Induction of Indoleamine 2,3-Dioxygenase in Vascular Smooth Muscle Cells by Interferon-γ Contributes to Medial Immunoprivilege

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Atherosclerosis, the leading cause of mortality and morbidity worldwide, is characterized by inflammation, injury, and remodeling of the vessel wall. Coronary atherosclerosis results from atheromatous plaques that accumulate over decades, while an accelerated form of atherosclerosis may occur within months to years in transplanted hearts termed graft atherosclerosis. Immunohistological analyses of atherosclerotic lesions have revealed that the leukocytic infiltrate of the arterial wall is not uniform. Infiltration by T cells and macrophages predominates in the intima and adventitia, whereas the media is relatively spared (1–4). Similar findings of a relatively bland media have also been noted in experimental models of atherosclerosis and graft arteriosclerosis (5–7). The mechanism(s) for medial immunoprivilege is unknown, although it has been proposed that elastic laminae found in that arterial layer may prevent leukocyte trafficking (8).

Atherosclerosis and graft arteriosclerosis are characterized by leukocytic infiltration of the vessel wall that spares the media. The mechanism(s) for medial immunoprivilege is unknown. In a chimeric humanized mouse model of allograft rejection, medial immunoprivilege was associated with expression of IDO by vascular smooth muscle cells (VSMCs) of rejecting human coronary artery grafts. Inhibition of IDO by 1-methyl-tryptophan (1-MT) increased medial infiltration by allogeneic T cells and increased VSMC loss. IFN-γ-induced IDO expression and activity in cultured human VSMCs was considerably greater than in endothelial cells (ECs) or T cells. IFN-γ-treated VSMCs, but not untreated VSMCs nor ECs with or without IFN-γ pretreatment, inhibited memory Th cell alloresponses across a semipermeable membrane in vitro. This effect was reversed by 1-MT treatment or tryptophan supplementation and replicated by the absence of tryptophan, but not by addition of tryptophan metabolites. However, IFN-γ-treated VSMCs did not activate allogeneic memory Th cells, even after addition of 1-MT or tryptophan. Our work extends the concept of medial immunoprivilege to include immune regulation, establishes the compartmentalization of immune responses within the vessel wall due to distinct microenvironments, and demonstrates a duality of stimulatory EC signals versus inhibitory VSMC signals to artery-infiltrating T cells that may contribute to the chronicity of arteriosclerotic diseases. The Journal of Immunology, 2007, 179: 5246–5254.

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In other more classical sites of immune privilege in the body, initial notions of passive physical barriers have been supplanted by more recently discovered active biological processes. Medawar described the brain and anterior chamber of the eye as immunologically privileged sites due to an absence of lymphatics (preventing afferent immune responses) and blood vessels (preventing efferent immune responses), respectively (9). More recently, the immunoprivileged status of the brain and eye has been ascribed to immune deviation due to a number of factors in the local microenvironment, including neuropeptides, TGF-β, and Fas ligand (10, 11). Multiple cooperative systems also sanction the immune privilege of the fetus cohabiting within the mother. A unique mechanism that contributes to the immunoprivilege of the placenta is the expression of IDO by trophoblast cells (12). IDO, an IFN-γ-inducible, intracellular enzyme, catalyzes the first and rate-limiting step in oxidative catabolism of the essential amino acid, tryptophan along the kynurenine pathway (13). Treatment of pregnant mice with 1-methyl-tryptophan (1-MT), a pharmacologic agent that inhibits IDO activity, causes T cell-mediated rejection of allogeneic, but not syngeneic, fetuses (14). The immunomodulatory effects of IDO result from tryptophan depletion in the microenvironment which prevents T cell proliferation, promotes T cell apoptosis, induces T cell ignorance, anergy, or deviation, and generates regulatory T cells (15).

