Inhibition of *Chlamydia pneumoniae* Replication in HEp-2 Cells by Interferon-γ: Role of Tryptophan Catabolism

Sheetal J. Mehta, Richard D. Miller, Julio A. Ramirez, and James T. Summersgill

Interferon-γ (IFN-γ) induces tryptophan catabolism in HEp-2 cells, possibly via stimulation of host cell indoleamine-2,3-dioxygenase activity, in a dose-dependent (12.5–1600 U/mL) fashion after 24 h, resulting in a 99% conversion to its metabolites at 1600 U/mL. Replication of *Chlamydia pneumoniae* isolates A-03 and BAL-16 was inhibited in HEp-2 cells following treatment with 50 and 100 U/mL IFN-γ, respectively; however, addition of excess L-tryptophan (200 μg/mL) to monolayers infected with *C. pneumoniae* resulted in unrestricted growth of both isolates up to 1600 U/mL IFN-γ. *C. pneumoniae* could be recovered from IFN-γ–treated monolayers, indicating the potential for this bacterium to undergo an altered life cycle, in vitro, analogous to that described in detail for *Chlamydia trachomatis*. The ability of *C. pneumoniae* to persist in host tissue despite an immunologic response would be an important attribute in order to cause or exacerbate chronic infections.

*Chlamydia pneumoniae* causes acute respiratory disease, including pneumonia, bronchitis, sinusitis, and pharyngitis [1,2], and has been implicated in chronic disease states, such as atherosclerosis [3], asthma [4], and sarcoidosis [5]. The role of *C. pneumoniae* in atherosclerosis is unclear; however, numerous studies have reported detection of this bacterium in atheromatous lesions [6–8], including a study from this laboratory on the isolation of *C. pneumoniae* from coronary atheroma of a patient with severe coronary artery disease [9]. A second isolate from a carotid artery atheroma has recently been reported [10]. The ability of *C. pneumoniae* to cause persistent respiratory infections in humans has been documented [11–13]; however, the mode of survival in host tissue, despite appropriate antibiotic therapy, is not known.

Interferon-γ (IFN-γ)–mediated activation of host cells to restrict multiplication of chlamydiae has been proposed as a mechanism for establishing persistent *Chlamydia trachomatis* infection in vivo. Byrne and colleagues [14, 15] have presented a detailed description of IFN-γ–mediated persistence of *C. trachomatis* in cell culture. This mechanism involves the induction of host cell indoleamine-2,3-dioxygenase (IDO) with subsequent degradation of tryptophan to its metabolites, thus depriving the bacterium of levels of this amino acid sufficient for growth. In vitro, this results in the formation of large aberrant replicative forms, representing an interruptive stage in the normal chlamydial life cycle. Removal of the IFN-γ pressure, accompanied with the return of normal tryptophan levels, allows for the resumption of the normal chlamydial life cycle. This is an intriguing theory of a mechanism for chlamydial survival in host tissue and may explain the ability of this organism to cause chronic infection with the resulting inflammatory changes noted in *C. trachomatis* disease.

The ability of *Chlamydia* species to survive in host tissue would be a necessary attribute for this bacterium to either cause or exacerbate the inflammatory reactions seen in atheromatous plaques and other chronic infections. We have previously shown that growth of *C. pneumoniae* is restricted in HEp-2 cells pretreated with IFN-γ and that the addition of tumor necrosis factor-α may enhance the inhibitory activity of IFN-γ [16]. The present study sought to further characterize this cytokine effect in terms of establishing the role of tryptophan catabolism in the growth restriction of a coronary and a respiratory isolate of *C. pneumoniae*. An attempt was also made to determine if *C. pneumoniae* could be recovered from IFN-γ–treated monolayers, thus indicating the potential to undergo an interruptive life cycle, in vitro, similar to that seen with *C. trachomatis*.

