Cytokines Induce Indoleamine 2,3-Dioxygenase Expression in Human Atheroma-Associated Cells: Implications for Persistent *Chlamydomphila pneumoniae* Infection

Jessica B. Sakash,^{1,2} Gerald I. Byrne,³ Andrew Lichtman,¹ and Peter Libby^{2*}

Vascular Research Division, Department of Pathology,¹ and Leducq Center for Cardiovascular Research,² Brigham and Women's Hospital, Boston, Massachusetts 02115, and Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin 53706³

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This study shows that vascular smooth muscle cells express significantly higher levels of gamma interferon-inducible indoleamine 2,3-dioxygenase (IDO) activity than endothelium or mononuclear cells. Since IDO activity is linked to persistent *Chlamydomphila pneumoniae* infection, our results suggest that smooth muscle cells may be an important reservoir of that organism in atherosclerosis.

Ample evidence indicates that T cells respond to local antigens and produce gamma interferon (IFN- γ) in atheroma (16). Recent attention has focused on the association of *Chlamydomphila pneumoniae* with lesion progression (5, 19, 25). *C. pneumoniae* localizes in human atherosclerotic lesions as assessed by electron microscopy (30) and by PCR and immunostaining (17, 18). Furthermore, *C. pneumoniae* can infect and proliferate within endothelial cells, smooth muscle cells, and macrophages in vitro (14, 15).

The ability of *C. pneumoniae* to survive chronically in vascular host tissue may derive from its capacity to establish a persistent infection. The persistent form of *C. pneumoniae*, characterized by enlarged abnormal forms, differs structurally and metabolically from the elementary and reticulate bodies (3, 10, 22). This obligate intracellular pathogen, like other chlamydial species, requires tryptophan for growth (7, 27). The mammalian enzyme indoleamine 2,3-dioxygenase (IDO) catabolizes tryptophan and can lower the concentration of this amino acid in host cells. Interestingly, IFN- γ , a cytokine present in atherosclerotic plaques, can regulate IDO expression in some cells. Numerous studies have investigated the effects of various cytokines on IDO regulation in multiple cell lines (4, 8, 11, 23, 24, 27, 33). IFN- γ -induced IDO activity can induce the persistent form of *C. pneumoniae* infection in cultured cells (26). This study compared the effects of IFN- γ on IDO activity in different types of human vascular wall cells, including saphenous vein endothelial cells (SVEC), saphenous vein smooth muscle cells (SVSMC), aortic smooth muscle cells (ASMC), and peripheral blood mononuclear cells (PBMC). SVEC, SVSMC, and ASMC were isolated as previously described (20). PBMC were isolated from healthy donors by plateletpheresis (6), followed by adherence to plastic culture flasks (2 h at 37°C) (21).

SVEC, SVSMC, ASMC, and PBMC were plated at a cell density of 1.5×10^5 cells/cm² in 96-well plates. Confluent

monolayers were overlaid with media containing IFN- γ (Endogen) from 0 to 800 U/ml. In some experiments, tumor necrosis factor alpha (TNF- α) (0 to 1,000 U/ml) (Endogen) was added 24 h later. After 72 h of incubation at 37°C, the medium was replaced with [³H]tryptophan pulse media containing 0.05 mM L-tryptophan (Sigma) and 1 μ Ci of L-5-[³H]tryptophan (New England Nuclides)/ml in Hanks balanced salt solution (Gibco BRL). Plates were incubated an additional 4 h at 37°C, after which the supernatants and cell lysates prepared by 10% trichloroacetic acid extraction were collected and frozen until analysis. Each data point was determined in triplicate for two different cell donors.

Catabolism of tryptophan to N-formylkynurenine and kynurenine was measured in culture supernatants and cell lysates by paper chromatography as described previously (28). With the exception of tryptophan oxidase in hepatocytes, IDO is the only enzyme that degrades tryptophan to kynurenine (32). The percentage of specific catabolism of tryptophan was calculated as previously described (8) using

FIG. 1. IFN- γ induction of tryptophan catabolism in vascular wall cells. Data represent means $\pm$ standard deviations of triplicate determinations from one of three experiments with similar results. The SVSMC and ASMC values were significantly greater than those for either PBMC or SVEC ($P < 0.05$ by analysis of variance) at all IFN- γ concentrations.