In the present study, we investigated whether medial immunoprivilege in graft arteriosclerosis results from an anti-inflammatory factor produced by the vessel wall. We find that the IFN-γ-inducible...
expression of IDO by human vascular smooth muscle cells (VSMCs) inhibits allogeneic T cell activation, proliferation, and accumulation in vitro and in vivo.

### Materials and Methods

#### Artery grafting

Segments of human epicardial coronary arteries from explanted hearts of cadaveric organ donors or cardiac transplant recipients were interposed into the infrarenal aortae of female, 8- to 12-wk-old, non-leany (serum IgG <1 μg/ml) SCID/beige mice (Taconic Farms) using an end-to-end microsurgical anastomotic technique as described (7). Human subject protocols were approved by the Yale Human Investigations Committee and the New England Organ Bank and animal procedure protocols were approved by the Yale Animal Care and Use Committee. At 1 wk postoperatively, certain animals received an adoptive transfer of 3 × 10^5 human PBMCs i.p. which were obtained by apheresis of healthy volunteers and isolated by density centrifugation. In selected experiments, mice received either 1-methyl-DL-tryptophan pellets s.c. that released 200 mg over 10 day periods from 1 to 4 wk postoperatively or similar doses of placebo pellets (Innovative Research of America). Retro-orbital blood samples were collected at 2 wk after reconstitution and analyzed for human CD3+ T cells and mouse CD45+ leukocytes as described (7). In other experiments, mice received Ad5.CMV-human IFN-γ or Ad5.CMV-LacZ (Qiagen) at 1 × 10^7 plaque-forming units i.v. at 1 wk postoperatively and circulating human IFN-γ was confirmed by ELISA (R&D Systems) after 2 wk (data not shown).

#### Graft analysis

Artery grafts were procured at 5 wk postoperatively (4 wk after PBMC or cytokine treatment) and analyzed by immunohistochemistry using mouse anti-human CD45RO, α-smooth muscle actin (α-SMA; DakoCytomation), and IDO (Chemicon International). Binding of secondary Ab (Jackson Immunoresearch) was detected with peroxidase/3-amino-ethyl carbazole kits (Vector Laboratories). Cell counting of nuclei surrounded by positive immunostaining was performed under high magnification and averaged from 5 cross-sections for each graft. The areas of vascular compartments were measured by computer-assisted microscopy and image software as previously described (7).

#### Cell isolation

Human endothelial cells (ECs) were isolated by enzymatic harvesting from umbilical cord veins and serially cultured in M199 medium (containing t-tryptophan at 49 μmol/L) supplemented with 20% FBS, 2 mmol/L L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Invitrogen), 50 μg/ml fibroblast growth factor-1 (Collaborative Research), and 100 μg/ml porcine intestinal heparin (Sigma-Aldrich). Human aortic or coronary artery VSMCs were isolated by explant outgrowth and serially cultured in M199 medium supplemented with 20% FBS, L-glutamine, and antibiotics. No phenotypic differences were detected between the two types of VSMCs and vascular cell cultures were used at passage 3 to 4.

Human CD4+ T cells were isolated by positive selection using Dynabeads (Dynal Biotech) and further purified by depleting recently activated T cells and naive T cells with anti-mouse IgG Dynabeads and mouse anti-human HLA-DR and CD45RA Abs (BD Pharmingen) at 10 μg/ml. Isolated cells were >95% CD45+ CD4+ T cells by FACS analysis (data not shown). T cells were cultured in RPMI 1640 medium (containing t-tryptophan at 24.5 μmol/L) supplemented with 10% FBS, L-glutamine, and antibiotics.