**Materials and Methods**

*Cell line.* HEp-2 cells (CCL-23) were obtained from the American Type Culture Collection (Rockville, MD) and grown in Iscove’s modified Eagle medium (BioWhittaker, Walkersville, MD) supplemented with 10% fetal bovine serum, nonessential amino acids, HEPES buffer, 10 μg/mL gentamicin, 25 μg/mL vancomycin, and 12.5 μg/mL nystatin. Cells were grown in 75-cm² flasks (Costar, Cambridge, MA), harvested with trypsin-EDTA, washed, and resuspended at 3 × 10⁶ cells/mL.

*Bacterial isolates.* *C. pneumoniae* A-03 (ATCC VR-1452) is a clinical isolate that was obtained from the coronary artery of a patient during heart transplantation at the Jewish Hospital Heart and Lung Institute (Louisville) [9]. *C. pneumoniae* BAL-16, a
respiratory isolate from a patient with pneumonia, was obtained from Margaret Hammerschlag (Health Sciences Center at Brooklyn, Brooklyn, NY). Both isolates were passaged in HEp-2 cell monolayers in medium containing 1.0 μg/mL cyclohexamide and then harvested, aliquoted, and stored at ~70°C until needed.

Major histocompatibility class II response of HEp-2 cells to IFN-γ. Flat-bottomed, 24-well tissue culture plates (Costar) with coverslips were seeded with 1.0 mL of HEp-2 cell suspension and allowed to adhere for 24 h prior to use in experiments. Cells were then treated with 100 U/mL IFN-γ (Promega, Madison, WI) and incubated for 24, 48, and 72 h. At each time period, cells from three wells were washed, trypsinized, harvested, and pelleted. Cells (200 μL) and PBS-azide (800 μL) were added to each microcentrifuge tube. These tubes were centrifuged, and 20 μL of HLA-DR–phycoerythrin antibody (HLA-DR–PE; Becton Dickinson, Sparks, MD) was added to the pellets and mixed. Phosphate buffer (800 μL) was added, and the tubes were centrifuged. The percentage of HEp-2 cells expressing major histocompatibility complex class II molecules was detected by flow cytometry. Unlabeled cells were used as a control.

Effects of IFN-γ on C. pneumoniae replication in HEp-2 cells. Aliquots (100 μL) of C. pneumoniae A-03 and BAL-16 stock preparations (10^7 inclusion-forming units [ifu]/mL) were added to wells of 24-well plates containing a coverslip with a confluent HEp-2 cell monolayer. Plates were then centrifuged at 800 g for 1 h, followed by an additional 30-min incubation at 37°C. Wells were aspirated and covered with fresh medium containing 0–1600 U of IFN-γ/mL (Promega). All plates were incubated for 72 h at 37°C, and in some experiments, supernatants were aspirated and monolayers assayed for tryptophan catabolism measurements as described below. Coverslips were then removed, stained with a Chlamydia species–specific anti-lipoplyascharride, fluorescent isothiocyanate–labeled antibody reagent (Pathfinder; SanoDiagnostics Pastuer, Chaska, MN), and examined by epifluorescence microscopy (200×) for the presence or absence of inclusion body formation. Identical experiments were also done in the presence of excess (200 μg/mL L-tryptophan or D-tryptophan (Sigma, St. Louis)). All experiments were repeated four times.

Measurement of tryptophan catabolism. Catabolism of tryptophan to its metabolites in infected and uninfected HEp-2 cells was measured by paper chromatography as described by Pfefferkorn [17]. Aliquots (100 μL) of supernatant, applied in 10-μL increments, were spotted on Whatman (Maidstone, UK) 3-mm filter paper. The filter paper was then placed into a chromatography jar containing 0.1 N HCl as the eluent and allowed to migrate, and the paper was air-dried overnight. Sample spots (1.0 cm each) were excised, placed in scintillation vials, and counted. The percent specific catabolism of tryptophan to its metabolites, N-formylkynurenine and kynurenine, was calculated using the formula 

\[
\frac{\text{cpm}_{\text{sample metabolite}} - \text{cpm}_{\text{pulse metabolite}}}{\text{cpm}_{\text{pulse tryptophan}}} \times 100,
\]

where cpm_{sample metabolite} is counts per minute (cpm) present in control sample, cpm_{pulse metabolite} is cpm present in tryptophan metabolite fraction, and cpm_{pulse tryptophan} is cpm of tryptophan present.

