’s salt solution supplemented with HEPES (total volume, 250 μl), sometimes with an unlabeled putative competitor substrate, were performed at 37°C. Labeled amino acids were from Nycomed Amersham (Little Chalfont, United Kingdom), except Trp, which was from NEN Life Science Products (Hounslow, United Kingdom). The concentrations of Trp, His, Lys, and AIB used were 10, 50, 500, and 100 μM, respectively, at 0.1 or 0.3 (Lys only) μCi ml⁻¹. Competitor substrates (Sigma) were added for a time consistent with the initial rate of amino acid entry. Incubations were terminated by rapidly rinsing the monolayers twice with ice-cold 0.9% saline, which contained 12.5 mM L-Trp when Trp transport was being investigated. After the trays were drained, monolayers were dissolved in 250 μl of 0.1 M NaOH for 2 h and the soluble radioactivity of 200-μl samples in 3 ml of scintillation fluid was counted in a Canberra Packard 2700TR liquid scintillation spectrometer.

An estimation of the amount of cell protein in six replicate wells was made at each time point in the experiments. Monolayers were washed twice with ice-cold phosphate-buffered saline (2 ml) and dissolved in 0.1 M NaOH for protein analysis, using biocinchonic acid (Sigma). The intracellular water volume was estimated from the steady-state distribution of the nonmetabolizable hexose 3-O-methyl-d-[14C]glucose, as described by Kletzien et al. (27). The value obtained for McCoy cells after a 45-min incubation at 37°C in Earle’s salt solution with 5 mM 3-O-methyl-d-[14C]glucose (Amersham; 1 μCi ml⁻¹) was 4.8 μl mg of protein⁻¹. Amino acid uptake was expressed as picomoles of amino acid per microliter of intracellular water.

More than 90% inhibition of L-Trp uptake occurred when 10 mM either 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH) or L-Leu, substrates of system L (9), was added during Trp transport at the initial rate (Table 1). This provides evidence that Trp was entering the cells predominantly via transport system L. This was supported by the reduced ability of AIB to inhibit transport, although some Trp may have been taken up by system A/ASC transporters. Some amino acids could have been entering via system T, since ϕ-Trp also inhibited the transport of L-Trp by 80%, although contamination of ϕ-Trp by as little as 1% L-Trp could have accounted for the inhibition.

Almost complete inhibition of L-His uptake occurred in the presence of 20 mM L-Gln or L-Asn, both of which are competitive substrates of transport system N (28) (Table 1). This suggests that L-His was also transported via system N. Corre-

**TABLE 1. Inhibition analysis of transport systems in McCoy cells**

<table>
<thead>
<tr>
<th>Transport system (amino acid substrate)</th>
<th>Competitor (conc in mM)</th>
<th>% Inhibition</th>
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<tr>
<td>L (tryptophan)</td>
<td>BCH (10)</td>
<td>95 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>L-Leu (10)</td>
<td>94 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>AIB (10)</td>
<td>40 ± 15</td>
</tr>
<tr>
<td></td>
<td>ϕ-Trp (10)</td>
<td>80 ± 7.2</td>
</tr>
<tr>
<td>N (histidine)</td>
<td>L-Gln (20)</td>
<td>97 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>L-Asn (20)</td>
<td>92 ± 2.1</td>
</tr>
<tr>
<td>y+ (lysine)</td>
<td>L-Arg (10)</td>
<td>92 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>L-Orn (10)</td>
<td>89 ± 8.5</td>
</tr>
<tr>
<td>A/ASC (AIB)</td>
<td>meAIB (10)</td>
<td>98 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>L-Cys (10)</td>
<td>95 ± 3.5</td>
</tr>
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</table>

* Competitive substrates of each transport system were added at the same time as labeled amino acids for a period consistent with the initial rate of transport.
* Values are means ± standard deviations for at least two experiments with triplicate determinations in each.
amino acid into DK20-infected cells was consistently greater than that into mock-infected cells at the 16-h time point (Fig. 1b and c; a comparable uptake curve was also obtained for Lys). Moreover, both the initial rate of influx and the approximate asymptote value for each amino acid were greater for infected cells than for mock-infected cells (Table 2). In most cases, the differences were moderate but significant (P < 0.05), with increases during infection ranging from 1.2 to 1.4 times the values for uninfected cells for both initial rates and asymptotes. Such differences in amino acid transport might be sufficient to increase the numbers of infectious chlamydiae present in inclusions.