#### Cell culture

Coculture experiments were performed by placing 2 × 10^4 ECs or VSMCs in gelatin-coated wells of 24-well culture plates and where indicated treated with IFN-γ (Biosource International) at 100 ng/ml for 3 days, washed in medium, and both IFN-γ-treated ECs and VSMCs were confirmed to express class II MHC Ags before every experiment by FACS

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**Table 1. TRP and KYN plasma levels**

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<th>No PBMC +1-MT</th>
<th>PBMC +1-MT</th>
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<tr>
<td>TRP (μmol/L)</td>
<td>85.3 ± 4.3</td>
<td>81.4 ± 6.9</td>
<td>79.3 ± 5.8</td>
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<td>KYN (μmol/L)</td>
<td>0.45 ± 0.07</td>
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<td>0.54 ± 0.11</td>
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</tr>
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<td>KYN/TRP (×10^-3)</td>
<td>5.30 ± 0.72</td>
<td>6.03 ± 0.80</td>
<td>6.70 ± 1.23</td>
<td>9.16 ± 1.54</td>
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* SCID/beige mice bearing human coronary artery grafts were reconstituted or not with 3 × 10^5 human PBMCs i.p. after 1 wk and treated or not with 1-MT at 20 mg/day for an additional 4 wk. Tryptophan (TRP) and kynurenine (KYN) plasma levels were determined at 5 wk postoperatively. Data represent mean ± SEM (n = 3–5 in each group). Comparisons were by ANOVA, and differences between the groups did not reach statistical significance.
analysis (BD Biosciences) using a FITC-labeled mouse anti-human DR Ab (Immunotech). T cells were labeled with 250 nM CFSE (Molecular Probes) for 20 min, and 10^6 CD45RO^-/CD4^- T cells in 1 ml of supplemented RPMI 1640 medium was added to wells containing vascular cells. The cultures were maintained in 5% CO2 at 37°C for up to 9 days. T cell proliferation was assessed by FACS analysis of CFSE dilution after counterstaining with PE-labeled mouse anti-human CD4 Ab (Immunotech System). IL-2 supernatant levels were measured by ELISA (eBioscience) according to the manufacturer’s instructions. Certain coculture experiments were performed in advanced RPMI 1640 medium (an enriched formulation that enables cell growth in low serum concentrations) that was custom-ordered tryptophan-free (Invitrogen) and supplemented with 0.5% FBS and different doses of L-tryptophan, L-kynurenine, 3-hydroxy-DL-kynurenine, or 3-hydroxyanthranilic acid (Sigma-Aldrich).

**FIGURE 2.** Inhibition of IDO increases medial infiltration and VSMC loss. CD45RO (A), α-SMA (B), and IDO (C) expression were analyzed by immunohistochemistry in human coronary artery grafts of SCID/beige mouse recipients 4 wk after PBMC reconstitution and treatment with either placebo (left panels) or the IDO inhibitor, 1-MT (right panels). A similar analysis for α-SMA expression in artery grafts from unreconstituted hosts was also performed (B insets). The bar represents 100 μm for all panels. Medial CD45RO^-/α-SMA^- cells (D) and α-SMA^- cells (E) were counted in placebo-treated (open symbols) or 1-MT-treated (filled symbols) PBMC-reconstituted animals. Data are means ± SEM, n = 6, * p < 0.01 vs paired control grafts (t test).

**FIGURE 3.** IFN-γ induces IDO expression and activity in VSMCs. A, IDO expression was analyzed by immunohistochemistry in human coronary artery grafts of SCID/beige mouse recipients 4 w after i.v. infection with Ad-LacZ (left panel) or Ad-IFN-γ (right panel). The bar represents 100 μm for both panels. B, IDO protein expression was also analyzed by Western blotting of cultured VSMCs treated with IFN-γ for various times and at different doses and compared with the expression of β-actin loading control. IDO (C) and TrpRS transcripts (D), normalized to GAPDH mRNA, were determined by real-time RT-PCR in cultured ECs (open bars) and VSMCs (closed bars) after treatment with IFN-γ at different doses for 6 h. Levels of tryptophan (TRP; E and F), kynurenine (KYN; G and H), and a ratio of KYN/TRP (I and J) were determined by HPLC from the supernatants of cultured ECs (open bars) and VSMCs (closed bars) treated with IFN-γ at 30 ng/ml for various times (left panels) or at different doses for 48 h (right panels). Data represent single values and are representative of three independent experiments.
In experiments using the Transwell system, $2 \times 10^5$ ECs or VSMCs were placed in gelatin-coated 0.4-μm pore size membrane inserts (BD Biosciences) above the cell cocultures in an additional 0.5 ml of medium. In certain Transwell experiments, 1-methyl-D-tryptophan or L-tryptophan (Sigma-Aldrich), adjusted to pH 7.4, were added at 200 μmol/L on day 1, and 24.5 μmol/L daily, respectively. HPLC Tryptophan and kynurenine concentrations of plasma samples and culture supernatants were determined by HPLC. Tryptophan was monitored by its native fluorescence at 285 nm excitation and 360 nm emission wavelength, and kynurenine was detected by UV absorption at 365 nm wavelength in the same chromatographic run.