Effects of tryptophan-depleted medium on C. pneumoniae growth. Tryptophan-depleted medium was prepared by the addition of individual amino acids, excluding tryptophan, according to the recipe for Iscove’s modified Eagle medium. This was then supplemented with 10% fetal bovine serum, which had been previously dialyzed for 48 h to remove all tryptophan. Nonessential amino acids and antibiotics were then added as described above.

An aliquot was resupplemented with 16.0 μg/mL L-tryptophan, representing the normal concentration in control medium. Aliquots (100 μL) of C. pneumoniae A-03 and BAL-16 stock preparation (10^7 ifu/mL) were added to wells of a 24-well plate containing a coverslip with a confluent HEp-2 cell monolayer. Plates were then centrifuged at 800 g for 1 h and followed by an additional 30-min incubation at 37°C. Wells were then aspirated, replaced with fresh tryptophan-depleted medium containing 1.0–16.0 μg/mL L-tryptophan, and incubated for 72 h at 37°C. Monolayers were then fixed for 10 min in methanol, stained, and examined by epifluorescence microscopy (200×) for the presence or absence of inclusion bodies.

Recovery of C. pneumoniae from treated HEp2 cells. Recovery of C. pneumoniae from HEp-2 cells grown in tryptophan-depleted medium was done in 96-well plates (Costar, Cambridge, MA) seeded with HEp-2 cells and incubated for 24 h at 37°C. Aliquots (100 μL) of C. pneumoniae A-03 and BAL-16 stock preparation (10^7 ifu/mL) were added to these monolayers. All plates were then centrifuged at 800 g for 1 h, followed by an additional 30-min incubation at 37°C. Wells were aspirated and replaced with fresh medium containing 1.0–16.0 μg/mL L-tryptophan. Plates were incubated for 72 h at 37°C, after which all monolayers were supplemented with fresh growth medium containing normal concentrations of L-tryptophan. At 48, 72, and 96 h of incubation at 37°C, wells were aspirated and monitored for the presence or absence of inclusion bodies.

In one set of experiments, at the end of the 96-h incubation period, plates were then incubated onto a fresh HEp-2 monolayer for detection of infectivity. After further incubation for 48 h, monolayers were then fixed for 10 min in methanol, stained, and examined by epifluorescence microscopy (200×) for the presence or absence of inclusion bodies.

Recovery experiments of C. pneumoniae from IFN-γ–treated HEp-2 cells were similar to those described above, with the exception of the addition of IFN-γ, 0–1600 U/mL, to normal growth medium prior to inoculation with C. pneumoniae A-03 or BAL-16.

Results

IFN-γ–induced tryptophan catabolism in HEp-2 cells. Examination of the expression of major histocompatibility complex class II molecules by HEp-2 cells when treated with IFN-γ indicated that after 72 h of incubation with 100 U/mL IFN-γ, 99.0 % of HEp-2 cells expressed this antigen (data not shown). To determine that tryptophan catabolism can be induced by IFN-γ, HEp-2 cells were treated with increasing concentrations (0–1600 U/mL) of IFN-γ, and tryptophan conversion to its metabolites was measured by paper chromatography. As shown in figure 1, there was a linear response of catabolism over the concentration range. At 100 U/mL
of an excess amount of L- or D-tryptophan (200 μg/mL). As seen in table 1, supplementation with D-tryptophan had no detectable effect on the IFN-γ–mediated inhibition of growth. In contrast, L-tryptophan completely reversed the inhibitory effect of IFN-γ treatment, and C. pneumoniae could replicate up to 1600 U/mL.

Growth of C. pneumoniae in tryptophan-depleted medium. To study direct involvement of tryptophan in the inhibition of C. pneumoniae growth in HEp-2 cells, experiments were done in tryptophan-depleted medium resupplemented with increasing concentrations of L-tryptophan. Table 2 indicates that C. pneumoniae did not grow in tryptophan-depleted medium or in medium resupplemented with 1.0–11.5 μg/mL L-tryptophan. However, on addition of L-tryptophan at ≥12.0 μg/mL, C. pneumoniae replication was detectable by inclusion body formation. Both isolates A-03 and BAL-16 showed identical requirements for concentrations of L-tryptophan in the medium.