In these studies, it was observed that labeled amino acids appeared to be greatly concentrated inside McCoy cells during transport. His, Trp, Lys, and AIB were concentrated inside these cells by more than 30-, 25-, 15-, and 50-fold, respectively. This supported our finding of a marked intracellular concentration of amino acids during infection of infected or uninfected McCoy cells in medium supplied with the concentrations of amino acids found in plasma (17). It is possible that McCoy cells might have an enhanced concentrative capability as transformed cells; transformation has been reported to lead to increases in the transport activity of a number of cell lines, in particular transport system A (2, 5, 14, 22, 35). Hence, any induction of amino acid transport during chlamydial infection could be masked and would not be detected under these conditions.

The results of this study thus suggest that McCoy cells incubated in normal medium are very effective at accumulating amino acids. It may therefore be more appropriate to look for chlamydial induction of amino acid transport in other cell types, particularly nontransformed cells. It is also possible that regulatory mechanisms for acquisition of nutrients may be switched off when the organisms are exposed to an abundance of nutrients within the host cell, despite competition with the host for amino acids.

It is possible that chlamydiae might induce nutrient uptake via a pathway similar to that described for malaria infection (26), i.e., a pore or channel instead of a conventional amino acid transporter. The enhanced permeability pathway detected during malaria infection has broad substrate specificity and does not saturate at physiological concentrations, consistent with its being a channel rather than a carrier (24). It has also been shown to be anion selective and has functional properties resembling those of chloride channels in other cell types (25).

An investigation of the presence of such a pathway during chlamydial infection would be worthy of further study. Furthermore, changes in amino acid transport might instead occur at the inclusion membrane, which has been reported to be passively impermeable to small molecules (19). Studies have indicated the presence of Chlamydia-derived proteins localized in the inclusion membrane (34, 41), and the traffic of host Golgi lipids to chlamydiae within the inclusion (15, 16) may also affect inclusion membrane composition.

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**TABLE 2. Initial influx rates and asymptote values of amino acids transported into McCoy cells infected with strain DK20 of C. trachomatis**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Initial influx (nmol/mg of cell water/min)</th>
<th>Asymptote value (μM)</th>
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<tbody>
<tr>
<td>L-His</td>
<td>infected cells: 1,425 ± 73*; mock-infected cells: 1,122 ± 58</td>
<td>infected cells: 2,427 ± 140*; mock-infected cells: 1,971 ± 23</td>
</tr>
<tr>
<td>L-Trp</td>
<td>infected cells: 410 ± 30*; mock-infected cells: 302 ± 25</td>
<td>infected cells: 395 ± 6*; mock-infected cells: 304 ± 16</td>
</tr>
<tr>
<td>L-Lys</td>
<td>infected cells: 618 ± 41*; mock-infected cells: 455 ± 25</td>
<td>infected cells: 7,509 ± 197*; mock-infected cells: 6,204 ± 46</td>
</tr>
<tr>
<td>AIB</td>
<td>infected cells: 420 ± 11*; mock-infected cells: 311 ± 14</td>
<td>infected cells: 5,411 ± 373; mock-infected cells: 4,865 ± 165</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations for triplicate determinations in one experiment. Linear regression fits to the influx data were used, and correlation coefficients were always >0.99. *, significantly different from values for mock-infected cells (P < 0.05).
REFERENCES


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