Western blotting

IFN-γ-treated VSMCs were lysed in radioimmunoprecipitation assay lysis buffer (20 mM Tris (pH 7.5), 1% Nonidet P-40, and Roche Complete protease inhibitor mixture). Equal amounts of protein per sample were separated by SDS-PAGE, transferred electrophoretically to a nitrocellulose membrane (Bio-Rad), and immunoblotted with primary Abs to β-actin or IDO (Chemicon) followed by HRP-conjugated secondary Abs (Jackson ImmunoResearch). Detection of the bound Ab by ECL (Pierce Biotechnology) was performed according to the manufacturer’s instructions.

Quantitative PCR

Total RNA was isolated from IFN-γ-treated and DNase-treated cells using NucleoSpin RNA II kits (Clontech Laboratories). Bulk reverse transcription with random hexamer primers was performed according to the Multi- tscribe RT system protocol (Applied Biosystems). RT-PCR were prepared with TaqMan 2 × PCR Master mix and predeveloped assay reagents for IDO, TrpRS, and GAPDH (Applied Biosystems). An iCycler and its system interface software (Bio-Rad) were used to run samples and analyze data. All cDNA samples were run in duplicate and a DNase-treated RNA sample processed without the reverse transcriptase enzyme was used as the negative control. The expression level of each target was normalized to that of GAPDH.

Statistical analyses

Student’s t test and one-way ANOVA were performed using the Prism software program (GraphPad Software). Differences with $p < 0.05$ were considered to indicate statistical significance.

Results

Medial Sparing by Allogeneic T cells is Associated with IDO Expression by VSMCs

We have previously reported an experimental model of graft arteriosclerosis of human coronary arteries interposed into the infrarenal aorta of SCID/beige mouse recipients reconstituted with allogeneic human PBMCs (7). The alloimmune-mediated arterial injury and remodeling is characterized by intimal and adventitial accumulation of effector T cells with relative sparing of the media (Fig. 1A). Enumeration of graft CD45RO cells confirmed a 5-fold greater density of memory T cells in the adventitia than the intima, which in turn had a 5-fold heavier inflammatory infiltrate than the media (Fig. 1D). The mildly inflamed media did not demonstrate a significant loss of VSMCs (Fig. 1, B and E) and the total area of the media remained unchanged (7) despite the variable medial thinning that occurred in association with the PBMC-induced...
outward vascular remodeling (increase in vessel diameter). We examined whether the media expressed immunoregulatory molecules that play a role in other immunoprivileged sites. Graft infiltration by allogeneic T cells was associated with the induction of IDO expression, particularly within the media (Fig. 1C), although the diffuse pattern of IDO immunostaining in frozen sections did not allow for further analysis of cellular expression details. Graft rejection and local up-regulation of IDO in the vessel wall did not perturb systemic levels of tryptophan and kynurenine in the xenogeneic hosts (Table I).