Table 1. Growth of C. pneumoniae in IFN-γ–treated HEp-2 cell monolayers. To determine the effect of IFN-γ treatment of HEp-2 cells on replication of C. pneumoniae, isolates A-03 and BAL-16 were grown in the presence of increasing concentration of IFN-γ (table 1). Both isolates of C. pneumoniae were inhibited from forming detectable inclusion bodies in IFN-γ–treated HEp-2 cells. C. pneumoniae isolate A-03 failed to produce detectable inclusions at 50 U/mL IFN-γ, whereas the minimum inhibitory concentration for BAL-16 was 100 U/mL. These results were confirmed during three separate experiments.

To determine the effect of excess tryptophan on C. pneumoniae replication, similar experiments were done in the presence of an excess amount of L- or D-tryptophan (200 μg/mL). As seen in table 1, supplementation with D-tryptophan had no detectable effect on the IFN-γ–mediated inhibition of growth. In contrast, L-tryptophan completely reversed the inhibitory effect of IFN-γ treatment, and C. pneumoniae could replicate up to 1600 U/mL.

Growth of C. pneumoniae in tryptophan-depleted medium. To study direct involvement of tryptophan in the inhibition of C. pneumoniae growth in HEp-2 cells, experiments were done in tryptophan-depleted medium resupplemented with increasing concentrations of L-tryptophan. Table 2 indicates that C. pneumoniae did not grow in tryptophan-depleted medium or in medium resupplemented with 1.0–11.5 μg/mL L-tryptophan. However, on addition of L-tryptophan at ≥12.0 μg/mL, C. pneumoniae replication was detectable by inclusion body formation. Both isolates A-03 and BAL-16 showed identical requirements for concentrations of L-tryptophan in the medium.

Recovery of C. pneumoniae from tryptophan-depleted and IFN-γ–treated HEp-2 monolayers. To determine if C. pneumoniae could be cultured from monolayers in which growth had been restricted by tryptophan depletion or IFN-γ treatment, C. pneumoniae–infected monolayers were resupplied with normal growth medium. As shown in table 3, replication was not observed, nor was recovery possible, from tryptophan-depleted medium or tryptophan-depleted medium resupplemented with 1.0–7.0 μg/mL L-tryptophan. L-tryptophan, added at 8.0–11.5 μg/mL, was unable to support initial growth of C. pneumoniae; however, recovery from these monolayers was possible following 72 and 96 h of incubation in normal growth medium. The resulting inclusions formed by these recovered bacteria were not infectious when applied to fresh HEp-2 monolayers. Addition of L-tryptophan to tryptophan-depleted medium at concentrations ≥12.0 μg/mL gave results similar to those seen with control medium, in that inclusion formation was seen 48 h after the addition of fresh medium and the inclusions that formed at 96 h contained infectious elementary bodies (EB).
Table 2. Role of tryptophan in supporting growth of C. pneumoniae in HEp-2 cells.

<table>
<thead>
<tr>
<th>Tryptophan (µg/mL)</th>
<th>Growth at 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤11.0</td>
<td>+</td>
</tr>
<tr>
<td>11.5</td>
<td>+</td>
</tr>
<tr>
<td>12.0</td>
<td>+</td>
</tr>
<tr>
<td>13.0</td>
<td>+</td>
</tr>
<tr>
<td>14.0</td>
<td>+</td>
</tr>
<tr>
<td>15.0</td>
<td>+</td>
</tr>
<tr>
<td>16.0</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE. – denotes no inclusions detected; + denotes inclusions detected in >90% of monolayer.

* HEp-2 cells grown in control medium infected with C. pneumoniae.
† HEp-2 cells grown in tryptophan-depleted medium resupplemented with tryptophan at normal levels (16.0 µg/mL) infected with C. pneumoniae.
‡ HEp-2 cells grown in tryptophan-depleted medium infected with C. pneumoniae.