* Corresponding author. Mailing address: Leducq Center for Cardiovascular Research, Brigham and Women's Hospital, 221 Longwood Ave., Boston, MA 02115. Phone: (617) 732-6628. Fax: (617) 732-6961. E-mail: plibby@rics.bwh.harvard.edu.

FIG. 2. Levels of tryptophan catabolites in cell lysates and supernatants of cultures of ASMC (A) and SVEC (B) treated with 0 and 150 U of IFN- γ per ml for 72 h. One of three experiments with similar results is shown, and each data point represents the mean $\pm$ standard deviation of triplicate determinations.

the following equation: % specific catabolism = $(\text{cpm}_{\text{test}} - \text{cpm}_{\text{spontaneous}}) / (\text{cpm}_{\text{test}} - \text{cpm}_{\text{spontaneous}}) \times 100$.

The data in Fig. 1 show that IFN- γ induced approximately 45 and 50% of the catabolism of the total tryptophan in ASMC and SVSMC, respectively, while maximal IFN- γ -induced tryptophan catabolism in PBMC and SVEC was much lower, 25 and 7%, respectively. Supernatants of phytohemagglutinin-activated PBMC or CD4⁺ T cells also induced about six times as much IDO activity in ASMC as in SVEC (data not shown). We examined tryptophan catabolites in cell lysates to explore the possibility that the supernatant data reflected differences between cell types in their ability to transport IDO-generated tryptophan catabolites out into the medium, rather than differences in intracellular IDO activity. Analysis of cell lysates indicates that SVEC treated with 150 U of IFN- γ per ml retain more IDO-generated tryptophan catabolites than they transport into the medium, while the opposite is true for ASMC (Fig. 2). However, the increased levels of tryptophan catabolites in SVEC lysates compared to ASMC lysates do not quantitatively account for the difference between the catabolite levels found in the supernatants of ASMC and SVEC. Fur-

FIG. 3. The synergistic effect of TNF- α on tryptophan catabolism in ASMC and SVEC treated with 200 U of IFN- γ per ml. Data represent the means $\pm$ standard deviations of triplicate determinations from one of two experiments with similar results. No significant activity was seen in TNF- α -treated cells in the absence of IFN- γ . *, $P < 0.05$ by Student's t test.

thermore, Western blot analysis of lysates of IFN- γ -treated ASMC, SVEC, and PBMC indicated that IDO protein levels in these cells correlated with the levels of enzyme activity shown in Fig. 1 (data not shown). The untreated cells did not contain IDO protein. IFN- γ induction of IDO-specific mRNA was detectable in all three cell types by reverse transcription-PCR analysis, and untreated cells did not contain IDO-specific mRNA (data not shown).

Several studies have documented the various ways in which TNF- α and IFN- γ can act synergistically (1, 9, 12, 13, 29). Because TNF- α is found in atheromata and SMC both produce and respond to TNF- α (34), we investigated whether this cytokine could contribute to IDO induction. TNF- α alone did not significantly affect IDO induction (data not shown). However, in the presence of IFN- γ , TNF- α synergistically enhanced tryptophan catabolism in ASMC ($P < 0.05$), but not in SVEC (Fig. 3). This result agrees with previous studies of TNF- α and IFN- γ induction of IDO mRNA in epithelial cells (2) and IDO activity in macrophages (11). Furthermore, the two cytokines synergize to inhibit *C. pneumoniae* replication in Hep-2 cells (31).

The potential pathological effects of chronic infection of the vessel wall with *C. pneumoniae* underscore the importance of understanding the regulation of this organism's unique persistent developmental form. Studies of *Chlamydia trachomatis* persistence have pointed to a pivotal role of the lymphokine IFN- γ in augmenting the levels of the enzyme indoleamine 2,3-dioxygenase (32). This mammalian enzyme catabolizes the host tryptophan supply, inducing conversion of the bacterium to its persistent form (4). Therefore, our novel finding of the sensitivity of SMC to IFN- γ - and TNF- α -regulated IDO expression being greater than that of endothelium and monocytes suggests that intimal SMC in atherosclerotic lesions may provide a haven for persistent *C. pneumoniae* infection.

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