**Inhibition of IDO increases medial infiltration and VSMC loss**

To determine whether IDO was necessary for medial immunoprivilege, we treated pairs of SCID/beige mice that received adjacent segments of human coronary arteries and the same allogeneic human PBMCs with either a placebo or 1-MT. Host reconstitution by human T cells was not affected by 1-MT compared with placebo (6.8 ± 1.7 vs 5.0 ± 0.9% human CD3+ cells/mouse CD45+ cells, respectively, p = 0.4790). Treatment with 1-MT increased medial infiltration and decreased the expression of the VSMC marker, α-SMA (Fig. 2, A and B). VSMC loss did not occur in 1-MT-treated recipients in the absence of PBMCs (Fig. 2B insets). Cell counting verified a significant increase in medial T cell infiltration and VSMC loss resulting from 1-MT treatment (Fig. 2, D and E). There was also a trend to a higher density of intimal T cells in 1-MT-treated animals compared with placebo-treated controls, but the differences were more modest than that of medial infiltration (26 vs 83% increase, respectively) and did not reach statistical significance (data not shown). IDO expression within the graft was not modulated by 1-MT (Fig. 2C), and circulating levels of tryptophan and kynurenine did not differ between the treatment groups (Table I).

**IFN-γ induces IDO expression and activity in VSMCs**

We further investigated the regulation and activity of IDO in human vascular cells. Exposure to the T cell-derived cytokine, IFN-γ, in the absence of allogeneic PBMCs, was sufficient to induce the expression of IDO by graft VSMCs in vivo (Fig. 3A). IFN-γ also induced the expression of IDO protein and mRNA in coronary artery or aorta VSMCs in a time- and dose-dependent fashion (Fig. 3, B and C). Quantification of transcripts by real-time RT-PCR demonstrated that the IFN-γ-mediated induction of IDO in VSMCs was considerably greater than in umbilical vein ECs. Similarly, the up-regulation of tryptophanyl-tRNA synthetase (TrpRS), an enzyme required for tryptophan incorporation in protein synthesis, was also far greater in VSMCs than ECs (Fig. 3D). There was minimal, if any, induction of IDO and lesser up-regulation of TrpRS in peripheral blood CD4+ Th cells compared with ECs (data not shown). We confirmed that the greater induction of IDO expression in VSMCs than in ECs correlated with orders of magnitude greater IDO activity as measured by tryptophan depletion, kynurenine production, and a ratio of metabolite to precursor (Fig. 3, E–J).

**IFN-γ-treated VSMCs do not activate allogeneic memory T helper cells**

We compared the capacity of cultured vascular cells to activate allogeneic human T cells. We have previously reported that IFN-γ-treated ECs express MHC class II molecules, but not IFN-γ-treated, MHC class II Ag-expressing VSMCs, induced CD4+ T cell proliferation as assessed by [3H]thymidine uptake (16). We confirmed these results using untreated or IFN-γ-pretreated vascular cells (Fig. 4A) and CFSE-labeled, memory CD45RO+CD4+ T cells in a coculture system. A subset of alloreactive CD45RO+CD4+ T cells proliferated in response to IFN-γ-pretreated, MHC class II Ag-expressing ECs as determined by a progressive increase in the CFSElow population of T cells after 7, 8 (data not shown), and 9 days (Fig. 4B). In contrast, untreated ECs, untreated VSMCs, and IFN-γ-pretreated VSMCs, which express comparable levels of MHC class II molecules as IFN-γ-pretreated ECs, did not activate allogeneic memory Th cells (Fig. 4B). Similarly, IFN-γ-pretreated ECs, but not VSMCs, stimulated IL-2 production by CD45RO+CD4+ T cells after 2 days of coculture (Fig. 4C). The lack of IL-2 secretion and T cell proliferation by IFN-γ-pretreated VSMCs was associated with greater tryptophan depletion and kynurenine production than similarly treated ECs (Fig. 4, D–F).