Similar experiments to determine if C. pneumoniae could be recovered from IFN-γ–treated HEp-2 cells (table 4) indicated that monolayers infected with A-03 were initially inhibited at 50 U/mL and that 100 U/mL IFN-γ was required to inhibit BAL-16. Upon the addition of fresh growth medium, neither isolate produced detectable inclusion bodies after 48 h of incubation; however, inclusions were seen following 72 and 96 h of incubation. Of interest, both isolates were capable of replication at all IFN-γ concentrations at 72 or 96 h after the addition of fresh medium; however, inclusions containing infectious EB were seen only up to 200 U/mL IFN-γ.

Table 3. Recovery of C. pneumoniae from HEp-2 cells grown in tryptophan-depleted medium.

<table>
<thead>
<tr>
<th>Tryptophan (µg/mL)</th>
<th>Recovery of inclusion formation at various hours after addition of fresh medium</th>
<th>Infectivity of inclusion at end of 96 h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0–7.0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.0–11.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>≥12.0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE. – denotes no inclusions detected; + denotes inclusions detected in >90% of monolayer.

* 96 h after addition of fresh medium, monolayers were harvested and inoculated onto fresh HEp-2 monolayers for detection of infectivity.
† HEp-2 cells grown in control maintenance medium infected with C. pneumoniae.
‡ HEp-2 grown in tryptophan-depleted medium resupplemented with normal levels of tryptophan (16 µg/mL) infected with C. pneumoniae.
³ HEp-2 grown in tryptophan-depleted medium infected with C. pneumoniae.

Table 4. Recovery of C. pneumoniae from IFN-γ–treated HEp-2 cells.

<table>
<thead>
<tr>
<th>IFN-γ (µg/mL)</th>
<th>Growth at 48 h</th>
<th>Inclusion formation at various hours after addition of fresh medium</th>
<th>Infectivity of inclusion at end of 96 h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>50</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>200</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>400</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>800</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1600</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NOTE. – denotes absence of inclusion bodies; + denotes presence of inclusion bodies in >90% of monolayer.

* 96 h after addition of fresh medium, monolayers were harvested and inoculated onto fresh HEp-2 monolayers for detection of infectivity.
† HEp-2 cells grown in control medium infected with C. pneumoniae.

Discussion

Chlamydiae are common pathogens, causing a broad spectrum of human infections, and evidence has emerged supporting an association of C. pneumoniae with coronary artery disease. The role of C. pneumoniae in atherosclerosis is not known; however, it has been found in early (fatty streaks) and late (fibrous plaques) lesions by numerous investigators. The demonstration of C. pneumoniae in atherosclerotic lesions does not in itself prove a causal relation between the organism and atherosclerosis; however, the ability of C. pneumoniae to play an important role in this chronic inflammatory process would necessitate survival of the organism in host tissue for an extended period of time. Byrne and colleagues [14, 15] suggested an in vitro model of C. trachomatis persistence in cell culture by activation of IFN-γ–mediated host cell tryptophan catabolism with a subsequent reduction in intracellular pools of tryptophan. This provides a stimulus for C. trachomatis to undergo an altered life cycle, resulting in the ability of the bacterium to enter into a viable but nonculturable state and persist in such a state until the IFN-γ pressure is removed. It is postulated that this may represent the mechanism of chlamydial persistence in host tissue in vivo. Thus, it was of interest to investigate whether C. pneumoniae likewise possessed a susceptibility to the degradation of host cell tryptophan similar to that seen with C. trachomatis.
Tryptophan catabolism in HEp-2 cells is induced in a dose-dependent fashion by treatment with IFN-γ, and this stimulation leads to a potent restriction of *C. pneumoniae* intracellular replication. Catabolism of tryptophan in HEp-2 cells appears to be t-tryptophan specific, in that growth inhibition studies in the presence of excess d-tryptophan had no effect, whereas excess t-tryptophan completely reversed the effect of IFN-γ–mediated growth inhibition of both isolates tested here. This would suggest that IFN-γ treatment induces an l-tryptophan–specific IDO activity in HEp-2 cells and that IDO induction is responsible for *C. pneumoniae* inhibition, presumably through reductions in available intracellular tryptophan levels. However, no direct evidence of IDO induction was provided in this study.