**IFN-γ-treated VSMCs inhibit memory T cell activation by allogeneic ECs**

We next examined whether IFN-γ induces an inhibitor of T cell activation by VSMCs using a Transwell system. IFN-γ-pretreated VSMCs suspended within a semipermeable membrane insert markedly inhibited the proliferation of CD45RO+CD4+ T cells cocultured with IFN-γ-pretreated, allogeneic ECs (Fig. 5, A and B). The expression of IDO by IFN-γ-pretreated VSMCs inhibited T cell alloresponses by ECs, although the inhibition of IFN-γ-pretreated VSMCs was not as marked as that of IFN-γ-pretreated ECs (Fig. 5, C and D). In contrast, untreated ECs, untreated VSMCs, and IFN-γ-pretreated ECs, which express comparable levels of MHC class II molecules as IFN-γ-pretreated VSMCs, did not inhibit the proliferation of alloreactive CD45RO+CD4+ T cells (Fig. 5, E and F).
FIGURE 6. Suppressor activity of VSMCs is dependent on IFN-γ-induced IDO. No cells, untreated (−), or IFN-γ-pretreated (+) VSMCs were placed within semipermeable membrane Transwell inserts overlying CFSE-labeled CD45RO+/CD4⁺ T cells cocultured with allogeneic untreated (−) or IFN-γ-pretreated (+) ECs in the absence (open bars) or presence (filled bars) of 1-MT. After 9 days, the cells at the bottom of the wells were labeled with CD4-PE and analyzed by flow cytometry (A and B). Supernatants were also removed after 2 days and analyzed for IL-2 (C) and kynurenine/tryptophan (KYN/TRP) ratios (D). Similarly, the cells were cultured across Transwells in the absence (open bars) or presence (filled bars) of l-tryptophan (TRP) and analyzed for T cell proliferation as evidenced by CFSE dilution after 9 days (E). Data are means ± SEM; n = 5; *, p < 0.01 all vs EC− control; †, p < 0.05 VSMC+ vs EC−; ††, p < 0.01 VSMC+ vs VSMC−; and †††, p < 0.01 1-MT vs vehicle (ANOVA).

Suppressor activity of VSMCs is dependent on IFN-γ-induced IDO

We then tested if induction of IDO activity by IFN-γ was necessary for the contact-independent immunosuppressive effect of VSMCs. Strikingly, addition of 1-MT reversed the inhibitory effect across a semipermeable membrane of IFN-γ-pretreated VSMCs on the proliferation of CD45RO⁺/CD4⁺ T cells cocultured with IFN-γ-pretreated, allogeneic ECs below (Fig. 5C). Inhibition of T cell activation by IFN-γ-pretreated VSMCs was associated with significant tryptophan depletion and kynurenine production (Fig. 5D).

Tryptophan depletion is not sufficient for T cell anergy to allogeneic VSMCs, but prevents T cell alloreponses to ECs

We also investigated whether IFN-γ-inducible IDO activity was responsible for the absence of T cell alloreponses to MHC class II Ag-expressing VSMCs. Treatment with 1-MT or supplementation with 1-tryptophan did not result in proliferation of CD45RO⁺/CD4⁺ T cells cocultured with IFN-γ-pretreated, allogeneic VSMCs (Fig. 7, A and B).

Finally, we examined if tryptophan depletion or metabolite production was more important in IDO-mediated immunosuppression using the coculture system. Tryptophan supplementation increased the proliferation and IL-2 secretion of alloreactive, memory Th cells in a dose-dependent fashion in tryptophan-deficient custom medium (Fig. 7, C and D). In contrast, kynurenine treatment did not significantly affect T cell clonal expansion or cytokine production in tryptophan-replete conventional medium (Fig. 7, E and F). Similarly, other tryptophan metabolites, such as 3-hydroxykynurenine and 3-hydroxyanthranilic acid, did not influence the activation of CD45RO⁺/CD4⁺ T cells cocultured with IFN-γ-pretreated, allogeneic ECs (data not shown).

Discussion

We describe the regulation of adaptive immune responses by vascular cells through IFN-γ-inducible expression of IDO in human VSMCs that prevents T cell activation and clonal expansion in response to allogeneic ECs in vitro. This bidirectional interaction inhibits the accumulation of T cells within the medial compartment in vivo in a humanized model of graft arteriosclerosis that is dependent on IFN-γ responses (7). We have identified an anti-inflammatory factor expressed by VSMCs that we had predicted from earlier work (5, 16). However, because IDO inhibition was not sufficient to enable T cell activation in response to IFN-γ-pretreated VSMCs in coculture, an additional contact-dependent inhibitory molecule may be expressed on the cell surface of VSMCs or alternatively VSMCs lack a nonredundant, unidentified costimulatory molecule. We have not as yet investigated for possible immune deviation, although we have previously reported that...
CD4+ T cells initially exposed to IFN-γ-pretreated VSMCs subsequently proliferated with a similar pattern and magnitude to ECs from the same donor vs freshly isolated T cells and that serologic neutralization of TGF-β did not relieve the suppressive effects of VSMCs in coculture with T cells (16).

Our results suggest that the inhibition of T cell alloresponses to ECs by IDO-expressing VSMCs is predominantly due to tryptophan deprivation rather than generation of toxic metabolites. T cells were not activated by allogeneic EC-T cell cocultures were also performed in tryptophan-deficient medium supplemented with 10% or 0.5% serum and different concentrations of t-tryptophan (C) or in tryptophan-replete medium supplemented with 10% serum and different concentrations of kynurenine (D). IL-2 levels were also measured at 2 days after t-tryptophan (E) and kynurenine (F) supplementation. Data are means ± SEM; n = 3–8; *, p < 0.01 all vs EC− control (ANOVA).

Depletion of tryptophan in the microenvironment inhibits T cell activity without exerting overt negative effects on IDO-expressing regulatory cells (15). There are several possible reasons for the differential sensitivity to deprivation of an essential amino acid. First, activated T cells undergo massive clonal expansion and frequently dividing cells require a higher rate of protein synthesis. Second, the rate of transmembrane transport is a limiting step in tryptophan metabolism (23) and a putative high-affinity transporter of tryptophan, defined only as a biochemical activity at present, is

![Figure 7](http://www.jimmunol.org/Downloadedfrom)
expressed by APCs that may preferentially take up available amino acid under suboptimal extracellular concentrations (24). Third, tryptophan incorporation into protein biosynthesis by the aminoacyl-tRNA synthetase, TrpRS may also be induced by IFN-γ and compensate for a reduction in intracellular tryptophan (25). We found a direct correlation between IDO and TrpRS transcript induction by IFN-γ in vascular cells and CD4⁺ T cells and others have noted a similar differential regulation of IDO and TrpRS by IFN-γ in nonhemopoietic and myeloid vs lymphoid cell lines (26). Finally, paracrine IDO activity may induce different signaling effects in target cells, such as activation of the stress kinase, general control nondepressible-2 in T cells (27).

The inducible expression of IDO has been described in diverse cell types and IDO dysregulation has been implicated in various animal models of disease and several clinical disorders (25). In studies related to microbial infection, IFN-γ-inducible IDO activity was detected at significantly higher levels in human VSMCs than in ECs or PBMCs (28) and the IFN-γ-dependent resistance to certain pathogens in human vascular cells was dependent on IDO activity (29–32). In transplantation-related studies, IFN-γ-induced IDO expression and activity was highest in umbilical vein ECs and barely detectable in saphenous vein ECs or somatic artery ECs (33). In contrast to our findings, Beutelspacher et al. reported that addition of 1-MT to allogeneic T cell-umbilical vein EC cell cocultures did increase cellular proliferation (33); however their system differs from ours in a number of conditions, including the use of unfractionated T cells, pooled ECs from multiple donors, and assessment of proliferation by [3H]thymidine incorporation. The T cell suppressor activity by IFN-γ-treated ECs in this study was judged submaximal as overexpression of IDO in ECs further diminished T cell proliferation and also induced T cell apoptosis and anergy. Our findings of the relatively low expression and activity of IDO in ECs, compared with VSMCs, may explain why we see that stimulatory functions of umbilical vein ECs predominate over inhibitory interactions and result in net activation of allogeneic T cells (16). The paradigm of IDO overexpression has been successfully used in animal transplantation models to prolong pancreatic islet, lung, and coronary allograft survival (34–36), even though inhibition of IDO had no effect on allograft survival (33). We have recently described increased IDO activity as a marker of IFN-γ responses in patients with coronary atherosclerosis or acute rejection of allografts (37, 38), however the role of IDO in vascular inflammation was not determined in these or other studies. Our findings in human VSMCs, ECs, and arteries may not necessarily apply to murine systems due to species differences in vascular cell interactions with T cells. MHC class II Ag-expressing human ECs can directly activate allogeneic memory CD4⁺ T cells (39), whereas IFN-γ-treated murine ECs cannot (40). Conversely, murine VSMCs appear to be immunogenic and can activate T cells to produce IFN-γ and mediate vasculitis (41, 42).