Slight differences in IFN-γ susceptibility between the 2 isolates tested here were seen (50 U/mL for A-03 vs. 100 U/mL for BAL-16); however, no differences were seen when tryptophan resupplementation experiments were done in tryptophan-depleted medium. This may reflect distinct biologic features of each isolate. Alternatively, this could be explained either by lower sensitivities of detection with the tryptophan resupplementation assays or by IFN-γ possibly stimulating other undetected inhibition pathways in HEp-2 cells, of which A-03 is more susceptible. In addition, it appears that *C. pneumoniae* has a slightly decreased susceptibility to IFN-γ treatment of HEp-2 cells compared with that of *C. trachomatis* A/Har-13 in HeLa 229 cells, which required only 2.0 ng/mL (20.0 U/mL) for complete inhibition of growth [15]. Infectious *C. trachomatis* EB were not present at this concentration even at 72 h following the removal of IFN-γ.

*C. pneumoniae* requires exogenous supplies of l-tryptophan for infection of HEp-2 cells and subsequent formation of normal inclusion bodies. Concentrations of ≤11.0 µg/mL in the growth medium resulted in no formation of inclusion bodies after 72 h of incubation. At 8.0–11.0 µg/mL l-tryptophan, *C. pneumoniae* is restricted from completing its normal replicative cycle after 72 h of incubation. Upon resupplementation of normal levels of l-tryptophan, the bacterium appears to resume its normal replicative cycle, resulting in the formation of normal-appearing inclusions after 72 h of incubation. However, a concentration of ≥11.5 µg/mL is required for the formation of inclusions that contain infectious EB. This suggests strongly that *C. pneumoniae* can undergo an interruptive life cycle similar to that described for *C. trachomatis* and that the stimulus for this is l-tryptophan starvation. Ultrastructural studies of *C. pneumoniae*–infected HEp-2 monolayers are needed to confirm the exact nature of this stage.

*C. pneumoniae* can also be recovered from IFN-γ–treated HEp-2 monolayers following removal of the cytokine pressure and incubation for 72 h. Normal-appearing inclusions were seen at all concentrations of IFN-γ tested at 72 and 96 h after the removal of the cytokine, indicating a resistance of *C. pneumoniae* to the bactericidal activity of IFN-γ exposure. This bactericidal resistance was not seen in experiments done at low concentrations of t-tryptophan. It is not readily clear why normal-appearing inclusion bodies, containing noninfectious EB, would form. Perhaps, given the high concentrations of IFN-γ (i.e., 400–1600 U/mL) or the low tryptophan concentrations (<7.0 µg/mL) used in the recovery experiments, >96 h is required for a full recovery. It is also enticing to correlate t-tryptophan levels with levels of IFN-γ needed to inhibit replication; however, no direct measurements were made in these monolayers.

It is clear that *C. pneumoniae* exhibits susceptibilities to IFN-γ treatment and l-tryptophan concentrations similar to those described in detail for *C. trachomatis*, and this would appear to offer an in vitro correlate to what might occur in infected tissue. If *Chlamydia* species in general and *C. pneumoniae* in particular do participate in long-term, chronic, inflammatory-type disease states, they must possess some mechanism of survival in host tissue for extended periods of time. Although no replicative form of *Chlamydia* species other than those considered normal have been seen in host tissue to date, this does not preclude the possibility. Evidence continues to mount showing a strong physical association of *C. pneumoniae* and chronic infections, such as atherosclerosis, in humans. Studies in this laboratory are underway to determine whether growth-inhibitory characteristics of *C. pneumoniae* in response to IFN-γ pressure similar to those described here occur in a more relevant cell line, namely coronary artery smooth muscle cells. True causality must await further studies; however, the data presented here provide a potential mechanism of bacterial persistence in host tissue.

**Acknowledgment**

We thank Sam Welhausen for his assistance with the flow cytometry experiments.

**References**


17. Pfefferkorn ER. Interferon-γ blocks the growth of Toxoplasma gondii in human fibroblasts by inducing the host cell to degrade tryptophan. Proc Natl Acad Sci USA 1984;81:908–12.