The concept of medial immunoprivilege has been previously described in the context of host defense against pathogens unlike the classical definition of immune privileged sites in terms of allograft rejection. Infection with γ-herpesvirus 68 or cytomegalovirus causes vasculitis of elastic arteries in mice that is more severe and chronic in the absence of IFN-γ responses (43, 44). The persistent infection of VSMCs is due to inefficient clearance of virus associated with a failure of T cells and macrophages to enter the medial compartment and the investigators postulated that this may reflect a fundamental property of elastic laminae to restrict trafficking of leukocytes (8). Furthermore, genetic absence of IFN-γ receptors in vascular cells, but not leukocytes, and serologic neutralization of IFN-γ increased medial infiltration and necrosis (8). These findings were interpreted as demonstrating a protective role of IFN-γ in vascular infection and, interestingly, IDO has been shown to inhibit the replication of herpes simplex virus and cytomegalovirus (31, 45). Our results suggest an additional possible explanation of loss of an IFN-γ-inducible anti-inflammatory factor in medial VSMCs that normally inhibits the accumulation of T cells.

It is important to note that medial immunoprivilege is a relative phenomenon. Transmural arterial inflammation and medial necrosis can occur in robust acute rejection episodes (46), and the diagnosis of transmural arteritis portends a poor outcome in acute rejection of cardiac and renal allografts (47, 48). Similarly, panarteritic infiltration and medial destruction is characteristic of certain vasculitides (49, 50). It is not surprising that the media is capable of recruiting leukocytes under certain conditions as VSMCs may be induced by cytokines to express many immunogenic and proinflammatory molecules (5, 16). We hypothesize that medial immunoprivilege becomes manifest when a balance is achieved between the limited resistance of the media to inflammation vs the indolent IFN-γ-producing immune responses characteristic of atherosclerosis and graft arteriosclerosis. Although, other proinflammatory factors have been reported to induce vascular inflammation and the expression of IDO (25), we believe that the evidence for a pathogenetic role for IFN-γ in atherosclerosis and graft arteriosclerosis is particularly compelling (51). In keeping with our hypothesis for preferential IDO-mediated suppression of T cells by VSMCs, we have found a relatively uniform transmural infiltration of CD68⁺ macrophages in human coronary artery grafts in SCID/beige mice reconstituted with human peripheral blood CD34⁺ hematopoietic stem cells (unpublished observations).

In conclusion, our work extends the understanding of medial immunoprivilege from immune isolation to immune regulation and supports the concept of distinct immunological responses within separate vascular compartments and microenvironments. The chronicity of arteriosclerotic diseases may in part result from the duality of stimulatory EC signals versus inhibitory VSMC signals to artery-infiltrating T cells. Finally, enhancement of the natural resistance of the vascular wall to inflammation may represent a novel strategy for treatment of atherosclerosis and graft arteriosclerosis.

Disclosures
The authors have no financial conflict of interest.

References